Alterations of Autonomic Nervous System Activity in Children with Spina Bifida: A Case-Control Study

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Research Article

Keywords: Autonomic Nervous System, Spina Bifida, heart rate

Posted Date: April 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-436592/v1

Abstract

We compared heart rate variability parameters of patients with spina bifida with those of a control group during urodynamic study to evaluate the autonomic nervous system dysfunction of spina bifida. Continuous heart rate variability parameters were recorded during 3 successive periods (P0: 2 minutes before the start of filling; P1: start of filling to the first desire to void; P2: P1 to the end of filling or the start of voiding). Children with vesicoureteral reflux who underwent video-urodynamic study were established as a control group. We included 11 patients with spina bifida and 9 controls. At baseline, patients with spina bifida had lower values of RMSSD, pNN50, and HF, while LF/HF ratio was increased (5.04 ± 4.75 vs 0.67 ± 0.42, p = 0.014). During bladder filling, LF/HF values increased in the control group (P0 0.67 ± 0.42 vs P1 0.89 ± 0.34 vs P2 1.21 ± 0.64, p = 0.018) while it was decreased in spina bifida patients (P0 5.04 ± 4.75 vs P1 3.96 ± 4.35 vs P2 3.26 ± 4.03, p < 0.001). HF were significantly increased in spina bifida children during bladder filling (p = 0.002). In time domain, SDNN was increased only in control group during bladder filling. Parasympathetic activity domains were decreased in children with spina bifida at baseline. During the bladder filling phase, parasympathetic activity increased with fixed sympathetic activity in spina bifida group while the control group demonstrated a shifted balance toward sympathetic preponderance at the end of bladder filling. These findings may be related to the pathophysiology of neurogenic bladder in spina bifida.

Introduction

Spina bifida lies within the spectrum of neural tube defects caused by a failure of the caudal neural tube to fuse normally in early development, affecting the central nervous system and resulting in permanent disability. It is the most common birth defect with an average incidence of 1 to 10 in 1000 live births.[1] Magnetic resonance imaging can provide an early diagnosis and untethering may reduce potential lifelong disabilities. Spina bifida can result in severe disability of multiple organ systems, including neurological deficits, sphincter dysfunction, and limb deformities.[2]

Variable impact on the somatic, parasympathetic, and sympathetic innervation of the bladder affects the patient's ability to store and empty urine and can ultimately cause chronic kidney disease due to poor bladder dynamics. As the lower urinary tract (LUT) is closely coupled to the autonomic nervous system (ANS), [3] an indicator of ANS activity may offer objective information about bladder sensations and further understanding about underlying pathophysiology for specific diseases. Baselines and ANS activity changes during bladder filling has been studied in healthy controls or patients with lower urinary tract symptoms (LUTs) without neurological deficit.[4,5] However, there are no studies regarding how the ANS affects bladder pathophysiology in patients with spina bifida.

One of the most practical, reproducible, and non-invasive ways to monitor the ANS is heart rate variability (HRV). HRV measures the spontaneous change of the R-R interval in continuous electrocardiogram (ECG) as a response to physiologic demand which reflects the continuous interplay between sympathetic and parasympathetic influences.[6] Although HRV was initially used to evaluate autonomic function in

cardiovascular diseases, it has also been used to assess underlying dysfunction of ANS related to LUT disease in several studies.[5,7,8] Therefore, our objectives were (1) to compare ANS activity using HRV between children with spina bifida and a control group at baseline and (2) to investigate and compare responses to bladder filling during urodynamic study in both groups.

Results

There were no significant differences between the 2 groups with respect to baseline characteristics (Table 1). The control group consisted of 7 patients previously diagnosed with VUR and 2 patients who underwent examination to evaluate if VUR was present. Seven patients (63.6%) were in use of clean intermittent catheterization in the study group. Low bladder compliance less than 20 ml/cmHO was observed in 7 patients (63.6%) of the study group while none were present in the control group.

Table 1
Patient characteristics

	Control group (n = 9)	Spina bifida group (n = 11)	P value
Gender (male : female)	1 : 8	4 : 7	0.319
Median age, years (range)	7 (5–11)	11 (5–27)	0.295
Diagnosis, n (%)	VUR (7, 77.8%) Megaureter (1, 11.1%) Multiple cystic dysplastic kidney (1, 11.1%)	LMMC (8, 72.7%) MMC (3, 27.3%)	
CIC use, n (%)	0	8 (72.7)	0.005
Urodynamic parameters	211.11 ± 38.98	257.91 ± 119.91	0.246
Maximal cystometric capacity (ml)	93.28 ± 25.17	81.45 ± 33.97	0.398
MCC/EBC (%)	1 (11.1)	4 (36.4)	0.319
Detrusor overactivity, n (%)	0 (0)	7 (63.6)	0.005
Low bladder compliance (< 20 cm H_2O/ml), n (%)			

VUR, vesicoureteral reflux; LMMC, Lipomeningomyelocele; MMC, Meningomyelocele; CIC, clean intermittent catheterization; MCC, maximal cystometric capacity; EBC, estimated bladder capacity

Time domain parameters at baseline revealed that patients with spina bifida had a significantly lower mean RMSSD (25.64 vs 48.22 ms, p = 0.015) compared to control group (Table 2). They also had a lower pNN50 (7.33 vs 29.48%, p = 0.021). In frequency domain, HF was lower in patients with spina bifida (125

vs 776, p = 0.003), while there was no difference in LF. Children with spina bifida had a significantly higher LF/HF ratio at baseline compared to controls (5.04 vs 0.67, p = 0.014).

Table 2
Baseline autonomic profile of heart rate variability

	Control group (n = 9)	Spina bifida group (n = 11)	P value
Time domain	88.31 ± 10.00	96.84 ± 12.78	0.120
Heart rate (beats/minute)	50.61 ± 15.31	37.75 ± 19.10	0.120
SDNN (ms)	48.22 ± 22.54	25.64 ± 14.96	0.015
RMSSD (ms)	29.48 ± 22.82	7.33 ± 10.52	0.021
pNN50 (%)			
Frequency domain	987.20 ± 972.46	915.58 ± 1348.19	0.895
VLF (ms^2)	394.31 ± 176.79	523.99 ± 681.44	0.587
LF (ms^2)	776.87 ± 477.52	125.08 ± 104.39	0.003
HF(ms^2)	0.67 ± 0.42	5.04 ± 4.75	0.014
LF/HF			

SDNN, standard deviation of all NN intervals; RMSSD, root mean squared of successive differences NN intervals; pNN50, number of consecutive R-wave to R-wave intervals differing by more than 50 ms relative to the total number of intervals; VLF, very low frequency; LF, low frequency; HF, high frequency

Relative changes of HRV parameters from baseline to the bladder filling period were demonstrated in Table 3. Heart rate was unchanged during bladder filling in both groups. However, several parameters of HRV were increased or decreased with propensity during bladder filling. In time domain, a statistically significant difference was noted in SDNN values only in the control group (Fig. 1). LF values showed an increasing tendency with bladder filling in the control group. HF was only increased in patients with spina bifida, although the difference was not great (P0 = 125.08 ± 104.39 vs P2 = 223.10 ± 169.90, p = 0.002). The changes in LF/HF ratio was increased in the control group (P0 = 0.67 ± 0.42 vs P2 = 1.21 ± 0.64, p = 0.018) and decreased in the spina bifida study group (P0 = 5.04 ± 4.75 vs P2 = 3.26 ± 4.03, p < 0.001).

Table 3
Heart rate variability during bladder filling in the control group and the spina bifida patient group

	Variable	Period 0	Period 1	Period 2	P value
Control group	Time domain	88.31 ± 10.00	86.91 ± 9.79	85.90 ± 10.15	0.116
	Heart rate (beats/min)	50.61 ± 15.31	57.10 ± 16.54	63.66 ± 18.24	< 0.001
	SDNN (ms)	48.22 ± 22.54	53.05 ± 22.61	54.23 ± 21.70	0.167
	RMSSD (ms)	29.48 ± 22.82	32.75 ± 18.29	31.01 ± 15.89	0.676
	pNN50 (%)	987.20 ± 972.46	1242.94 ± 541.33	1515.71 ± 831.36	0.251
	Frequency domain	394.31 ± 176.79	710.90 ± 490.79	1431.53 ± 1493.67	0.073
	VLF (ms^2)	776.87 ± 477.52	924.33 ± 667.73	1222.59 ± 1074.91	0.208
	LF (ms^2)	0.67 ± 0.42	0.89 ± 0.34	1.21 ± 0.64	0.018
	HF (ms^2)				
	LF/HF				
Spina bifida group	Time domain	96.84 ± 12.78	‘	85.05 ± 27.68	0.395
	Heart rate (beats/min)	37.75 ± 19.10	88.81 ± 28.47	42.99 ± 18.64	0.465
	SDNN (ms)	25.64 ± 14.96	40.42 ± 15.60	29.19 ± 14.60	0.180
	RMSSD (ms)	7.33 ± 10.52	25.84 ± 11.90	10.69 ± 10.70	0.182
	pNN50 (%)	915.58 ± 1348.16	7.53 ± 8.28	1188.77 ± 1036.70	0.615
	Frequency domain	523.99 ± 681.44	1065.32 ± 861.12	496.39 ± 534.65	0.556
	VLF (ms^2)	125.08 ± 104.39	449.91 ± 455.76	223.10 ± 169.90	0.002
	LF (ms^2)	5.04 ± 4.75	164.52 ± 145.08	3.26 ± 4.03	< 0.001
	HF (ms^2)		3.96 ± 4.35		
	LF/HF				

SDNN, standard deviation of all N-N intervals; RMSSD, root mean square of successive differences of successive NN normal intervals; pNN50, number of consecutive R-wave to R-wave intervals differing by more than 50 ms relative to the total number of intervals; VLF, very low frequency; LF, low frequency; HF, high frequency

Discussion

To our knowledge, this is the first study to objectively compare the baseline profile of ANS activity and responses to bladder filling in children with or without spina bifida using spectral analysis of cardiovascular signal variability methodology. Several findings in our study are noteworthy. First, the baseline profile of HRV was altered in spina bifida patients in terms of decreased values related with the peripheral nervous system (RMSSD, pNN50, HF) and increased LF/HF ratio representing sympathetic dominance. The response to bladder filling also significantly differed between the two groups, most pronounced in the LF/HF ratio (increasing value in the control group vs decreasing value in the spina bifida group with bladder filling). These results provide an explanation of the underlying neuropathology in spina bifida.

There are a variety of urological consequences depending on the severity of the fusion abnormality and location of the lesion. An upper motor neuron lesion with detrusor overactivity and detrusor sphincter dyssynergy are most likely to develop, but an acontractile detrusor and sphincter denervation are also seen as a result of spinal cord tethering.[9,10] The mainstay in treatment of spina bifida is still combination of pharmacological agents and clean intermittent catheterization, which has had limited success.[11] Current treatment strategies have focused on preventing the consequences of neurogenic bladder rather than trying to understand the disease, therefore the understanding of the disease has not yet improved.

Since the first report of changes in HRV by Hon and Lee,[12] HRV has been used extensively as a quantitative marker of ANS activity. Previous studies demonstrated that ANS dysfunction proven by HRV reflects the underlying pathology of LUTs.[5,8] The most frequently studied LUTs associated with HRV is overactive bladder (OAB), which is controversial as the results, as well as study settings, of how ANS differs from healthy controls were heterogenous. Hubeaux et al. reported a predominance of parasympathetic activity with the bladder emptied and a preponderance of sympathetic activity at the end of bladder filling in women with OAB syndrome.[13] Choi and Kim observed a decrease in HRV indexes, including HF, SDNN, RMSSD, in women presenting with OAB compared to healthy women.[14] In children with bladder bowel dysfunction, HF was significantly lower than healthy controls at baseline.[15]

In healthy subjects, LF/HF ratio showed a stable sympathovagal balance until first desire to void, while the balance demonstrated a shift towards sympathetic activation before strong desire to void.[4] This response is thought to be caused by a similar mechanism to the vesicovascular response that is mediated by sympathetic nerves, such as the hypogastric nerve.[16] Unlike previous studies, LF/HF ratio increased until first desire to void and continued to rise until the end of filling in our study. This result may be due to the younger age group in our study not being capable of expressing exact bladder sensation and also the wide variation of HRV value. During bladder filling, contrary to the control group, only HF increased without increasing LF in the spina bifida group, resulting in a decrease in LF/HF ratio. These results suggest ANS dysfunction, predominantly a sympathetic ANS dysfunction, in spina bifida. Given that the thoracolumbar sympathetic efferent pathways in the hypogastric and pelvic nerves induce an inhibition of detrusor muscle and an excitation of the bladder base and urethra, the alterations observed in sympathetic activity during bladder filling may lead to a sensation of urgency and a problem in urine

storage. In our study, neurogenic detrusor overactivity (NDO) was seen in only 4 patients (36.3%); however, on past urodynamic studies, NDO was observed in all except 1 patient. These urodynamic results may be associated with the decreased LF/HF ratio during bladder filling.

In addition, SDNN in time domain was significantly increased with bladder filling in the control group, whereas in remained unchanged in spina bifida patients. Given that SDNN represents overall HRV,[17] the unchanged value of SDNN in spina bifida may be due to decreased neurotransmission of ANS during bladder filling. With extrapolation from the enteric nervous system, which has similar nerve distribution as the bladder, neural loss and decreased nerve fiber density in the myenteric plexus was seen in spina bifida patients, which correlated with severity of bowel dysfunction.[18] These alterations may be caused by reaction to disrupted extrinsic innervation, resulting in trans-neuronal degeneration. Therefore, we infer that these evidences may be related to an overall decreased HRV value during bladder filling in spina bifida, but they cannot explain the sympathetic predominance at baseline.

Recently, autonomic cardiovascular function was evaluated in wheelchair-using children with MMC, and RMSSD at rest were reduced compared to the control group, which agrees with our results of reduced RMSSD, pNN50, and HF in the spinal bifida group at baseline.[19] The pathology underlying reduced vagal tone and increased LF/HF ratio in spina bifida patients is uncertain. In patients with thoracic spinal cord injuries, higher heart rates and reduced vagal tone has been documented.[20] This may reflect compensation for decreased stroke volume and compensatory reductions in vagal tone to maintain autonomic balance.[21,22] Deteriorating vascular properties (small diameter, low flow, and high shear stress) were present in patients with spina bifida[23], and these results suggest that decreased vagal tone and sympathetic predominance in spina bifida may occur via similar mechanism demonstrated in spinal cord injury.

The main limitation of this study was the small number of subjects, therefore, HRV analysis was not performed for each subtype according to urodynamic study. Considering the large deviation of baseline HRV, a larger study is required to confirm our results. The second limitation is HRV measurements in our study were performed with a non-medical Bluetooth device, not with conventional ECG. However, recent studies have demonstrated that Bluetooth devices have provided an acceptable agreement for the measurement of HRV when compared with ECG. Therefore, the measurement method in this study has validity.[24,25] Although other criteria could be considered to see the change in HRV in terms of time, it is difficult to divide periods based on a specific time because each patient has different filling time inherent to their bladder capacity. Patients with spina bifida often lose their sense of bladder and demonstrate underactivity of bladder, which leads confusion in deciding proper point of each period. Despite these limitations, the criteria we applied to divide each period could be suboptimal method to observe the trends of HRV changes on bladder filling in both of control and spina bifida. Another concern is that HRV may be affected by emotional stress caused by the artificial setting of the study, however, the same setting was applied in both groups and the examination was conducted with sufficient time to adapt.

Another limitation may be that the control group consisted of children with VUR who may have abnormal bladder dysfunction that may be related to HRV, although this has not been studied.

At baseline, HRV parameters representing PNS activity are decreased in children with spina bifida compared to control group. During bladder filling, parasympathetic activity was relatively increased with a fixed sympathetic activity in spina bifida while control group demonstrated a shift in sympathetic/parasympathetic balance towards the sympathetic component. These results may be related to underlying neuropathology caused by spina bifida, which could be used to better understand and manage spina bifida.

Methods

Patient selection

The present study protocol was approved by the Yonsei University Health System Institutional Review Board (4-2015-0332). The study was performed in accordance with all applicable laws and regulations, good clinical practices, and the ethical principles described in the Declaration of Helsinki. A retrospective pilot study was carried out between July 2015 and March 2016 and this study confirmed the method of HRV measurement and identified the difference between control group and spina bifida patients. Subsequently, the prospective study was conducted from April 2016 to February 2017 to complement retrospective data after IRB approval of study protocol. Participants were informed about the purpose and procedure of the study prior to participation and that they could withdraw from the study at any time without explanation. In both of a pilot and prospective study, informed consent was obtained from parents and also children through age-appropriate agreement document in the presence of legal representatives or parents. The inclusion and exclusion criteria of prospective protocol were retrospectively applied to the pilot data. Regardless of the timing of the study, HRV was measured with a fixed protocol during video urodynamic study.

All patients were evaluated via a combination of clinical history, physical examination, and questionnaire for LUTs. Inclusion criteria for the study group consisted of patients older than 4 years old who underwent detethering surgery due to spina bifida, either due to a lipomeningomyelocele (LMMC) or meningomyelocele (MMC). The control group contained patients who underwent a video urodynamic study to evaluate vesicoureteral reflux (VUR) or other anomalies in upper urinary tract. Exclusion criteria were as follows: medication of anticholinergics and a-blocker within one month; diagnosis of neurological disorders affecting the autonomic nervous system except spina bifida; diagnosis of hypertension or arrhythmia; diabetes; history of previous urological surgery including augmentation cystoplasty; anatomical abnormalities in bladder and urethra; having had coffee, tea, cigarettes and other foods which could affect autonomic nervous system before examination. In control group, patients who had LUTs such as daytime incontinence or urgency were also excluded.

In sixteen of pilot cases, four patients were retrospectively excluded following criteria due to LUTs requiring medication. Five cases with spina bifida were also excluded due to history of augmentation cystoplasty and taking anticholinergics. The remaining seven patients were recruited retrospectively. From April 2016, twenty patients were prospectively screened for the study and 13 patients were enrolled. A total of 20 children (9 in the control group and 11 in the study group) were included for analysis.

Urodynamic study and HRV measurement

Urodynamic study was performed using a recommended technique by the International Children's Continence Society.[26] We used 6-Fr double-lumen catheters for the urethra and 12-Fr fluid filled balloon catheters for the rectum. A saline solution was warmed to body temperature for infusion at filling rates of 5–10% of a known or predicted capacity. The expected bladder capacity was estimated by using the following formula (in mL): [30 + (age in years×30)]. In children with spina bifida, bladder filling was terminated under the following conditions: child has a strong urge to void, continuous leakage is observed, detrusor pressure reaches greater than 40 cm H_2O, or the patient felt a sensation of bladder fullness or abdominal discomfort.[27] The patients were in the supine position and were not under anesthesia during the examination. Surface electrodes were used and EMG patch were positioned symmetrically, perineally, left and right of the anus.

In the control group, bladder filling was continued until children felt a strong desire to void and could no longer delay micturition. Maximal cystometric capacity is the bladder volume at the end of the filling phase or when "permission to void" is given.[26]

To prevent the anxiety caused by examination from affecting baseline HRV levels, patients were given sufficient stability after setting up the examination. After setting and calibration of urodynamic 3 channels, continuous HRV data was obtained during three periods of the bladder filling (Fig. 2).

Period 0 (P0): two minutes before the start of bladder filling

Period 1 (P1): from the start of bladder filling until patients feel the first desire to void

Period 2 (P2): from the end of P1 until the end of bladder filling

The present study compares R-R intervals derived from a tablet computer using the R-R intervals derived from the Polar H7 chest belt heart rate monitor as reference (Polar Electro Oy, Kempele, Finland), which has been already validated.[28] The R-R values were transferred to the computer via the Elite HRV application (Elite HRV LLC, NC, US) and exported for HRV analyses using the Kubios HRV standard version 3 software (Kubios Oy, Kuopio, Finland). Time and frequency domain measures were assessed according to current guidelines.[17] Comparable to most studies, time domain measures were 1) the standard deviation of all NN intervals (SDNN), 2) the root mean squared of successive differences of successive R-R normal intervals (RMSSD), 3) the successive percentage of R-R interval differences greater than 50 ms (pNN50). RMSSD and pNN50 are correlated with high frequency (HF), which is a

response to changes in parasympathetic activity. Frequency domain measured power spectra with calculation of the integral of 1) very low frequency (VLF; ≤0.04 Hz) 2) low frequency (LF; 0.04–0.15 Hz), which is the marker of primarily sympathetic cardiac modulation with some parasympathetic influence, 3) HF (0.15–0.4 Hz), which is a marker of parasympathetic modulation, and 4) LF/HF ratio, which is an indicator of the autonomic balance between the sympathetic and parasympathetic nervous systems.[17]

Statistical analysis

Data are expressed as mean ± standard deviation. The Mann-Whitney U test was used in comparison of mean values as a nonparametric approach and the t-test was used for parametric variables. Coefficient of variation (CoV) (CoV = SD/mean) were calculated to measure the dispersion of a probability distribution. Changes of HRV during bladder filling were compared in each group using repeated measures analysis of variance (ANOVA). The compound symmetry assumption was studied with the Mauchly sphericity test. After observing the significance of the Mauchly sphericity test and seeing the lack of spherical or posite symmetry in repeated measurements of HRV parameters, the Pillai trace multivariate test was adopted. The mean value of each HRV parameter in different time periods was compared with paired t-test. All computations involved standard software (SPSS v19 for Windows; IBM Corp, Armonk, NY, USA), with significance set at $p \leq 0.05$.

Declarations

Acknowledgements

This study was supported by a faculty research grant of Yonsei University College of Medicine (6-2016-0155)

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Figures

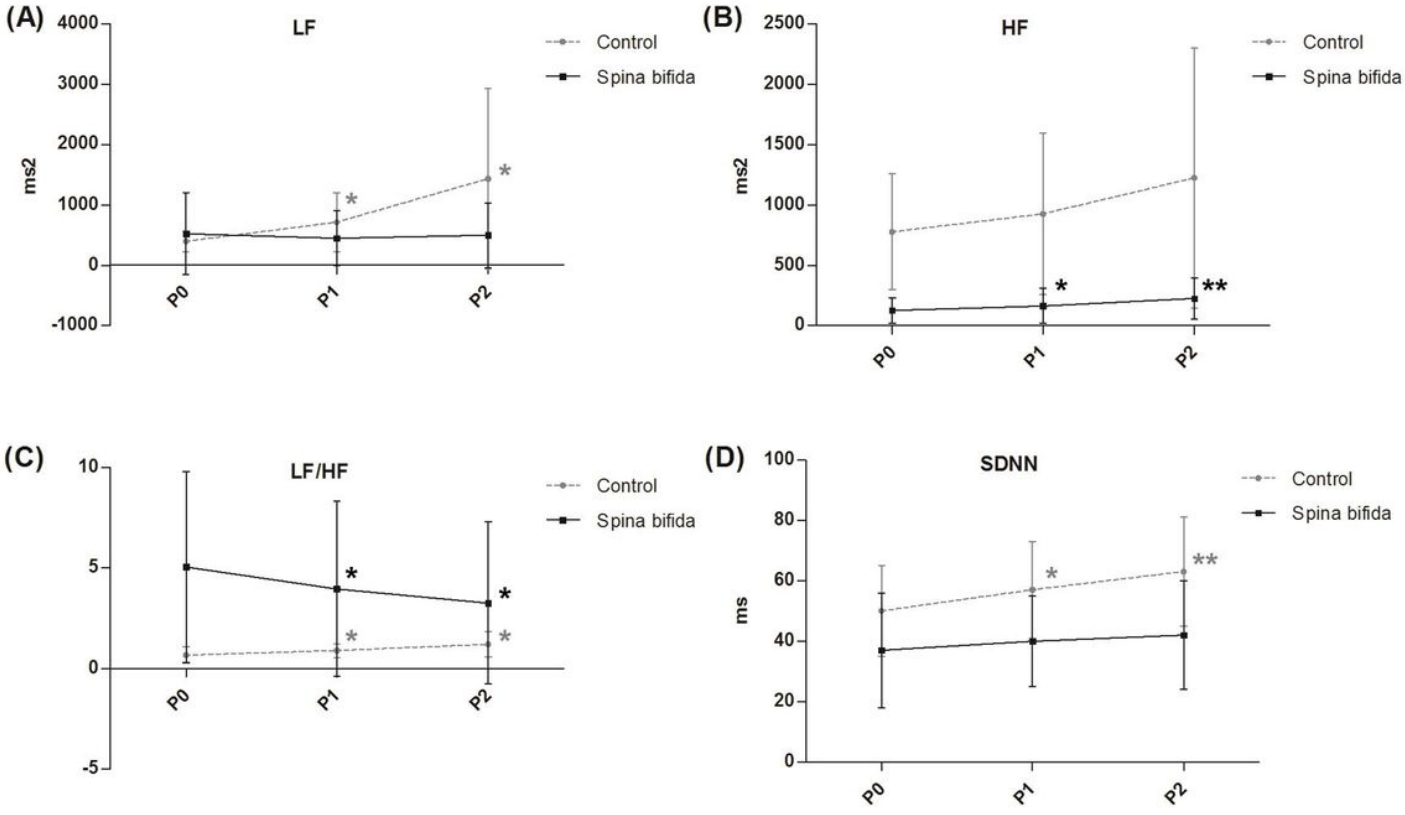

Figure 1

Changes of heart rate variability during bladder filling phase in patients with spina bifida and control group * p<0.05 compared only with P0 by paired t-test, ** p<0.05 compared with P0 and P1 by paired t-test

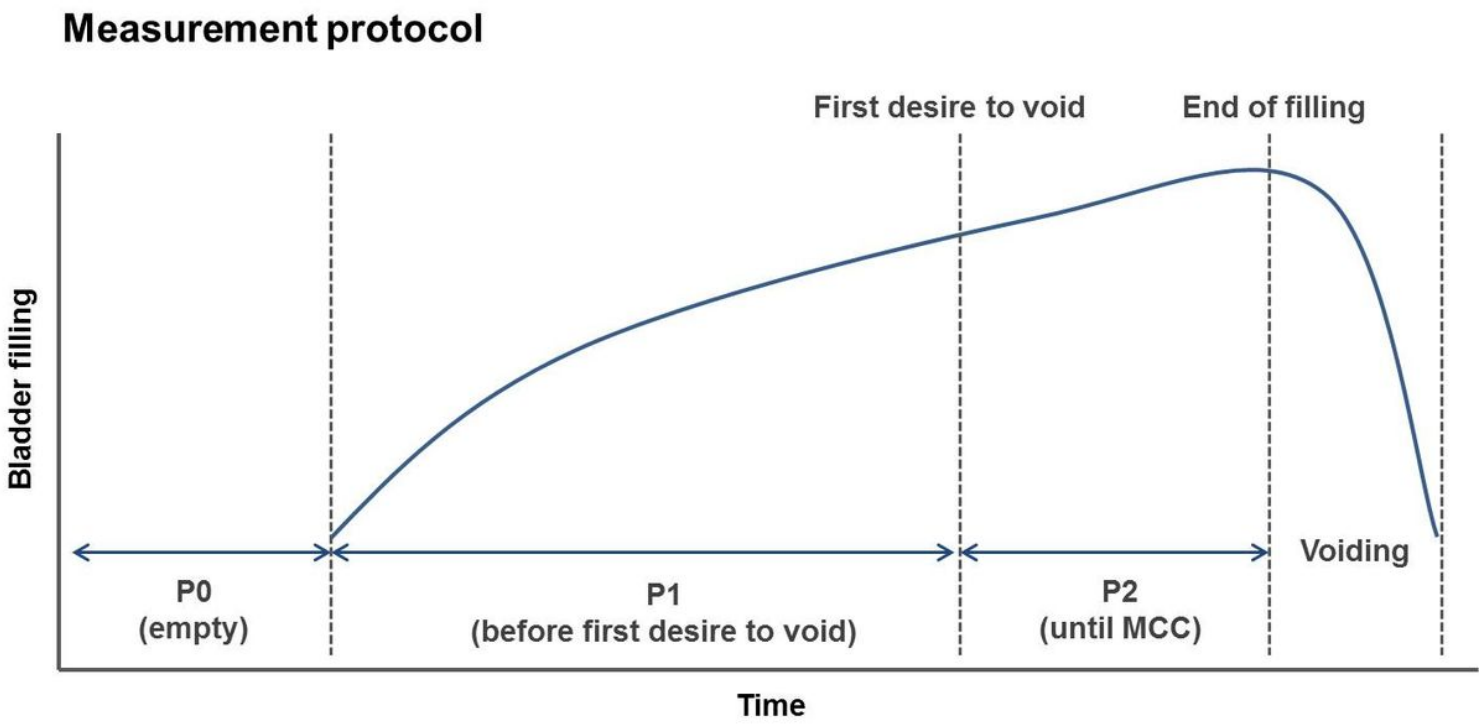

Figure 2

Measurement protocol of heart rate variability