Ageing International (2024) 49:249–262
https://doi.org/10.1007/s12126-023-09538-5

Clinical Significance of Salivary Alpha-amylase Activity in Patients With Parkinson's Disease

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Accepted: 4 November 2023 / Published online: 11 December 2023

Abstract

Salivary alpha-amylase (sAA) is known as a stress indicator for the general adult population. However, its clinical significance has not been established in patients with neurodegenerative diseases. The subjects consisted of the PD group (n = 146), the control (C) group (n = 109) and the age-matched control (AC) group (n = 79). We explored what clinical indicators are associated with sAA in patients with Parkinson's disease (PD). Results of factor analysis and stepwise multiple linear regression analysis demonstrated that NRS remained as the only factor related to sAA ($p < 0.05$). However, sAA was strictly related to age in the two control groups ($p < 0.01$). There was no significant difference in sAA between the PD and AC groups ($p < 0.05$). The results indicated that sAA could be an indicator of mental stress in patients with PD. In contrast, in the C group, sAA seemed to be mainly influenced by sympathetic tone associated with ageing.

Keywords Parkinson's disease · Salivary alpha-amylase · sAA · Aging · Daily stress · Normal subjects

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Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the slow progression of characteristic motor symptoms. In recent years, attention has been focused on non-motor symptoms such as psychiatric symptoms, cognitive deficits, and autonomic dysfunction. Psychiatric symptoms, including depression, apathy, fatigue, and impulse control disorders have a significant impact on the quality of life in PD (Kadastik-eerme et al., 2015; Valentino et al., 2018; Baig et al., 2019). Neuropsychiatric symptoms are more common in patients with Parkinson's disease including depression (58%), apathy (54%), anxiety (49%) and hallucinations (44%) (Aarsland et al., 2007). Autonomic dysfunction has emerged as an important aspect of nonmotor dysfunction, which covers a broad spectrum that includes cardiovascular, gastrointestinal, urological, sexual, and thermoregulatory dysfunctions in both the early and later stages of PD (Pfeiffer, 2020). Both sympathetic and parasympathetic involvements are present in PD, and pathology involves both peripheral and central components of the autonomic nervous system (Djaldetti et al., 2009).

Recent technological advances have increased healthcare professionals' interest in biomarkers for the diagnosis and prediction of therapeutic efficacy. Salivary biomarkers have gained wide popularity as they facilitate easy, non-invasive and rapid collection of samples compared to blood and urine samples (Kauffmann & Lamster, 2002; Rohleder et al., 2004; Yamaguchi et al., 2004). Salivary alpha-amylase (sAA) has emerged as a surrogate marker of acute stress corresponding to sympathetic activation (Ali & Nater, 2020); sAA levels increase acutely in response to physical (Chatterton et al., 1996) as well as psychological stressors (Rohleder et al., 2004; Yamaguchi et al., 2004; Nater et al., 2006; Nater et al., 2005). However, sAA may be affected by other factors such as diurnal variation, gender, and age (Almela et al., 2011; Ali & Nater, 2020). There are even reports of decreased sAA in youths with depression (Jezova et al., 2020). In healthy subjects or patients, sAA alone has not yet become a specific clinical indicator of any kind, and several studies have concurrently measured salivary biomarkers other than sAA (Ali & Pruenssner, 2012; Chaturvedi et al., 2018). Therefore, sAA is influenced not only by autonomic nervous system activity but also by a variety of other factors. Currently, it is unknown how sAA changes in neurodegenerative diseases presenting with autonomic neuropathy. We investigated the clinical significance of sAA in PD patients. What clinical indicators does sAA reflect in PD patients? Does sAA decrease or increase in patients with PD complicated by autonomic dysfunction? Do sAA values of PD patients differ from those of healthy subjects? Does sAA reflect mental stress in healthy subjects and the patients? The present study is designed to answer these questions.

Material and Methods

Subjects

Participants were enrolled into two groups: control (C) and PD groups. Participants in the control group were recruited by a letter posted at Tokushima Hospital. Patients and their families who were hospitalized at Tokushima Hospital, Kamojima Hospital,

and older adults care facilities were also invited to participate in the study. The eligibility criteria for control subjects were 1) no obvious dementia, a Mini-Mental State Examination (MMSE) score of 24 or higher, 2) no complications of underlying disease, and 3) aged from 20 to 90 years. Patients with PD were recruited from inpatients or outpatients at Tokushima National Hospital. The eligibility criteria for such patients were 1) fulfilling the diagnostic criteria for PD according to the Parkinson's Disease Society of the UK Brain Bank (Hughes et al., 1992), 2) willingness to participate in this study, and 3) no obvious dementia and an MMSE score of 24 or higher. In addition, the absence of recent troubles (e.g., death of an acquaintance, quarrel with a close relative) that come to mind immediately was a common inclusion criterion for both groups. The study was conducted from September 2019 to December 2020. Due to the different age distribution between the C and the PD groups, we classified the subjects in the C group who were in the same age group as the PD group into the age-matched control (AC) group after the inclusion was complete.

Salivary Alpha-Amylase (sAA) Assay

We used the Salivary Amylase Monitor (Nipro Co., Japan), a medical device that received medical approval from the Ministry of Health, Labour, and Welfare (Japan) in 2007. The salivary amylase assay kit consists of a disposable test strip and the main unit of the device. Saliva was sampled by directly immersing a saliva-sampling strip in saliva under the tongue for 30 s. The strip was immediately placed in an automatic saliva transfer system, and saliva was transferred to the alpha-amylase test paper on the reverse side of the strip sleeve by compression. The alpha-amylase test paper contained the substrate 2-chloro-4-nitrophenyl-4-O-b-D-galactopyranosylmaltoside. The enzyme reaction started upon transfer by compression, and the level of free 2-chloro-4-nitrophenyl was optically measured after 20 s. When a test strip containing at least 28 µl of saliva is inserted into the assay device, alpha-amylase activity is displayed after 20 s (Nakano & Yamaguchi, 2011).

Subjective Stress Assessment

The present study employed the Numerical Rating Scale (NRS) as subjective stress assessment in the PD, C, and AC groups. Originally used as a subjective pain scale, the NRS has been used as a measure of fear and mental fatigue (Lu et al., 2020; Morimoto et al., 2019). The scale ranges from 0 to 10, where 0 represents no stress and 10 represents the worst possible stress (Karvounides et al., 2016). We adopted the NRS as an indicator of chronic and general stress in daily life. We asked them how much stress they were feeling now, if the strongest stress they had ever experienced was 10. In addition, the Self-rating Depression Scale (SDS) and the General Health Questionnaire (GHQ) 12 were also examined in the PD group. The SDS is a screening test used to assess the degree of depression in patients. Responses to the 20-question questionnaire are assessed using a four-item scale. Scores range from 20 to 80, with higher scores indicating a greater depressive state (Zung, 1965). The

GHQ is a useful test for understanding, assessing, and screening for minor psychiatric disorders and is used in a variety of settings. The original version of the GHQ had 60 items, while the modified version has a variety of shorter items. We adopted the 12-item version of the GHQ, which is easy to test and widely used in various fields such as medical care, the public sector, and welfare (Matsuzaki et al., 2007; Goldberg et al., 1997). We used the 0-0-1-1 GHQ scoring method to calculate the total score of the GHQ12. On a scale from 0 to 12, higher scores indicate lower mental health scales (Doi & Minowa, 2003).

Cognitive Function Assessment

The Mini Mental State Examination (MMSE) and Frontal Assessment Battery (FAB) were employed to assess cognitive functioning. The MMSE is a widely used assessment of overall cognitive performance and consists of five sections (orientation, registration, attention and calculation, recall, and language). Scores range from 0 to 30, with 23 indicating possible dementia (Folstein et al., 1975). FAB was used to assess performance in executive functioning. The FAB consisted of six subtests (similarities, lexical fluency, Luria motor sequences, conflicting instructions, go-no-go test, and prehension behavior). Scores range from 0 to 18, with lower scores indicating a higher degree of executive dysfunction (Dubois et al., 2000).

Unified Parkinson's Disease Rating Scale (UPDRS)

The UPDRS includes subscales of mental function, activities of daily living, and motor function; total scores on the scale range from 0 to 176, with higher scores indicating a more severe disease with PD (Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease, 2003).

Difference between supine and standing systolic blood pressure (orthostatic hypotension (OH) score).

We used the OH score as an indicator of cardiovascular sympathetic function. The Schellong test was employed to determine OH in patients whose systolic blood pressure decreased by 20 mmHg or more in a standing position. Since orthostatic hypertension has been reported to be associated with a variety of other clinical conditions such as essential hypertension, dysautonomia, and type 2 diabetes mellitus (Kario, 2013; Fessel & Robertson, 2006). Therefore, OH scores below -20 were treated as missing values in patients with orthostatic hypertension.

Outcome and Statistical Analyses

In PD we set nine candidate factors to search for factors associated with sAA, including Age, NRS, SDS, GHQ12, MMSE, FAB, UPDRS, Duration of disease, and OH score. We tried to identify the factors that co-grouped with sAA by factor analysis of all 10 variables. Next, we performed a multiple linear regression analysis to identify

factors associated with sAA within the identified group. We then set up two control groups (C/AC groups) for comparison with PD and obtained three variables in the control groups, includingsAA, Age, and NRS.We compared the sAA, Age, and NRS scores between PD group and C/AC group using the Mann–Whitney U-test. In addition, the association of sAA with age and NRS was also analysed using multiple linear regression analyses in the C/AC groups. The collected data were analysed using SPSS Statistics for Windows, version 26.0 (IBM).

Results

The number of subjects who met the inclusion criteria for this study and participated in the study was 146 in the PD group. Table 1 shows the summary of clinical data in the PD group, which included Age (69.1 (67.8–70.4)) (mean (95% CI)), sAA (54.2 (47.0–61.4)), NRS (4.2 (3.9–4.5)), OH score (10.4 (6.6–14.2)), GHQ12 (2.7 (2.2–3.2)), MMSE (28.7 (28.4–29.0)), FAB (12.8 (12.3–13.3)), SDS (38.5 (37.1–39.9)), UPDRS (66.5 (61.5–71.5)), and Duration of disease (6.9 (6.2–7.6)). We assessed the structural validity of the clinical data analysis to classify them according to their characteristics. After removing the three variables with factor loadings greater than one, factor analysis was conducted on the remaining seven variables including FAB, MMSE, OH score, NRS, sAA, Age, and GHQ12 (Table 2). Since sAA belonged to the same group as NRS, Age and GHQ12, we conducted a stepwise linear multiple regression analysis with sAA as the dependent variable and NRS, Age, and GHQ12 as independent variables. Only NRS was found to be predictive of sAA ($\beta = 0.175$, 95%CI = 0.052 to 7.191, $p = 0.047$). Spearman's correlation showed that sAA and NRS were clearly related ($rs = 0.22$, $p = 0.009$) (Fig. 1A). The results of the regression analysis excluded GHQ12, in which the probability of F

Table 1 Descriptive data in Parkinson's disease (PD) group

Factors	N	mean	95%CI
Age	146	69.1	67.8–70.4
sAA	146	54.2	47.0–61.4
NRS	146	4.2	3.9–4.5
OH score[a,b]	103	10.4	6.6–14.2
GHQ12	129	2.7	2.2–3.2
MMSE[c]	113	28.7	28.4–29.0
FAB	113	12.8	12.3–13.3
SDS	110	38.5	37.1–39.9
UPDRS	114	66.5	61.5–71.5
Duration of disease	129	6.9	6.2–7.6

[a]Difference between supine systolic blood pressure and standing one (Orthostatic hypotension (OH) score)

[b]Elevated blood pressure of 20 mmHg or more after standing (orthostatic hypertension) was excluded

[c]Only those with a score of 24 or higher were be accepted

Table 2 Factor analysis of seven variables in PD (factor loading)

	Factor 1	Factor2	Factor3
FAB	.840	.017	.045
MMSE	.684	.001	-.130
OH score	-.049	-.890	-.010
NRS	-.140	-.061	.421
sAA	-.037	.043	.385
Age	.156	-.062	.304
GHQ12	-.052	.132	.283

was higher than 0.25. We therefore examined whether the relationship between sAA and NRS or Age was different between the C and PD groups.

Because the age distribution of patients in the PD and C groups was quite different (PD group, 47–87; C group, 22–87), we selected subjects from the C group with the same age range as the PD group and set up an AC group. The PD group consisted of 146 patients (72 men and 74 women) with a mean age of 69.1 years (67.8–70.4) (95% CI), whereas the C group consisted of 109 patients (38 men and 71 women) with a mean age of 57.5 years (54.2–60.8), and the AC group consisted of 76 patients (32 men and 44 women) with a mean age of 67.2 years (64.9–69.5) years. The results of Age, sAA, and NRS scores in the PD and C/AC groups are presented in Table 3.

Although the PD group were markedly older than the C group ($p < 0.0001$), they were similar to the AC group ($p = 0.3$). sAA of PD group (54.2 KU/L (61.4–47.0)) (mean (95% CI)) was higher than that of C group (43.3 KU/L (50.5–36.1)) ($p = 0.02$), but not AC group (48.7 KU/L (58.1–39.3)) ($p = 0.4$). The NRS of the PD group (4.2 (4.5–3.9)) was lower than that of the C group (4.9 (5.3–4.5)) ($p = 0.009$) and AC group (5.0 (5.5–4.5)) ($p = 0.007$). There was no significant difference between males and females in the sAA of each group (PD group, $p = 1.0$; C group, $p = 0.4$; AC group, $p = 0.5$) (data not shown). The results of the multiple linear regression analysis for sAA are presented in Table 4. sAA in the PD group was significantly related to NRS ($\beta = 0.203$, 95% CI = 0.832 to 7.254, $p = 0.014$) but not to age ($\beta = 0.114$, 95% CI = -0.247 to 1.429, $p = 0.165$). In contrast, sAA was strictly related to age in the C/AC groups (C group: $\beta = 0.361$, 95% CI = 0.397–1.179, $p = 0.000$) (AC group: $\beta = 0.336$, 95% CI = 0.459–2.238, $p = 0.003$) but not in the PD group. However, sAA was not significantly related to NRS in either group (C group: $\beta = -0.069$, 95% CI = -4.047–1.807, $p = 0.45$) (AC group: $\beta = -0.047$, 95% CI = -4.883–3.162, $p = 0.671$). The results of Spearman's correlation demonstrated that sAA significantly related to Age in the AC group (Fig. 1B) ($rs = 0.33$, $p = 0.005$).

Discussion

The nervous system's response to stress includes stimulation of the hypothalamus and brainstem, activation of the hypothalamic–pituitary–adrenal axis and the autonomic nervous system, which includes the efferent sympathetic–adrenal–medullary

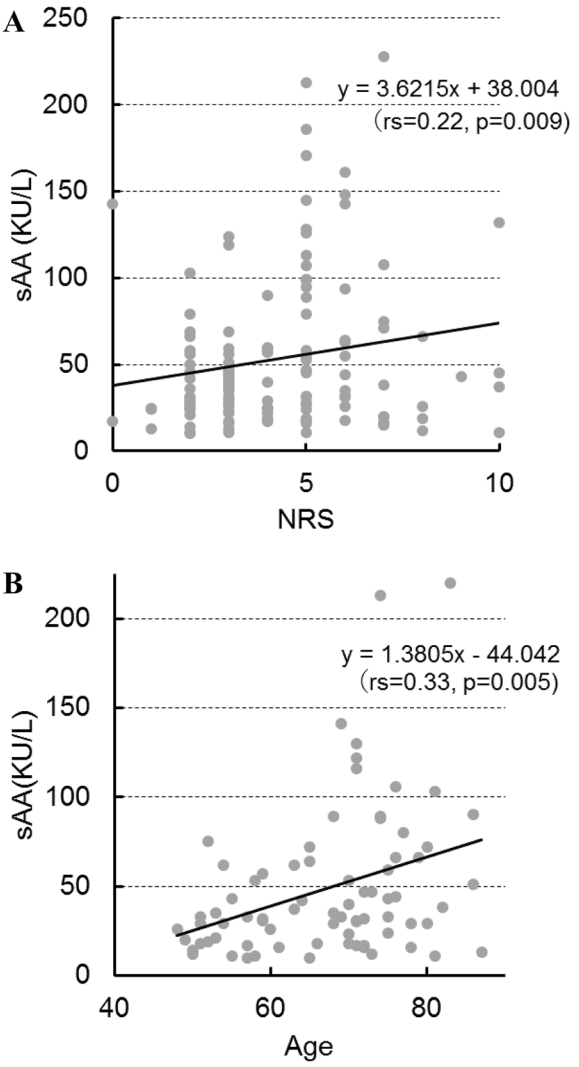

Fig. 1 Correlation of sAA and NRS in Parkinson's disease (PD) group (A) and that of sAA and Age in age-matched control (AC) group (B). **A** In PD group, sAA was correlated with NRS (rs = 0.22, p = 0.009). **B** In AC group, sAA was significantly correlated with Age (rs = 0.33, p = 0.005)

system. The salivary biomarkers of stress, anxiety or depression include cortisol, immunoglobulin A, lysozyme, melatonin, α-amylase, chromogranin A and fibroblast growth factor 2 (Choinowska et al., 2021). In addition, hair cortisol has received particular attention as a biomarker of chronic stress (Wang et al., 2015; Zhang et al., 2018; Russell et al., 2012; Van uum et al., 2008). The relationship between the above stress markers and the autonomic nervous system, hypothalamic–pituitary–adrenal axis and immune/inflammatory system has been extensively studied using various statistical methods (Lucas et al., 2017; Reeves et al., 2016; Sanada et al., 2020). Most of the studies have been done on mammals or healthy subjects, and few have been done on patients. The majority of the diseases studied are psychiatric diseases.

Table 3 Comparison of Age, sAA, and NRS in the PD group, control (C) group and age-matched control (AC) group

	C group	p^a	PD group	p^b	AC group
N	109		146		76
Age (mean)	57.5	< 0.0001	69.1	0.3	67.2
(95% CI)	54.2–60.8		67.8–70.4		64.9–69.5
sAA (mean)	43.3	0.02	54.2	0.4	48.7
(95% CI)	36.1–50.5		47.0–61.4		39.3–58.1
NRS (mean)	4.9	0.009	4.2	0.007	5.0
(95% CI)	4.5–5.3		3.9–4.5		4.5–5.5

[a]C group vs PD group

[b]PD group vs AC group

Recent biosensor technology has enabled the development of a small, inexpensive, and accurate instrument for measuring sAA. Using the device, sAA can be measured with only 28 µl of saliva, and the time from saliva collection to measurement is only one min. The levels of sAA were measured using the dry chemistry system. This system, developed in 2004, consisted of three devices, the salivary transcription device, a testing-strip and an optical analyzer (Yamaguchi et al., 2004). By adding maltose as a competitive inhibitor to a substrate Ga1-G2-CNP, a broad-range activity testing-strip was fabricated that could measure the salivary amylase activity with a range of 0–200 kU/l within 150 s. The calibration curve of the monitor for the salivary amylase activity showed R2 = 0.941, indicating that it was possible to use this monitor for the analysis of the salivary amylase activity without the need to determine the salivary volume quantitatively (Aoyagi et al., 2011). The hand-held monitor and the lab assay showed a significant positive correlation and the hand-held monitor suggested to be highly reliable for measuring the sAA level (Aoyagi et al., 2011). Using this system, there have been many reports that sAA activity was reportedly corelated to acute mental stress and sAA was significantly higher in the patients with schizophrenia and was significantly correlated to psychiatric symptoms (Inagaki et al., 2010; Matsubara et al., 2011; Arai et al., 2009).

Table 4 Association of sAA with Age and NRS using multiple linear regression model

Group	**Coefficient**	**Factors**	
		Age	NRS
PD	β	0.114	0.203
	95% CI	-0.247 to 1.429	0.832 to 7.254
	p	0.165	0.014
C	β	0.361	-0.069
	95% CI	0.397 to 1.179	-4.047 to 1.807
	p	0.000	0.450
AC	β	0.336	-0.047
	95% CI	0.459 to 2.238	-4.883 to 3.162
	p	0.003	0.671

Various biomarkers have been used for the early diagnosis of PD, but little is known about their psychological biomarkers (Lotankar et al., 2017). While sAA is certainly an indicator of acute stress with sympathetic activation, it is controversial as an indicator of chronic stress (Ali & Nater, 2020). Salivary secretion is mainly controlled by the parasympathetic nervous system. In contrast, sAA is mainly controlled by the sympathetic nervous system via norepinephrine. Therefore, sAA concentration depends on the activity of both the parasympathetic and sympathetic nerves (Xu et al., 2018). This dual control of autonomic nerves seems to complicate sAA secretion. PD is frequently complicated by various autonomic dysfunctions, and the most common one is orthostatic hypotension caused by a disorder of the vasomotor sympathetic nervous system (Pablo-fernandez et al., 2017). The clinical significance of sAA in neurodegenerative diseases, especially those with autonomic neuropathy, has been little studied. We investigated whether sAA is associated with psychological stress, autonomic neuropathy, or other factors in PD patients.

In the present study, we determined whether any of the various clinical parameters showed a similar pattern to sAA in patients with PD. The results of the factor analysis indicated that NRS score, Age, and GHQ12 were classified as the same factors as sAA. Results of multiple linear regression analysis demonstrated that the effect on sAA was higher for NRS, Age, and GHQ12 by order. The stepwise analysis left only the NRS score. The fact that sAA strictly correlates with NRS indicates that sAA is a good mental stress marker in PD. Notably, OH was first eliminated by factor analysis. Several autonomic dysfunctions become more prominent with disease progression in PD (Krämer et al., 2019; Shindo et al., 2003). The fact that OH is associated with sympathetic dysfunction would have led us to expect lower sAA levels in patients with PD and OH, but our results indicated that OH and sAA levels were unrelated. This suggests that the mechanism of sAA secretion is at least independent of the vasomotor sympathetic nervous system.

Next, we compared the sAA, Age, and NRS scores of the PD group with those of the C/AC group. The results revealed that the sAA of the PD group was not significantly different from that of the AC group, indicating that sAA level is independent of the presence of PD. The NRS score of the PD group was lower than that of the C/AC group. Subjects with PD were thought to have lower mental health than control subjects due to psychiatric symptoms such as depression and anxiety (Kadastik-eerme et al., 2015; Ma et al., 2018; Marsh, 2013), but unexpectedly, the present results showed better mental health in the PD group than in the C/AC group. Furthermore, the results indicated that sAA was significantly related to age. In contrast to PD, sAA was not correlated with NRS in the C/AC group. The correlation between sAA and age in the C/AC group may be influenced by physiological sympathetic activation in the ageing process (Seals & Esler, 2000). The increase in sAA with age, which is consistent with previous reports (Jezova et al., 2020; Xu et al., 2018; Strahler et al., 2010), in turn, indicates that sAA is independent of mental stress. Therefore, the fact that sAA is associated with subjective stress and not with aging may be a characteristic of patients with PD. In addition, the fact that sAA was not affected by age in PD patients may indicate that age-related sympathetic hyperactivity may interfere with various autonomic dysfunctions.

The present study has several limitations. First, we used only the NRS as the subjective stress scale and treated it as a chronic stress scale. Other chronic or acute stress scales as controls should also be employed. In addition, several assessments of mental functioning were performed in patients with PD, while only NRS was assessed in the control groups. Second, PD is known to be complicated by various dysfunctions in the autonomic nervous system. Autonomic dysfunction in PD patients was assessed only by OH, and autonomic functions other than the vasomotor sympathetic nervous system should also be evaluated. Third, we use only NRS as an indicator of daily stress and have not examined other stress indicators. Forth, the number of patients in the control group was smaller than that in the PD group, and the AC group should have included as many patients as the PD group.

In conclusion, the present results suggest that sAA may be an indicator of daily mental stress in PD. Sympathetic dysfunction, which is frequently complicated by PD, seems to be independent of sAA secretion. In contrast to PD, sAA was strictly related to age and not to mental stress in the control subjects, indicating that sAA level is regulated mainly by age-related sympathetic tone.

Acknowledgements We would like to thank the participants and their families for their contributions. We also thank H.Tatamidani, S.Shimada, R.Oshima, M.Matsuura, Y.Utsunomiya, Edagawa, Y.Kawamura, K.Taniguchi, and A.Tsukamoto for recruiting volunteers.

Authors' Contributions Y.M. wrote the manuscript and T.M. supervised and edited. Y.M., M.I., and T.M. designed the study and Y.M. and M.I. analyzed the data. T.T. contributed to the recruitment of volunteers as age-matched control. Y.M., M.I., N.S., and N.K. obtained data from subjects. H.S. provided valuable suggestions in the research design and completion of the manuscript. All authors have carefully read this submitted paper and agree with the contents of the manuscript.

Funding This study was supported by the Japan Society for the Promotion of Society (JSPS) KAKENHI Grant Number, 24500636.

Availability of Data and Material The data of this study are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declarations

Ethics Approval The study was conducted in accordance with the Harmonized Guidelines on Good Clinical Practice of the International Conference and the Declaration of Helsinki. The study approved by the Tokushima National Hospital Ethics Review Committee (2019-31-13) is registered in the UMIN Clinical Trials Registry (UMIN000041956).

Conflict of Interest All authors declare that they have no conflict of interest.

Consent to Participate All participants provided written informed consent before starting the study.

Informed Consent Written consent was obtained from all eligible patients who voluntarily agreed to participate in the study.

Ethical Treatment of Experimental Subjects (Animals and Humans) No experimental treatment was conducted on either human or animal subjects in this study.

Consent to Publish Patients signed informed consent regarding publishing their data.

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