J Neurol (2001) 248 : 684 – 689

ORIGINAL COMMUNICATION

Daniela Berg
Christiane Siefker
Georg Becker

Echogenicity of the substantia nigra in Parkinson's disease and its relation to clinical findings

Abstract Recently an increased echogenicity of the substantia nigra (SN) in patients with Parkinson's disease (PD) was demonstrated by transcranial ultrasound (TCS). In this study we set out to compare SN echogenicitiy with disease characteristics (time of onset, duration, toxin exposure) in a large patients sample. Patients' history and exposure to toxins were recorded from 112 PD patients who underwent a thorough neurological examination including assessment of disease stage according to Hoehn and Yahr and CURS (Columbia University Rating Scale). Personality was assessed according to the Freiburg Personality Inventory. In all patients the area of SN echogenicity was encircled and measured by TCS. All except 9 patients had hyperechogenic SN areas exceeding the mean plus standard deviation values of an age matched control group (0.19cm^2). The age of disease onset was lower in patients who displayed an area of SN echogenicity above this value. The area of SN echogenicity was larger contralateral to the side with more severe symptoms. None of the other characteristics correlated with ultrasound findings. We conclude that SN hyperechogenicity is a typical finding in PD. The cause of hyperechogenicity is so far unknown. Investigation of the underlying reason might disclose a pathogenic factor in PD.

Key words Parkinson's disease · Transcranial ultrasound · Substantia nigra hyperechogenicity · Vulnerability marker

Received: 13 November 2000
Received in revised form: 24 January 2001
Accepted: 19 February 2001

D. Berg, MD (✉) · C. Siefker · G. Becker, MD
Department of Neurology
University of Würzburg
Josef-Schneider-Str. 11
Tel.: +49-931-201 26 21
Fax: +49-931-201 34 89
e-mail:
daniela.berg@mail.uni-wuerzburg.de

Introduction

Until now, structural neuroimaging in Parkinson's disease (PD) has played only a limited role in the diagnosis of this common neurodegenerative disorder. Cerebral computed tomography (CT) appears normal in PD and magnetic resonance imaging (MRI) shows inconsistently either increased, normal or decreased signal intensity of the substantia nigra on T2-weighted images [1, 10, 15, 22]. Therefore, CT and MRI are mainly used to identify patients with symptomatic Parkinsonism, notably subcortical artherosclerotic encephalopathy or hydrocephalus, or atypical parkinsonian syndromes CE_4 [29]. In contrast to PD patients those with multi system atrophy (MSA) may exhibit a reduced signal intensity of the lentiform nucleus on T2-weighted MRI or atrophy of the brainstem and cerebellum [23, 24, 25, 28]. These findings, however, are by no means specific for this disorder and have also been described in typical PD patients [1]. Magnetic resonance spectroscopy (MRS) has been proposed in the differential diagnosis of parkinsonian syndroms [8, 12], but the diagnostic yield of MRS is limited as MRS findings in the various parkinsonian syndromes and classical PD overlap broadly [6, 18].

Transcranial sonography (TCS) is a new diagnostic technique that allows imaging of the brain parenchyma in two-dimensional black and white slices. In contrast to CT and MRI changes of the substanita nigra (SN) in PD patients may be detected by TCS. A previous study of our group demonstrated that a majority of PD patients exhibited a moderate to distinct increase in SN echogenicity compared with age matched controls [2]. We found a relation between the severity of illness and echogenicity

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of the SN in a relatively small sample of PD patients. Recently we were able to demonstrate that a significant number of healthy subjects (about 9 %) exhibit the same echo pattern of the SN as PD patients. Some of these healthy subjects with increased echogenicity had a subclinical impairment of the nigrostriatal system detected by [^{18}F]-Dopa PET examination [3]. This observation lead to the hypothesis that SN hyperechognicity might be a vulnerability marker of PD that can be detected by TCS prior to the onset of the disease. As the pilot study was performed in only a small patients' sample we conducted this study to compare SN echogenicitiy with disease characteristics in a larger sample of patients.

Patients and methods

After giving informed consent according to the declaration of Helsinki we included in this study 112 consecutive patients from the outpatients clinic of our hospital with definite PD based on the UK Parkinson's Disease Society Brain Bank criteria. All patients underwent detailed neurological and sonographic examinations, which were performed independently by two physicians blinded to the results of the other examination. Patient's medical and social history was taken carefully by a third examiner including the assessment of epidemiological data such as area of birth (from patients, their parents and grandparents), residential background, war time experience, episodes of great limitations (hunger), work and hobbies, exposure to toxic agents (particularly herbicides, pesticides and mercury containing solutions), use of alcohol, smoking or drugs, predominating nutrition, as well as family and medical history and medication. Some of these factors are known to be associated with the development of PD [19, 26]. Personality was assessed according to the Freiburg Personality Inventory (FPI) [11], testing 10 traits and 2 dimensions (extroversion/introversion; emotional stability/instability) of personality. All subjects underwent a thorough neurological examination, including the grading of Parkinson's disease according to Hoehn and Yahr [17]. The severity of the disease was assessed by the Columbia University Rating Scale (CURS) [21] while patients were on treatment. In addition, PD was classified according to the predominating symptoms as a tremor, rigid-akinetic or equivalent type of PD. Duration from first Parkinsonian symptoms leading to the diagnosis of PD and time of onset of dyskinesia, fluctuations and freezing were assessed as markers for the progression of the disease [5, 7].

For TCS examination a colour-coded, phased array ultrasound system equipped with a 2.5 MHz transducer was used (Elegra, Siemens Medical Systems, UG, Issaquah, United States). The examination was performed through a preauricular acoustic bone window with a penetration depth of 16 cm and a dynamic range of 45 dB. The SN was identified within the butterfly shaped structure of the mesencephalic brainstem, with scanning from both temporal bone windows (Figure). Signal brightness (echogenicity) is not quantifiable. Therefore assessment of SN echogenicity in the earlier study was semiquantitative [2]. To overcome this limitation and allow a more accurate comparison in this study a quantitative measurement of the area of SN echogenicity was applied by encircling and measuring the area of hyperechogenic signals in the SN region as described previously [3]. TCS examination was performed by an examiner blinded to the clinical data of the patient. Reproducibility of the sonographic measurement of the SN had been previously validated by encircling the SN of 104 subjects by two independent investigators. Sonographic measurements of signal extension proved adequately reproducible considering the smallness of the structure measured (right side: 1st examination: 0.12 (0.09;0.16) cm^2, 2nd examination: 0.12 (0.09;0.17) cm^2; Spearman rank correlation r=0.52, $p < 0.01$; left side 1st examination: 0.13 (0.09;0.15) cm^2, 2nd examination: 0.12 (0.09;0.17) cm^2; Spearman rank correlation r=0.70, $p < 0.01$).

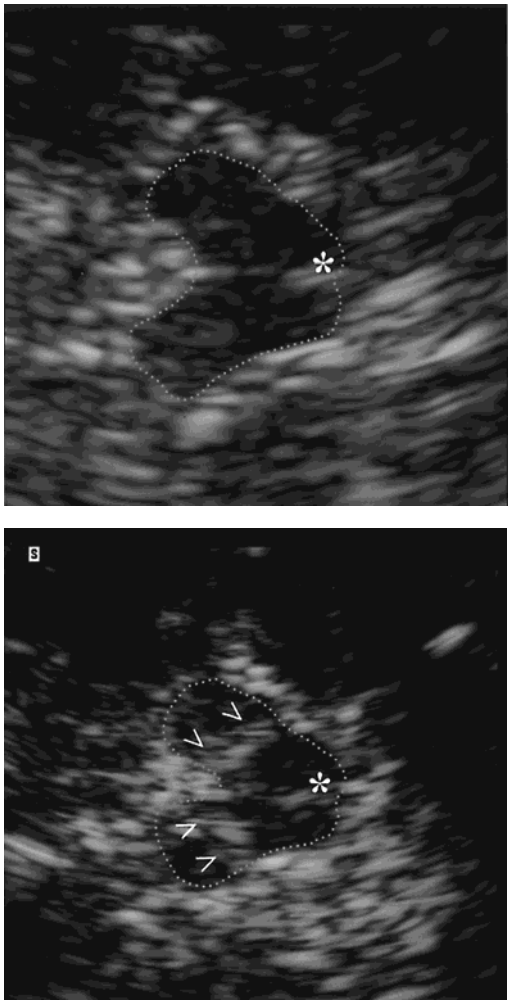

Fig. Ultrasound image of the mesencephalic brainstem in a healthy adult (A) and a patient with Parkinson's disease. (B) The butterfly shaped mesencephalic brainstem is encircled by dotted lines and surrounded by the hyperechogenic basal cisterns. The patient with Parkinson's disease exhibits linear hyperechogenic signals at the SN (<) on both sides which are not seen in the healthy control. * aqueduct.

Statistics

Descriptive statistics are given as median with lower and upper quartiles (25th and 75th percentile respectively). Results of SN echogenicity of PD patients were compared with measurements of hyperechogenic areas at the SN of 30 age matched controls examined by the same sonographer with the same ultrasound system [3]. The upper standard deviation of SN echogenicity in the controls group was used as the cut-off for further analyses. Intergroup comparison was performed by the Mann-Whitney U-Test. Correlation analysis was performed by Spearman rank correlation.

Results

TCS examination could be performed in 103 of the 112 subjects; 9 had no appropriate temporal acoustic bone windows and were therefore excluded from further analysis. In the remaining 103 subjects (42 female, 61 male, median age 63 (56;71) years) the mesencephalic brainstem was adequately displayed by TCS allowing measurements of the area of hyperechogenic signals at the SN (Figure). Median area of SN echogenicity was 0.24cm^2 (0.21; 0.27) for the right and 0.25cm^2 (0.21;0.28) for the left side. Hyperechogenic areas at the SN in PD patients exceeded those determined in 30 age-matched healthy controls, in whom the median area of SN echogenicity was 0.12cm^2 (0.08; 0.15) [3]. In 94 of the 103 patients the extent of the hyperechogenic signal at the SN was well above 0.19cm^2 at least on one side. This value represents the upper standard deviation of the control group. The area of SN echogenicity was significantly larger contralateral to the lateralisation of clinical symptoms (0.25 [0.23; 028] vs. 0.23 [0.20; 0.26]cm^2) (U-Test $p < 0.01$).

Neurological examination revealed an average Hoehn and Yahr score of 2 (2; 2,5) and CURS score of 16 (12; 21). In 24 patients tremor dominated the parkinsonian symptoms, 36 patients were classified as predominantly rigor-akinetic and 43 patients as equivalent with respect to their symptoms (Table1). Lateralisation to the right side was noticed in 46 patients, to the left side in 55 patients. In 2 patients no lateralisation could be detected. 23 patients had on/off fluctuations with a median onset of 7 (5; 11) years after the initial diagnosis, dyskinesia and freezing were detected in 20 and 5 patients (median time from first symptoms 7,5 (5,5; 11,5) and 13 (8; 13) years, respectively.) The actual medical treatment of the patients is outlined in Table 2.

Medical history revealed further neurological disorders in 7 patients, severe infections at any time since childhood in 47 patients and closed head trauma (CE_2) in 12 patients. Eight patients suffered from essential tremor, one patient had been on neuroleptics before PD onset. Thirteen patients reported to have smoked more than 10 packs per year (CE_3), 3 patients to have more than 2 alcoholic drinks daily. Family history was positive for PD associated symptoms in 16 patients and for essential tremor in 28 patients.

Epidemiological assessment revealed that 64 patients had been living predominantly in rural areas, 39 predominantly in cities. Ninety-one patients were born in Germany, 11 in Eastern Europe and one in China. Ten patients had been abroad for a time period of more than 2 months (7 in other European countries, 2 in Russia and 1 in Uruguay). Eighty-two patients were married, 13 widowed, 3 divorced and 5 unmarried. Fifty-three patients reported an exposure to toxins (11 to pesticides, 2 to herbicides, 12 to both, 16 to paint or solvents, 4 each to heavy metal, mercury or other chemicals). The results of the personality inventory are shown in Table 3.

Comparison of patients with SN echogenicity equal or below 0.19cm^2 on both sides with patients displaying an area of SN hyperechogeniciy of more than 0.19cm^2 on one or both sides revealed a younger age of disease onset of PD (54 [46; 63] vs. 65 [59; 72] years, U-test: p=0.019) in those with the larger area of SN echogenicity (Table 1). There was no difference in severity of the disease according to Hoehn and Yahr or CURS (U-Test p

Tab. 1 Clinical data of 103 PD patients grouped according to the TCS findings of the SN

	Whole group n=103	SN ≤ 0.19cm^2 on both sides n= 9	SN > 0.19cm^2 on or both sides n=94	p-value (U-test)
Age (years)	63 (56;71)	75 (68;77)	62 (53;70)	p=0.017
age at disease onset (years)	55 (46;64)	65 (59;72)	54 (46;63)	p=0.019
duration (years)	6 (3;10,5)	5 (3,75;8)	6 (3;11,5)	p > 0.05
H&Y score	2 (2;2,5)	2,5 (2;3)	2 (2;2,5)	p > 0.05
CURS	16 (12;21)	22 (12;24)	15 (12;20)	p > 0.05
Type (number of patients)				
Tremor dominating	24	2	22	
Rigid-akinitic	36	4	32	
Equivalent	43	3	40	
Number of patients with late motor complications, %				
Fluctuations	23	1 (11 %)	22 (23 %)	
Dyskinesia	20	1 (11 %)	19 (20 %)	
Freezing	5	5 (5 %)		
Years since onset of first symptoms				
Fluctuations	7 (5;11)	7	7,5 (5;11)	p > 0.05
Dyskinesia	7,5 (5,5;11,5)	7	8 (5;12)	p > 0.05
Freezing	13 (8;13)	13 (8;13)		

Data are given as median with lower and upper quartile (25th and 75th percentile respectively) or percentage. n number of patients, H&Y Hoehn and Yahr, CURS Columbia Univsersity Rating Scale.

Tab 2 Medication

Drug (mg)	Whole group n=103	SN ≤ 0.19cm² on both sides n= 9	SN > 0.19cm² on one or both sides, n=94	p-value (U-test)
Levodopa	400 (200; 600) n=79	275 (150; 400) n=6	400 (200; 600) n=73	p > 0.05
Bromocriptin	7.5 (6.25; 15) n=16	5 n=1	7.5 (7.5; 15) n=15	
Dopergin	0.7 (0.4; 1) n=4	0.2 n=1	8 n=3	
Ropinirol	4.5 (3; 6) n=17	3 n=1	4.5 (3; 6) n=16	
Pergolid	3 (3; 3.5) n=11		3 (3; 3.5) n=11	
Cabergolin	2 n=3		2 n=3	
Dihydroergocryptin	40 (30; 60) n=7		40 (30; 60) n=7	
Pramipexol	2.1 n=3	2.1 n=1	1.3 n=2	
Selegilin	10 (10; 10) n=25	7.5 n=2	10 (10; 10) n=23	
Amantadin	300 (300; 400) n=10		300 (300; 400) n=10	
Trihexyphenhidyl	4 n=2		4 n=2	
Metixen	10 n=1		10 n=1	
Apomorphin	5 n=1		5 n=1	
Budipin	60 (35; 60) n=24	60 n=2	55 (30; 60) n=22	

Number of patients treated (n) with the different substances given as median with lower and upper quartile (25th and 75th percentile respectively) according to the area of SN hyperechogenicity. Statistical analysis was only performed for levodopa as the groups were too small for the other substances

> 0.05). The time span between disease onset and first occurrence of motor fluctuations or dyskinesia was not significantly different between the groups, however the percentage of patients with fluctuations, dyskinesia or freezing was higher in the group of patients with SN hyperechogenicity above 0.19 cm² (Table 1). There was no difference in duration of the disease or amount of levedopa therapy between the groups (U-Test p > 0.05) (Table 2). The extent of hyperechogenic area at the SN was similar in the different PD types; the area of SN hyperechogenicity for the tremor dominating type was 0.23 (0.20; 0.26)cm² for the right and 0.26 (0.23; 0.28)cm² for the left side, for the rigid akinetic type for the right 0.23 (0.19; 0.26)cm², for the left 0.23 (0.21; 0.29)cm² and for the equivalent type for the right 0.24 (0.22;0.28)cm², for the left 0.25 (0.21; 0.29)cm² (U-test p > 0.05 for all groups). The percentage of individuals with SN echogenicity ≤ 0.19 cm² was about the same in the different groups (Table 1).

Tab. 3 Personality inventory (FPI-R), median with lower and upper quartile (25th and 75th percentile respectively)

	Median
Life satisfaction	5 (4;6)
Social orientation	5 (4;6)
Achievement orientation	4 (3;6)
Inhibitedness	6 (4;7)
Impulsiveness	5 (3;7)
Aggressiveness	4 (3;6)
Strain	5 (4;7)
Somatic complaints	5 (4;6)
Health concern	5 (4;7)
Frankness	5 (3;6)
Extroversion	4 (3;5)
Emotionally	6 (4;7)

Comparison of patients from rural areas or cities did not identify any difference concerning age of disease onset, severity or duration of PD or extent of SN echogenicity (U-test p > 0.05). Nor was there a significant difference for patients with family history of PD or essential tremor, exposure to toxins, smoking or infectious diseases (U-test p > 0.05). The personality inventory did not correlate with any of the parameters for disease onset, course or SN echogenicity (Spearman rank p > 0.05).

Discussion

This study confirms our previous findings of increased SN-echogenicity in PD patients [2]. The proportion of distinctly hyperechogenic SNs in the group of PD patients, however, was higher which is likely to reflect improvements in the ultrasound technology in the last five years. In 91 % of PD patients the extent of hypere-

chogenic signals at the SN was well beyond the standard deviation of an age matched control group while only 9 out of 103 PD patients (8.7 %) exhibited SN signal intensity within the normal range. Moreover, PD patients with a more extended hyperechogenic signal had an earlier disease onset. Additionally, these PD patients showed more often motor complications like fluctuations, dyskinesia and freezing when analysis was controlled for the duration of disease. These symptoms are regarded as signs of disease progression and may in part be related to the extent of neuronal loss at the SN [5, 7]. Moreover, the SN was found to be more hyperechogenic contralateral to the more affected side. Together these findings indicate that the echo pattern of the SN might be related to the extent of neuronal loss in the SN and to the onset and course of the disease. In view of these findings, the question rises whether factors leading to an increased tissue echogenicity at the SN might play a role in the pathogenesis of nigral injury or whether SN hyperechogenicity reflects the state of nigral degeneration.

The same echo pattern of the SN as seen in PD can be detected in about 9 % of healthy subjects [3]. Up to 60 % of these healthy subjects with SN hyperechogenicity exhibited a reduced F-Dopa uptake at the striatum on PET indicating a subclinical impairment of the nigrostriatal system. These findings imply that changes in echogenicity of the SN might be detected by TCS prior to the damage of nigral cells. Together with the finding that follow up examinations in PD patients and healthy subjects demonstrated an almost unchanged echo pattern of the SN (Becker and Berg, unpublished data) we speculate that increased echogenicity of the SN may be a susceptibility marker for nigral cell injury and that factors leading to an elevated SN echogenicity might also be of relevance in the pathogenesis of PD.

As not all PD patients display the echo feature of SN hyperechogenicity on TCS, this feature can only be regarded as one factor, important but not sufficient for the development of the disease. Additional factors must be proposed with different degrees of impact in the individual patient.

Exposure to exo-and endotoxins has been reported to be relevant in the development of nigral injury [19, 26]. In our study, we could not detect an association of the development of Parkinsonian signs and such toxins or other epidemiological data. However, such influences can not be ruled out as our study population was rather small and only a few, known factors associated with PD were assessed.

The reason for the increase in echogenicity is still unclear. Morphological changes occurring at the SN in PD which may lead to an alteration in tissue impedance and therefore SN echogenicity are several: Loss of pigmented neurons may result in tissue condensation, proliferation of microglia may increase cellular interfaces, and elevated heavy metal tissue content (especially an increase of iron) [9, 13, 16, 27, 28] may modify tissue impedance. According to recent findings iron is supposed to be a major source for the increased echogenicity of the SN; neurochemical and sonographic analyses of post mortem material revealed a close correlation between SN echogenicity and iron tissue content (Berg et al., unpublished). In addition, animal experiments have demonstrated more intense tissue echogenicity induced by increasing amounts of iron injected into the SN [4]. Therefore, we surmise that SN hyperechogenicity in PD might (at least in part) be due to disease-associated elevation of SN iron levels. Iron is supposed to play a pivotal role in the degeneration of SN neurons in PD, as it facilitates and accelerates neurodegenration by formation of free radicals and lipid peroxidation [14, 20, 30]. In the light of these observations, one may speculate that increased echogenicity of the SN might reflect higher tissue iron content which, on the other hand, could increase the oxidative stress within the SN resulting in a more rapid degeneration of SN neurons.

Our study demonstrates that TCS may serve as a valuable tool in the neuroimaging of PD providing easily available information in addition, to other neuroimaging data. Because of the lack of invasiveness and the relatively low cost, it is particularly useful for an application to a large number of patients. Further studies are required to determine whether differences in the echogenicity of the SN in PD patients may display differences in the genetic background or other pathogenetical factors of the disease.

Acknowledgement The authors to thank Wayne Ellswoth, Isaaqua, USA, Prof. K.V. Toyka and Prof. K. Reiners for their support and critical review of the manuscript. The study was supported by grants of the University of Würzburg.

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