To cite this article: Sahin EK, Uzunget SB. Heart rate variability is affected in young adult celiac patients, indicating autonomic dysfunction and increased cardiovascular risk. Turk J Clin Lab 2022; 4: 525-533.

Original Article

Heart rate variability is affected in young adult celiac patients, indicating autonomic dysfunction and increased cardiovascular risk

Genç yetişkin çölyak hastalarında bozulmuş kalp hızı değişkenliği otonomik bozukluk ve artmış kardiyovasküler risk

Kader Eliz Sahin[1], Sezen Baglan Uzunget*[2]

[1]Department of Cardiology. Adiyaman University Education and Research Hospital, Adiyaman, Turkey.
[2]Department of Cardiology, Liv Hospital Ankara,Turkey.

Abstract

Aim: Celiac Disease (CD) is a common autoimmune disease that can present at any age with varied symptoms. Cardiac involvement including vascular and arrhythmic disturbances has an important prognostic value in patients with autoimmune disorders. The aim of this study was to evaluate the effects of CD on cardiovascular autonomic functions by measuring the indices of heart rate variability (HRV).

Materials and Methods: Ninety-one CD patients and the ninety-three healthy volunteers were included in the study. We examined both time domain and frequency domain indices of HRV measured by 24-hour ambulatory electrocardiography.

Results: All of the time domain and frequency domain HRV parameters, with the exceptions of low frequency power and low frequency to high frequency ratio, were significantly lower in patients with CD compared to the control ($p < 0.01$). This difference retained for most of the HRV parameters when daytime and nighttime HRV parameters were compared separately. Correlation analysis revealed significant associations of HRV parameters with hemoglobin and ferritin levels and with duration of CD ($p < 0.01$). No correlation was found between HRV parameters and anti-tissue transglutaminase IgA and IgG levels which indicate the activity of the disease.

Conclusion: The study confirmed that patients with CD have reduced HRV, indicating the presence of objective autonomic dysfunction and increased cardiovascular risk.

Key words: autonomic dysfunction, Celiac disease, heart rate variability

Corresponding Author*: Sezen Baglan Uzunget, Liv Hospital Ankara, Kavaklıdere, Bestekar Cd No:8, 06680 Çankaya/Ankara
E-mail: sezenbaglan@hotmail.com
Orcid: 0000-0001-7608-3592
Doi: 10.18663/tjcl.1182275
Recevied: 03.10.2022 Accepted: 22.11.2022

TJCL Volume 13 Number 4 p: 525-533

Öz

Amaç: Çölyak Hastalığı, her yaşta çeşitli semptomlarla ortaya çıkabilen yaygın bir otoimmün hastalıktır. Vasküler ve aritmik bozuklukları içeren kalp tutulumu, otoimmün bozukluğu olan hastalarda önemli bir prognostik değere sahiptir. Bu çalışmanın amacı, kalp hızı değişkenliği (KHD) parametrelerini ölçerek, çölyak hastalığının kardiyovasküler otonomik fonksiyonlar üzerindeki etkilerini değerlendirmektir.

Gereç ve Yöntemler: Doksan bir çölyak hastası ve doksan üç sağlıklı gönüllü çalışmaya dahil edildi. 24 saatlik ayaktan elektrokardiyografi ile ölçülen kalp hızı değişkenliğinin hem zaman alanı hem de frekans alanı endekslerini inceledik.

Bulgular: Düşük frekanslı güç ve düşük frekans-yüksek frekans oranı hariç tüm zaman alanı ve frekans alanı KHD parametreleri, kontrole kıyasla çölyak hastalarında önemli ölçüde daha düşüktü ($p < 0.01$). Bu fark, gündüz ve gece KHD parametreleri ayrı ayrı karşılaştırıldığında, KHD parametrelerinin çoğu için korunmuştur. Korelasyon analizi, KHD parametrelerinin hemoglobin ve ferritin seviyeleri ve hastalık süresi ile önemli ilişkilerini ortaya çıkardı ($p < 0.01$). KHD parametreleri ile hastalığın aktivitesine işaret eden anti-doku transglutaminaz IgA ve IgG seviyeleri arasında korelasyon bulunmadı.

Sonuç: Çalışma, çölyaklı hastaların KHD'Yİ azalttığını doğruladı, bu bulgu objektif otonomik disfonksiyon ve artmış kardiyovasküler riskin varlığına işaret etmektedir.

Anahtar kelimeler: otonom disfonksiyon, çölyak hastalığı, kalp hızı değişkenliği

Introduction

Celiac disease (CD) is an autoimmune inflammatory disorder estimated to affect about 1 in 100 people worldwide. CD can develop at any age in genetically vulnerable people after consumption of gluten containing grains and manifests mainly with developmental, gastrointestinal and neurological complications 1. There are also increasing number of studies that have revealed cardiovascular system involvement in patients with CD. Diminished aortic elastic properties, impaired diastolic and systolic functions, increased risk of myo-pericarditis and heart rhythm disturbances, increased risk of stroke and ischemic heart disease have been reported in CD 2-4. Untreated adults with CD are shown to be at increased risk of premature atherosclerosis, as suggested to be associated with the presence of chronic inflammation caused by hypersensitivity to gluten and subsequently unfavorable biochemical results 3. Inflammation yields to changes in visceral perception and may induce hyper reaction and local and/or central sensitizations 5. Increased visceral sensory inflow may provoke autonomic nervous system (ANS) dysfunction and affects the heart 6. The cardiovascular part of ANS is responsible for the regulation of heart rate, blood pressure, and maintaining homeostasis. An impairment in ANS function is known to have a strong influence over heart and can lead to severely debilitating or even fatal cardiovascular conditions.

Heart rate variability (HRV) is one of the most feasible and widely used tools that indicate disturbances in the ANS and considered as a surrogate factor of the complex interactions between the brain and the cardiovascular system. 24-hour HRV measurement is accepted as a preferential method of assessing the cardiovascular risk, and its decreased values have been associated with in-hospital death, major complications after acute myocardial infarction, ventricular arrhythmias and sudden cardiac death in patients with heart failure 7,8. Moreover, decreased HRV has been reported to be predictive for mortality from all causes in general population 9.

After extensive search, we noticed that the data comparing cardiac autonomic involvement, in part HRV parameters, with CD patients and healthy subjects were quite limited and conflicting. We aimed in this study to evaluate HRV parameters obtained from 24-hour ECG Holter monitoring, as indicators of autonomic imbalance and predictors of increased cardiovascular risk in patients with CD. We also considered the potential correlations between clinical features of CD and HRV parameters.

Material and Methods

A total of one hundred and fifty-five ambulatory patients followed with diagnosis of CD were consecutively included at initial qualification of this cross-sectional study. CD was diagnosed based on a combination of clinical findings (positive serology for the following antibodies; anti-tissue transglutaminase IgA (tTG-IgA), anti-tissue transglutaminase IgG (tTG-IgG), anti-endomysium IgG and anti-gliadin IgA)) and intestinal biopsy results (presence of small intestinal villus atrophy on specimens). Exclusion criteria were set as follows: diabetes mellitus, hypertension, valvular heart disease, coronary artery disease, any known cardiac arrhythmia, left ventricular ejection fraction <50%, pericarditis, chronic kidney disease (GFR< 60 ml/min/1.72 m2), chronic liver disease and alcohol

intake, thyroid dysfunction, acute or chronic infection, chronic lung disease, insufficient Holter records and use of medications like neuropsychiatric drugs, analgesics and beta blockers.

After evaluation of exclusion criteria, 91 CD patients (52 females/39 males, mean age 28.8±6.79) were enrolled in the study. The control group involved gender and age matched 93 healthy volunteers who did not meet any of the exclusion criteria. A protocol consisting of a detailed medical history, physical examination, basic laboratory tests, 12-lead ECG, transthoracic echocardiography, and 24-hour ambulatory ECG was implemented for all participants. None of the participants had obvious symptoms of autonomic failure such as palpitations, blurred vision, abnormal sweating, nausea, light-headedness, dizziness, syncope, presyncope and balance problems. The patients were not receiving any medical treatment other than a strict gluten-free diet.

All study subjects provided signed informed consent after being informed about the study, Ethical approval was obtained from the Ethics Committee of our university with the decision number 2017/8-27.

Body mass index (BMI) was calculated by dividing weight in kilograms by the square of the height in meters. Brachial blood pressure measurements were taken twice using an automated device (Omron 705 IT electronic blood pressure monitor), and the average was calculated. Measurements were performed while the participant was sitting in recumbent position after at least 10 min rest. A comprehensive echocardiographic evaluation was performed for all participants using a Philips EPIQ 7 device (Philips Healthcare, Andover, MA, USA) following recommended protocols and criteria approved by European Association of Cardiovascular Imaging. The Simpson's method was used to calculate the left ventricular ejection fraction. Diastolic function was evaluated by using e' velocities and E/e' ratio. After 8 hours fasting period, blood samples were drawn from a vein in the forearm and were centrifuged at 3000 rpm for 15 min to separate sera. Complete blood count (CBC), thyroid stimulating hormone (TSH), fasting blood glucose, serum electrolytes, urea, creatinine, and lipid panel were measured. Autoantibody levels were checked to assess disease activity in CD patients.

Ambulatory Electrocardiography

Continuous ECG recordings of twenty-four hours were obtained using 3-channel digital recorders (DMS76a, Stateline, Nevada, USA). All patients had a similar daily routine. Recordings were analyzed by an experienced cardiologist who was totally blind to study population, using a standard software package (Cardioscan 11.0, DMS, NV, USA). Before analyses, data were manually reviewed and artefact beats were deleted. Ectopic beats were linearly inserted in the time series. Patients with a high rate (>5%) of ectopic beats or artifacts were discarded as exclusion criteria. 24-h average heart rate (HR) was recorded during 24- h electrocardiographic monitoring. Participants in both groups were instructed to have adequate rest and at least 8 hours of uninterrupted sleep the night before and on the day of the test. The period from 06:00 am to 10:00 pm was considered daytime and from 10:00 pm to 06:00 am as nighttime. Time and frequency domain indices of HRV were evaluated for 24 h and also for day and night periods separately.

Frequency domain analysis of HRV included total power (TP), high frequency (HF) component (0.15–0.40 Hz), low frequency (LF) component (0.04–0.15 Hz), and very low frequency (VLF) component (0–0.04 Hz), which were produced by Welch's Fast Fourier transform method. Normalized LF (LF norm) is the ratio between absolute value of the LF and difference between TP and VLF and normalized HF (HF norm) is the ratio between absolute value of the HF and difference between TP and VLF.

Time domain measures used for the analysis included the standard deviation of the normal-to-normal (NN) interval (SDNN) in seconds, the standard deviation of the average NN interval (SDANN) calculated over 5-min periods, the mean of the 5-min standard deviations of the NN intervals (SDNNI), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD) in milliseconds (ms), the number of adjacent NN intervals that differ from each other by more than 50 ms (NN50) as numbers and ratio of the number of interval differences of successive NN intervals of more than 50 ms to the total number of NN intervals (pNN50) as percentages. The integral of the density of the RR interval histogram divided by its height (Triangular Index) and triangular Interpolation of the NN Interval Histogram (TINN) were included as well. All analyses were performed according to the recommendations defined by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 10.

Statistical Analysis

All statistical analyses were performed using SPSS software (version 21.0 for Windows; SPSS Inc., Chicago, IL, USA). Normality of the data distribution was analyzed using the Kolmogorov-Smirnov test. Continuous data are presented as mean ± SD or median and interquartile ranges based on normality of variables. Differences among the groups were compared by independent samples t-test or Mann-Whitney U test according to normality of variables. Categorical variables were summarized as percentages and compared using chi-square test. Correlations between baseline biomarkers and HRV parameters were assessed with Spearman's and Pearson's correlation coefficients. Statistical significance of the findings was interpreted based on p values, and $p< 0.05$ was set as the level of statistical significance.

Results

Basic characteristics and laboratory findings of the study groups are presented in Table 1. Patients with CD were characterized by lower height, weight and BMI values compared to the control group. The mean known duration of the disease was 10±3.2 years in CD group. Serum lipid parameters, hemoglobin and serum ferritin levels were significantly lower in CD group compared to the control group. There were no statistically significant differences in majority of the echocardiographic parameters between the two groups (Table 2). Only significant difference among echocardiographic parameters was observed in é lateral and é mean parameters, however no diastolic dysfunction was observed in either group.

Table 1. Comparison of demographic data and laboratory findings of patients

	CD (+) (n=91)	Control (n=93)	P value
Age (years)	28.8 ± 6.7	29.2 ± 6.4	0.715[a]
Female (n, %)	52 (57.1%)	48 (51.6%)	0.451[b]
Height (cm)	167 ± 6.5	170 ± 6.4	0.001[a]
Weight (kg)	64 ± 8.4	70 ± 8.4	<0.001[a]
BMI (kg/m2)	22.8 ± 1.9	24.1 ± 1.7	<0.001[a]
Smoker (n, %)	18 (19.8%)	22 (23.7%)	0.524 [b]
SBP (mm Hg)	114 ± 9.8	116 ± 11.0	0.374[a]
DBP (mm Hg)	71 ± 6.7	74 ± 7.0	0.017[a]
tTG-IgG (IU/mL)	41.4(1.15-314.0)	-	
tTG-IgA (IU/mL)	100.0 (0.40-325.0)	-	
Duration of CD (years)	10 ± 3.2	-	
Total cholesterol (mg/dL)	150 ± 32.0	171 ± 25.5	<0.001[a]
Triglyceride (mg/dL)	97 ± 36.8	124 ± 50.7	<0.001[a]
HDL (mg/dL)	41 ± 11.4	50 ± 11.9	<0.001[a]
LDL (mg/dL)	89 ± 23.4	96 ± 23.0	0.049[a]
Fasting glucose (mg/dL)	89 ± 9.2	95 ± 8.9	<0.001[a]
Creatinine (mg/dL)	0.7 ± 0.13	0.8 ± 0.11	<0.001[a]
Sodium	139.31 ± 3.16	139.60 ± 3.25	0.534[a]
Potassium	4.23 ± 0.39	4.25 ± 0.42	0.657[a]
Calcium	9.35 ± 0.49	9.44 ± 0.44	0.187[a]
WBC (×109/L)	7.7 ± 2.2	7.3 ± 1.7	0.153[a]
Hemoglobin (g/L)	12.8 ± 2.42	14.5 ± 1.54	<0.001[a]
TSH (mU /L)	1.7 ± 1.1786	1.7 ± 0.948	0.655[a]
Ferritin (ng/mL)	8.3 (1.8 - 130.7)	35.0 (3.4 - 261.7)	<0.001[c]
CRP (mg/L)	0.2 (0.10 - 3.00)	0.2 (0.10 - 1.30)	0.584[c]

BMI: body mass index; CRP: C-reactive protein; DBP: diastolic blood pressure; HDL: high density lipoprotein; LDL: low density lipoprotein; SBP: systolic blood pressure; TSH: thyroid stimulating hormone, tTG-IgA: anti tissue transglutaminase A, tTG-IgG: anti tissue transglutaminase G, WBC; white blood cell
a; Mean ± SD in cases where the data were distributed normally, analyzed by student's t test.
b; analyzed by qi-square test.
c; Median (minimum-maximum) values were presented in cases where the data were not normally distributed, analyzed by Mann-Whitney U test.

Table 2. Comparison of echocardiographic measures of patients

	CD (+) (n=91)	Control (n=93)	P value
LA (mm)	31 ± 2.5	31 ± 2.4	0.226[a]
LVEDD (mm)	43 ± 1.9	44 ± 2.4	0.163[a]
LVESD (mm)	30 ± 1.9	30 ± 1.8	0.455[a]
LVEF (%)	61.1 ± 4.0	61.9 ± 4.1	0.212[a]
IVSd (mm)	8 ± 0.9	8 ± 0.8	0.656[a]
PWd (mm)	8 ± 0.9	8 ± 0.8	0.276[a]
E peak velocity (cm/s)	84 ± 13.2	85 ± 14.0	0.420[a]
A peak velocity (cm/s)	60 (40 - 140)	60 (40 - 140)	0.778[c]
E/A (ratio)	13±030	1.3 ± 0.29	0.284[a]
é septal velocity (cm/s)	10.7 ± 2.1	10.9 ± 1.5	0.543[a]
é lateral velocity (cm/s)	13.9 ± 2.3	15.0 ± 2.0	<0.001[a]
é mean velocity (cm/s)	12.3 ± 1.8	13.0 ± 1.5	0.008[a]
E/é mean	6.6 (4.0 – 12.0)	6.6 (4.5-10.0)	0.291[c]
Diastolic dysfunction (n, %)	1 (1.10%)	2 (2.15%)	0.573[b]
EDT (ms)	198 ± 27.08	198 ± 30.08	0.929[a]
IVRT (ms)	84 ± 11.3	87 ± 9.8	0.069[a]

A: late trans mitral flow velocity; E: early trans mitral flow velocity; é: mitral annular early diastolic velocity; EDT: E wave deceleration time; IVSd: interventricular septum thickness; IVRT: isovolumetric relaxation time; LA: left atrium diameter; LVEDD: left ventricular end-diastolic diameter; LVEF: left ventricular ejection fraction; LVESD: left ventricular end-systolic diameter; PWd: posterior wall thickness
a; Mean ± SD in cases where the data were distributed normally, analyzed by student's t test.
b; analyzed by qi-square test.
c; Median (minimum-maximum) values were presented in cases where the data were not normally distributed, analyzed by Mann-Whitney U test.

HRV parameters calculated for 24 h period were compared in Table 3. We found significantly lower values in all of the time domain HRV parameters of the patients with CD compared to the control. Also, TRI index, total power, VLF and HF parameters were significantly lower in CD patients compared to the control group. LF was also lower and LF/HF ratio was higher in CD patients, but these differences did not meet statistical significance.

A separate analysis for daytime and for night resting hours are shown in Table 4. In daytime activity, all frequency domain parameters and time domain parameters except AvgNN, pNN50, TINN were significantly lower in CD patients compared to the healthy control group. In the nighttime, in CD group, all of the time domain, and frequency domain parameters except LF/HF and LF norm ratio were statistically significantly lower compared to the control.

We also evaluated the circadian variations of HRV parameters (Table 5). In the control group with exceptions of SDNN, LF/HF ratio and LF norm values, time and frequency domain parameters were significantly lower in daytime activity compared to night resting hours. In CD group, AvgNN, SDSD, pNN50, Welch TP, VLF, HF and HF norm were significantly lower in daytime activity,

however, daytime decrease was not significant for RMSSD, NN51, Triangular index, TINN and LF parameters. Additionally, SDNN, LF/HF ratio and LF norm values statistically significantly lower in daytime in CD when compared to control.

Table 3. Comparison of HRV parameters in time and frequency domains between the CD(+) and the control group in a 24-hour period

	CD (+) (n=91)	Control (n=93)	P value
HR mean (min-1)	80 ± 9.35	76 ± 8.09	0.002[a]
HR minimum (min-1)	52 ± 5.42	48 ± 5.35	<0.001[a]
HR maximum (min-1)	143 ± 19.01	145 ± 16.00	0.486[a]
Time domain parameters			
SDNN (ms)	135.1 ± 25.22	163.4 ± 28.75	<0.001[a]
SDANN (ms)	120.7 ± 25.67	133.1 ± 31.50	0.004[a]
RMSSD (ms)	44.4 ± 11.54	53.1 ± 12.79	<0.001[a]
SDNNI (ms)	64.6 ± 10.11	76.0 ± 11.99	<0.001[a]
SDSD (ms)	44.4 ± 11.54	53.1 ± 12.79	<0.001[a]
NN50 (ms)	10713±6185.01	18794±7275.20	<0.001[a]
pNN50 (%)	14.8 ± 6.0	19.0 ± 7.9	<0.001[a]
Frequency domain parameters			
TRI index	19.8±5.3	22.4 ± 6.5	0.003[a]
Welch TP (ms2)	4350 ± 1059.50	5273 ± 1663.27	<0.001[c]
VLF (ms2)	2462 ± 839.27	3134 ± 1183.41	<0.001[a]
LF (ms2)	1185 ± 221.47	1268 ± 455.08	0.116[a]
HF (ms2)	702 ± 263.28	870 ± 426.73	0.002[c]
LF/HF ratio	1.8 ± 0.56	1.6 ± 0.8	0.126[c]

HF: high frequency power; HR: heart rate; LF: low frequency power; NN50: number of adjacent normal-to-normal (NN) intervals that differ from each other by more than 50 ms; pNN50: ratio of the NN50 to the total number of NN intervals; RMSSD: square root of the mean of the squared differences between adjacent NN intervals; SDANN: standard deviation of the average 5 min NN intervals; SDNN: standard deviation of NN intervals; SDNNi; mean of the 5 minute NN interval standard deviations; TRI index : triangular index; VLF: very low frequency power; TP: Welch total power
a; Mean ± SD in cases where the data were distributed normally, analyzed by student's t test.
c; Median (minimum-maximum) values were presented in cases where the data were not normally distributed, analyzed by Mann-Whitney U test.

Correlation analyses revealed presence of statistically significant ($p < 0.01$) correlations; between hemoglobin level and HR mean (R= -0.431), HR min (R= -0.360), SDNNI (R=0.369), Welch TP (R=0.422), VLF (R=0.376), LF (R=0.324); between ferritin level and HR mean (R= -0.410), HR min (R=-0.358), SDANN (R=0.322), SDNNI (R=0.436), Welch TP (R=0.419), VLF (R=0.408); and between duration of CD and HR mean (R=0.428), HR min (R=0.393), Welch TP (R= -0.373) and VLF (R= -0.3318). We found no correlation between tTG-IgA and tTG-IgG levels and any of the HRV parameters.

Discussion

We investigated HRV parameters among 91 CD patients and 93 healthy controls and found that almost all of the time domain and frequency domain HRV parameters, with the exceptions of LF and LF/HF ratio, were significantly lower in patients with CD compared to the control. This difference sustained for most of the HRV parameters when daytime and nighttime HRV parameters were compared separately. Correlation analysis disclosed significant associations of HRV parameters with hemoglobin, ferritin levels and with duration of CD. Nevertheless, no correlation was observed between HRV parameters and anti-tissue transglutaminase IgA and IgG levels.

Numbness, burning or tingling sensations, paresthesia, sweating, palpitations, lightheadedness, gastroparesis, incomplete bladder emptying, and orthostatic hypotension are well defined symptoms and findings in CD patients all of which are indicators of autonomic imbalance 11. Gluten sensitivity, in charge of the manifestations observed in CD, has been linked to a substantial number of multifocal motor neuropathies, small fiber sensory polyneuropathies and idiopathic axonal neuropathies 12. Considering this, the vagus, the body's longest and most complex cranial nerve, and the maestro responsible for immune defense mechanisms as well as regulating brain gut axis and heart rate, must have had its fair share of gluten-related damage. In this study we found significantly lower values in all of the time domain and most of the frequency domain HRV parameters of the patients with CD compared to the control. Although the ANS related symptoms in CD have been previously described, our results are important in terms of defining the autonomic involvement of the heart by using HRV parameters with prognostic and objective value.

Only a few publications based on small patient groups have referred to ANS impairment in patients with CD. Most of these studies used cardiovascular autonomic tests which measure blood pressure or heart rate response to stimuli such as valsalva maneuver, handgrip, isometric exercise, acoustic stress and/or deep breathing, rather than using spectral HRV analysis. Giorgetti et al. compared results of cardiovascular autonomic tests in 8 untreated CD patients with 13 healthy control subjects, and they revealed that the parasympathetic component was relatively predominant but their results were not statistically significant and did not change after 6 and 12 months of gluten-free diet 3. Penny et al reported a higher prevalence of (4%) serology and biopsy-proven CD in patients with Postural Orthostatic Tachycardia Syndrome, a dysautonomic condition 13. Usai et al reported autonomic neuropathy in 19% and different degrees of autonomic dysfunction in 45 % of 27 untreated CD patients with upper digestive functional disorders. We suppose that they might have underestimated the real prevalence of dysautonomia in CD patients since the study was composed of a small number of subjects 14.

Table 4. Comparison of HRV parameters between the CD(+) and the control groups during daytime and nighttime periods

	CD (n=91)	Control (n=93)	P value
Time domain parameters of daytime			
AvgNN (ms)	718 ± 87.96	740 ± 88.72	0.090[a]
SDNN (ms)	51.5 ± 18.81	60.7 ± 25.38	0.006[a]
RMSSD (ms)	34.2 (16.79 - 72.72)	37.9 (16.33 - 82.09)	0.003[c]
SDSD (ms)	34.2 (15.80 - 72.82)	38.0 (15.31 - 82.18)	0.003[c]
NN51 (ms)	4630 (608 - 13937)	7265(635 - 15045)	<0.001[c]
pNN50 (%)	12.1 (5.44 - 29.47)	14.1 (1.46 - 41.48)	0.153[c]
TRI index	8.9 ± 1.80	10.9 ± 2.05	<0.001[a]
TINN(ms)	295.7 ± 54.82	311.9 ± 58.51	0.055[a]
Frequency domain parameters of daytime			
TP (ms2)	3524 (1508.53 - 7432.60)	4721 (1257.89 - 075.74)	<0.001[c]
VLF (ms2)	2192 (931.30 - 4548.72)	2554 (894.67-6237.62)	<0.001[c]
LF (ms2)	993 ± 325.17	1383 ± 423.85	<0.001[a]
HF (ms2)	466 (157.96 - 1702.59)	677 (161.18-2023.51)	<0.001[c]
LF/HF ratio	2.0 (0.78 - 3.97)	1.7(0.53 - 4.91)	0.037[c]
LF norm (%)	72.5 ± 7.90	70.0 ± 8.13	0.034[a]
HF norm (%)	27.5 ± 7.90	30.0 ± 8.13	0.034[a]
Time domain parameters of nighttime			
AvgNN (ms)	870 ± 94.10	915 ± 90.18	0.001[a]
SDNN (ms)	60.3 ± 10.94	67.3 ±13.78	<0.001[a]
RMSSD (ms)	37.2 (19.93 - 63.78)	55.0 (5.39 - 112.50)	<0.001[c]
SDSD (ms)	40.3 (22.96 - 66.87)	55.1(17.30 - 112.66)	<0.001[c]
NN51 (ms)	5230 (641 - 15176)	9841(562 - 19246)	<0.001[c]
pNN50 (%)	19.6 (6.00 - 41.72)	29.8(1.13 - 54.48)	<0.001[c]
TRI index	9.0 ± 1.68	11.7 ± 2.24	<0.001[a]
TINN(ms)	296.8 ± 40.47	348.0 ± 61.43	<0.001[a]
Frequency domain parameters of nighttime			
TP (ms2)	4367 (1717.90 - 8226.91)	6963 (1601.58 - 13302.29)	<0.001[c]
VLF (ms2)	2561 (524.40 - 6676.64)	3596 (1035.04 - 8072.57)	<0.001[c]
LF (ms2)	1028 ± 333.01	1719 ± 681.76	<0.001[a]
HF (ms2)	642 (252.15 - 1739.02)	1239 (342.54 - 4120.68)	<0.001[c]
LF/HF ratio	1.6(0.42 - 4.78)	1.3(0.49 - 4.29)	0.067[c]
LF norm (%)	62.8 ± 10.82	57.2 ± 11.13	0.001[a]
HF norm (%)	37.2 ± 10.82	42.8 ± 11.13	0.001[a]

HF: high frequency power; HR: heart rate; LF: low frequency power; NN50: number of adjacent normal-to-normal (NN) intervals that differ from each other by more than 50 ms; pNN50: ratio of the NN50 to the total number of NN intervals; RMSSD: square root of the mean of the squared differences between adjacent NN intervals; SDANN: standard deviation of the average 5 min NN intervals; SDNN: standard deviation of NN intervals; SDNNi; mean of the 5 minute NN interval standard deviations; TINN: triangular interpolation of the NN interval histogram; TRI index : triangular index; VLF: very low frequency power; TP: Welch total power
a; Mean ± SD in cases where the data were distributed normally, analyzed by student's t test.
c; Median (minimum-maximum) values were presented in cases where the data were not normally distributed, analyzed by Mann-Whitney U test.

Przybylska-Felus et al. recently showed that both LF and HF parameters, obtained from 30 min ECG recordings, were lower in a CD cohort of 25 patients than in controls. They identified disturbed autonomic balance in 14 (56%) of CD patients 15. However, Kuusela reported that the correlation between resting values obtained from short-term measurements and indices obtained from 24-hour observations was unsatisfactory 16. 24-hour monitoring of HRV provides more valid measurements of VLF, total power, and LF/HF-domain indices compared to ultra-short-term and short-term monitoring. Furthermore, from frequency-domain indices, only 24-hour HRV measurements achieve prognostic power for morbidity and mortality. Again, 24-hour SDNN values have prognostic power such as predicting future heart attack risk while short term SDNN values do not 17. SDNN is affected by both sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) activity and is highly correlated with VLF, LF and total frequency power. Lower SDNN values have been

Table 5. Comparison of HRV parameters between daytime and nighttime periods

	DAYTIME	NIGHTTIME	P value
CELIAC (+)			
AvgNN (ms)	718 ± 87.96	870 ± 94.10	<0.001[a]
SDNN (ms)	51.5 ± 18.81	60.7 ± 25.38	<0.001[a]
RMSSD (ms)	34.2 (16.79 - 72.72)	37.2 (19.93 - 63.78)	0.080[c]
SDSD (ms)	34.2 (15.80 - 72.82)	40.3(22.96 - 66.87)	<0.001[c]
NN51 (ms)	4630 (608 - 13937)	5230(641 - 15176)	0.157[c]
pNN50 (%)	12.1 (5.44 - 29.47)	19.6(6.00 - 41.72)	<0.001[c]
TRI index	8.9 ± 1.80	9.0 ± 1.6	0.642[a]
TINN(ms)	295.7 ± 54.82	296.8 ± 40.47	0.882[a]
Welch TP (ms2)	3524 (1508.53 - 7432.60)	4367 1717.90 - 8226.91)	0.002[c]
VLF (ms2)	2192 (931.30 - 4548.72)	2561 (524.40 - 6676.64)	0.009[c]
LF (ms2)	993 ± 325.17	1028 ± 333.01	0.484[a]
HF (ms2)	466 (157.96 - 1702.59)	642 (252.15 - 1739.02)	<0.001[c]
LF/HF ratio	2.0 (0.78 - 3.97)	1.6 (0.42 - 4.78)	<0.001[c]
LF norm (%)	72.5 ± 7.90	62.8 ± 10.82	<0.001[a]
HF norm (%)	27.5 ± 7.90	37.2 ± 10.82	<0.001[a]
CONTROL			
AvgNN (ms)	740 ± 88.72	915 ± 90.18	<0.001[a]
SDNN (ms)	60.3 ± 10.94	67.3 ± 13.78	<0.001[a]
RMSSD (ms)	37.9 (16.33 - 82.09)	55.0 (5.39 - 112.50)	<0.001[c]
SDSD (ms)	38.0 (15.31 - 82.18)	55.1 (17.30 - 112.66)	<0.001[c]
NN51 (ms)	7265 (635 - 15045)	9841 (562 - 19246)	0.012[c]
pNN50 (%)	14.1(1.46 - 41.48)	29.8 (1.13 - 54.48)	<0.001[c]
TRI index	10.9 ± 2.05	11.7 ± 2.24	0.012[a]
TINN (ms)	311.8 ± 58.15	348.0 ± 61.4	<0.001[a]
Welch TP (ms2)	4721 (1257.89 - 075.74)	6963 (1601.58 - 13302.29)	<0.001[c]
VLF (ms2)	2554 (894.67 - 6237.62)	3596 (1035.04 - 8072.579)	<0.001[c]
LF (ms2)	1383 ± 423.85	1719 ± 681.76	<0.001[a]
HF (ms2)	677 (161.18 - 2023.51)	1239 (342.54 - 4120.68)	<0.001[c]
LF/HF ratio	1.7 (0.53 - 4.91)	1.3 (0.49 - 4.29)	0.002[c]
LF norm (%)	70.0 ± 8.13	57.2 ± 11.13	<0.001[a]
HF norm (%)	30.0 ± 8.13	42.8 ± 11.13	<0.001[a]

HF: high frequency power; HR: heart rate; LF: low frequency power; NN50: number of adjacent normal-to-normal (NN) intervals that differ from each other by more than 50 ms; pNN50: ratio of the NN50 to the total number of NN intervals; RMSSD: square root of the mean of the squared differences between adjacent NN intervals; SDANN: standard deviation of the average 5 min NN intervals; SDNN: standard deviation of NN intervals; SDNNi; mean of the 5 minute NN interval standard deviations; TINN: triangular interpolation of the NN interval histogram; TRI index: triangular index; VLF: very low frequency power; Welch TP: Welch total power
a; Mean ± SD in cases where the data were distributed normally, analyzed by student's t test.
c; Median (minimum-maximum) values were presented in cases where the data were not normally distributed, analyzed by Mann-Whitney U test.

associated with more frequent and complex arrhythmias, as well as a higher risk of developing arterial hypertension and progression to atherosclerotic lesions 18,19. The pNN50 is closely correlated with vagal activity, still the RMSSD is the primary time-domain measure used to estimate the PNS mediated changes reflected in HRV 17. The low levels we observed in all these HRV parameters support the presence of impaired vagal activity as well as impaired SNS activity in CD.

Among the frequency parameters of HRV; LF and HF bands are usually assumed to correspond to cardiac sympathetic and parasympathetic activities, respectively 20. However, considering the complex nonlinear interactions between the sympathetic and parasympathetic parts of the autonomic nervous system, this would be a very simplistic approach 21. There is accumulating evidence that demonstrates cardiac 22. The other frequency parameter, VLF rhythm is generated by heart's intrinsic nervous system (PNS) and the frequency and amplitude of its oscillations are modulated by the 23. VLF power has been found to be more strongly associated with all-cause mortality than LF or HF power. Low VLF power also

has been shown to be associated with high inflammation and arrhythmogenic cardiac death.

In our results; compared to the control, significantly lower values of total, VLF, LF and HF powers, in day and night recordings, and significantly higher LF/HF ratio in daytime measurements also support that both the sympathetic and the parasympathetic components of the cardiac autonomous nervous system are affected in CD patients. These findings are important to demonstrate that CD patients have prognostic consequences concerning ANS control of heart rate.

We performed correlation analyses to investigate the factors associated with the decrease in HRV in CD patients. Immunological injuries come to the fore to explain pathophysiology of gluten neuropathy alongside nutritional deficiencies and toxic injuries. However, our correlation analyses revealed no association between any of the HRV parameters with the blood levels of either tTG-IgA, the most sensitive and specific blood test which shows activity of CD or tTG-IgG which is an alternative test in people who have IgA deficiency. In our cohort, mean duration of CD diagnosis was 10 ±3.27 years and correlation analyses revealed significant positive correlations between CD duration with HRV parameters. In other words, our results showed that the relationship between ANS deterioration and CD duration was greater than that with current activity of the disease. While there are publications showing that a gluten-free diet retrieves symptoms and adverse features, there are also reports asserting that it has no benefit and that longer-term inflammation result in irreversible changes 24. Luostarinen et al. showed that the axonal neuropathy might be present subclinical in CD, without evidence of malabsorption and often persisted even with good adherence to a gluten free diet 25. They also reported an increased occurrence of axonal neuropathy even in well treated CD patients. Their findings are consistent with our results showing that prolonged duration of CD may have negative effects on heart rate variability, without an evidence of clinical disease activity.

The difference between CRP levels in CD and control groups was not significant in this study, which can be attributed to the sample size as well as to the difficulty in ruling out subtle inflammatory state in the control group at all. Electrolyte disturbances are common in CD and their relation to decreased HRV is controversial 26, we observed no significant difference in between the electrolyte levels of the two groups.

We found significantly lower hemoglobin and serum ferritin levels in CD patients compared to the control and also demonstrated mild to moderate correlations between HRV parameters and hemoglobin and ferritin levels. Relation between anemia and decreased HRV was reported previously. Gehi et al. showed that a decrease in hemoglobin level of 1 g/dl in patients with stable coronary heart disease was associated with an increased probability of having low HRV, independent of possible confounding factors 27. Yokusoglu et al. previously described altered ANS activity in iron deficiency anemia and suggested that there would be a relation between anemia and the increased sympathetic activity that is triggered by the hypoxia perceived through carotid bodies 28. Thus, treatment of iron deficiency anemia is of extra importance in CD.

As for the diurnal variation, since vagal activity is dominant during sleep of a healthy person, HRV is expected to be higher typically during nighttime. According to our findings, although nighttime increases in the control group were statistically more significant, most of the time domain and frequency domain parameters were higher during the nighttime in both CD and control groups thus not allowing to assert presence of a diurnal deterioration in the autonomic activity of CD patients.

This study has some limitations, especially the relatively small sample size. However, despite the small sample size, our results are noteworthy as it is one of the studies with the highest number of subjects among studies evaluating autonomic function in CD patients. Another limitation is that the present study only investigated HRV parameters from 24-hour ambulatory ECG, which can be affected by daily physical activity, and did not examine autonomic function tests evaluating sudomotor and baroreflex functions. In addition, most of the patients in the study were diagnosed at adult age and had relatively short known disease durations.

Conclusion

We have demonstrated significant difference between CD and control groups, in 24-hour time domain and frequency domain parameters that are prognostic for cardiovascular events. Autonomic control of the heart rate is significantly affected by the involvement of the gut brain axis in CD, therewithal, iron deficiency anemia and hypolipidemia might also have contributed to some extent. Since increased duration of disease correlates with impairment in ANS control of heart rate, we suggest that early diagnosis and onset of treatment of CD with special attention on iron deficiency and anemia is of great importance to prevent irreversible autonomic neurological damage and thus cardiovascular adverse outcomes.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding sources

None

Ethical approval

Approval for the study was obtained from the local ethics committee (Decision number: 2017/8-27)

Acknowledgments

We owe MD, Assoc Prof. IO a dept of gratitude and we would like to thank him for referring and providing us guidance for patients with celiac disease.

References

1. Rubio-Tapia A, Murray JA. Celiac disease. Curr Opin Gastroenterol. 2010;26(2):116-122.
2. Mormile R. Celiac disease and atherosclerosis: An immunologic puzzle to be solved? Immunol Lett. 2016;180:75-76.
3. De Marchi S, Chiarioni G, Prior M, Arosio E. Young adults with coeliac disease may be at increased risk of early atherosclerosis. Aliment Pharmacol Ther. 2013;38(2):162-169.
4. Ciaccio EJ, Lewis SK, Biviano AB, et al. Cardiovascular involvement in celiac disease. World J Cardiol. 2017;9(8):652-666.
5. Buéno L, Fioramonti J, Garcia-Villar R. Pathobiology of visceral pain: molecular mechanisms and therapeutic implications. III. Visceral afferent pathways: a source of new therapeutic targets for abdominal pain. Am J Physiol Gastrointest Liver Physiol. 2000;278(5):G670-676.
6. Giorgetti GM, Tursi A, Iani C, et al. Assessment of autonomic function in untreated adult coeliac disease. World J Gastroenterol. 2004;10(18):2715-2718.
7. Nolan J, Batin PD, Andrews R, et al. Prospective study of heart rate variability and mortality in chronic heart failure: results of the United Kingdom heart failure evaluation and assessment of risk trial (UK-heart). Circulation. 1998;98(15):1510-1516.
8. Huikuri HV, Stein PK. Clinical application of heart rate variability after acute myocardial infarction. Front Physiol. 2012;3:41.
9. Sessa F, Anna V, Messina G, et al. Heart rate variability as predictive factor for sudden cardiac death. Aging (Albany NY). 2018;10(2):166-177.
10. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation. 1996;93(5):1043-1065.
11. Chin RL, Latov N. Peripheral Neuropathy and Celiac Disease. Curr Treat Options Neurol. 2005 Jan;7(1):43-48.
12. Hadjivassiliou M, Grünewald RA, Kandler RH, et al. Neuropathy associated with gluten sensitivity. J Neurol Neurosurg Psychiatry. 2006 Nov;77(11):1262-6.
13. Penny HA, Aziz I, Ferrar M, et al. Coeliac Disease is More Common in Subjects with Postural Orthostatic Tachycardia Syndrome Compared to Healthy Controls. Gut. 2016;65:165-166.
14. Usai P, Usai Satta P, Lai M, et al. Bassotti G. Autonomic dysfunction and upper digestive functional disorders in untreated adult coeliac disease. Eur J Clin Invest. 1997;27(12):1009-1015.
15. Przybylska-Felus M, Furgala A, Zwolinska-Wcislo M, et al. Disturbances of autonomic nervous system activity and diminished response to stress in patients with celiac disease. J Physiol Pharmacol. 2014;65(6):833-841.
16. Kuusela T. Methodological aspects of heart rate variability analysis. In: Kamath MV, Watanabe MA, Upton ARM, editors. Heart Rate Variability (HRV) Signal Analysis. Boca Raton, FL: CRC Press .2013; 9–42.
17. Shaffer F, McCraty R, Zerr CL. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. Front Psychol. 2014;5:1040.
18. Colivicchi F, Bassi A, Santini M, Caltagirone C. Cardiac autonomic derangement and arrhythmias in right-sided stroke with insular involvement. Stroke. 2004;35(9):2094-2098.
19. Dekker JM, Crow RS, Folsom AR, et al. Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: the ARIC Study. Atherosclerosis Risk In Communities. Circulation. 2000;102(11):1239-1244.
20. Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. Circulation. 1991;84(2):482-92.
21. Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. Front Physiol. 2013;4:26.
22. Taylor JA, Myers CW, Halliwill JR, et al. Sympathetic restraint of respiratory sinus arrhythmia: implications for vagal-cardiac tone assessment in humans. Am J Physiol Heart Circ Physiol. 2001;280(6):H2804-2814.
23. Shaffer F, Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. Front Public Health. 2017 Sep 28;5:258.
24. Mayassi T, Ladell K, Gudjonson H, et al. Chronic Inflammation Permanently Reshapes Tissue-Resident Immunity in Celiac Disease. Cell. 2019;176(5):967-981.
25. Luostarinen L, Himanen SL, Luostarinen M, et al. Neuromuscular and sensory disturbances in patients with well treated coeliac disease. J Neurol Neurosurg Psychiatry. 2003;74(4):490-494.
26. Wen TL, Chung-Kwe W, Yang IF, Yang TF. Relationship between electrolytes and heart rate variability parameters in end-stage renal failure patients before and after hemodialysis. Anadolu Kardiyol Derg. 2007;7 (Suppl 1):142-4.
27. Gehi A, Ix J, Shlipak M, et al. Relation of anemia to low heart rate variability in patients with coronary heart disease (from the Heart and Soul study). Am J Cardiol. 2005;95(12):1474-1477.
28. Yokusoglu M, Nevruz O, Baysan O, et al. The altered autonomic nervous system activity in iron deficiency anemia. Tohoku J Exp Med. 2007;212(4):397-402.