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Original Article

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ABSTRACT

Objective: Inflammatory bowel disease (IBD) has effects on neural cardiovascular control mechanisms. The aim of this study was to evaluate the effect of IBD status on cardiovascular autonomic functions by measuring heart rate variability (HRV) parameters with 24-hour holter electrocardiogram (ECG) recording.
Design: A prospective analytical case control study
Setting: Yıldırım Beyazıt University Medical Faculty and Ataturk Education and Research Hospital Ankara, Turkey
Subjects: Sixty-seven patients with IBD and 51 matched control subjects were included in the study
Intervention: All participants underwent a 24-hour Holter recording to assess HRV parameters.
Main Outcome Measure: The study population was separated into 3 groups of active disease, remission and control group to analyse the effect of disease activity status on the HRV parameters.
Results: No difference was determined between the IBD and control groups in respect of any HRV parameters. Significant differences were determined between the active IBD patients and the remission group in terms of the HRV measurements of SDNN5, Triangular index, AVG, SDVLF and SDdef. The measurements of SDNN and PNN50 were found to be significantly different between the active IBD patients and the control group.

Conclusions: The results of this study demonstrated for the first time that the active phase of IBD is associated with cardiac autonomic abnormalities compared to both a control group and IBD patients in remission. Patients with IBD should be followed up closely for cardiovascular events as they appear to be at risk for cardiovascular diseases and arrhythmia, particularly during the active phase of the disease.

KEYWORDS: Heart rate variability, inflammatory bowel diseases, autonomic nervous system, Chronic inflammation

INTRODUCTION

Inflammatory bowel disease (IBD), with the two main forms of Crohn's disease (CD) and ulcerative colitis (UC), is an autoimmune disease characterized by exacerbation and remission periods of inflammation in the gastrointestinal tract. Currently, the etiology of IBD remains unknown. Previous studies have shown that IBD patients are at increased risk of myocardial infarction (MI), atrial fibrillation (AF), stroke, heart failure, hospitalization, and cardiovascular death [1-5].

Heart rate variability (HRV) is the fluctuation in the time intervals between adjacent heartbeats. Assessment of HRV is based on analysis of consecutive normal R-R (NN) intervals and may provide quantitative information on the modulation of cardiac vagal and sympathetic nerve input. This is a non-invasive, practical and reproducible test which can be used to assess the autonomic nervous system (ANS) modulation functions under physiological and pathological conditions[6-9]. Changes in HRV patterns provide a sensible and advanced indicator of health involvement. A higher HRV is a signal of good adaptation and characterizes a healthy person with efficient autonomic mechanisms. Conversely, a decrease in HRV is thought to reflect inability or attenuation of the ANS and changes in the sinoatrial node response, which may indicate the presence of physiological malfunction in the patient, require further investigations to establish a specific diagnosis. Moreover, severe cardiovascular disease may also be related to a reduced HRV[10].

The aim of this study was to examine cardiac autonomic functions in a large patient cohort of IBD, including both those in active and remission periods of the disease, using HRV measurements. To date, the association of HRV and IBD activity status has not been evaluated using 24-hour Holter electrocardiogram (ECG) recording. Thus, it was planned to investigate whether HRV parameters are impaired in IBD patients compared to a control group and to evaluate the relationship between IBD disease activity status and HRV parameters.

SUBJECTS AND METHODS

Patients

This prospective study included a total of 67 consecutive patients with IBD (53 patients with UC, 14 patients with CD, aged 18-50 years) and a healthy control group of 51 subjects. Patients were recruited from the Department of Gastroenterology, Ataturk Education and Research Hospital, Ankara, between December 2013 and October 2015. The IBD diagnosis was confirmed with established criteria of clinical, radiological, endoscopic and histological findings. A detailed medical history was taken, including disease

duration and medications, and all patients underwent a routine physical and echocardiographic examination. Surrogate markers of disease activity were defined as hospitalizations with IBD as the primary diagnosis, initiation of biological anti–tumor necrosis factor treatment, and claimed prescription of glucocorticoids. With the combined use of these markers and disease activity scores using the Crohn's Disease activity index (CDAI) and the Mayo index [11-13], the disease stages of remission and flare-up were defined.

Exclusion criteria were age <18 years, structural heart disease, overt cardiovascular disease on the basis of abnormal echocardiographic findings, diabetes mellitus, hypo- or hyperthyroidism, pulmonary disease and neoplastic or chronic systemic diseases, previous gastrointestinal surgery, or the use of medications such as beta-blockers that could interfere with HRV, anti-arrythmic drugs, digitalis or central sympatholytic agents, antihistaminic agents, benzodiazepines, or antidepressants. The control group was formed of healthy individuals with no complaints of organic or functional disease and who were not taking any medications at the time of evaluation.

Echocardiography

All the echocardiographic evaluations were made by a single, experienced cardiologist, blinded to patient's data. The echocardiographic examinations were applied using a Vingmed System 7 (Vivid 7, GE, Horten, Norway) with a 2.5- to 3.5-MHz transducer. The left ventricular (LV) systolic and diastolic functions were analyzed using standard two-dimensional (2D) echocardiography, M-mode echocardiography, and pulsed-wave (PW) echocardiography according to the latest guidelines[14]. The LV ejection fraction (LVEF) was calculated with the biplane modified Simpson method.

Heart rate variability measurement

A 24-hour Holter recording was applied to all study participants to evaluate the HRV parameters. Holter ECG was performed using a 3-channel digitized recorder (Custo flash 500, Custo Med, Ottobrunn, Germany). Reviewing and editing of the data was performed by an experienced physician blinded to the study population. To be acceptable for the study evaluation data suitable for analysis were required from a period of 23 hours. Recordings were repeated in cases where these criteria were not met. HRV analysis was performed on measurements, which were standardised and had been validated for the evaluation of autonomic control of the heart[15].

There are three methods for quantifying HRV. These are time domain analysis, frequency domain analysis and the geometric method. In time domain analysis, the intervals between adjacent normal R waves (normal to normal, NN intervals) are measured over the period of recording. In the time domain method, SDNN (the standard deviation of all NN intervals during a 24-hour period) is the most commonly used time domain measurement of HRV. SDANN5 (the standard deviation of the 5-minute average of NN intervals) measures long-term fluctuations. The most common variables calculated as differences between normal R-R intervals are rMSSD (the square root of the mean squared differences of successive NN intervals).and pNN50 (proportion of differences between successive intervals >50 ms). RMSSD is an estimate of high-frequency variations in short-term RR recordings and, therefore, reflects parasympathetic

regulation of the heart. The time domain HRV indices measured in this study were SDNN, SDNN5 (the mean of the deviation of 5 min NN intervals over the entire recording) SDANN5, rMSSD, pNN50 and the HRV Triangular index.

Frequency-domain measurements estimate the distribution of absolute or relative power into four frequency bands. Frequency domain parameters include total power (TP), very low frequency (VLF), low frequency (LF), and high frequency (HF). HF reflects the parasympathetic outflow, and TP reflects overall autonomic activity, although the physiological explanation of the VLF component is less defined. The LF power is modulated by both sympathetic and parasympathetic outflows as well as by other factors, including baroreceptor activity. In the frequency domain analysis of this study, examination was made of AVG (Average value of all RR intervals), SDTF(Standard deviation of TF over 24 hours), SDVLF(Standard deviation of VLF over 24 hours), SDHF (Standard deviation of HF over 24 hours), and SDdef (Standard deviation in the predefined frequency range).

The descriptions and clinical meanings of the HRV parameters calculated in this study are presented in Table 1 and Table 2.

Clinical and Laboratory Assessments

Venous blood samples were taken from all participants after a 12-hour fast. High-sensitive C-reactive protein (Hs-CRP) was calculated using a nephelometric method. All laboratory analyses were performed using autoanalyzers. Blood pressure measurements were taken 3 times with the patient seated and following a 5-min rest period and the average measurement was used in the analyses. Hypertension (HT) was defined as blood pressure >140/90 mm Hg or the use of antihypertensive agents. Diabetes mellitus (DM) was defined as a fasting plasma glucose level >126 mg/dL or glucose level >200 mg/dL at any time of measurement, or the use of antidiabetic drugs. Dyslipidemia was defined as total cholesterol level of 260 mg/dL or LDL level 160 mg/dL or the use of lipid-lowering agents.

Statistical analysis and ethics

All statistical analyses were made using SPSS statistical software (IBM SPSS Statistics for Windows, Version 20.0, IBM Corp., Armonk, NY, USA). Continuous variables were stated as mean ± standard deviation (SD) and categorical variables as number (n) and percentage (%). The normality of the distribution of continuous variables was analyzed using the Shapiro-Wilk test. The significance of the differences in the measurements obtained for the control and patient groups was analyzed using the Student's t-test or the Mann-Whitney U test. ANOVA was applied for multiple comparisons between groups. For quantitative values, the Kruskal Wallis test was used for the comparison of patients with active disease, remission and the healthy control group. To identify pairs of groups with significant differences in quantitative parameters, Bonferroni adjustment for multiple comparisons was used. The Pearson´s correlation analysis was performed for variables with normal distribution and Spearman´s rank correlation was used for variables with non-normal distribution. Tests of significance were two-tailed and a value of $p<0.05$ was accepted as statistically significant.

The study was conducted in compliance with the latest version of the Declaration of Helsinki. Written informed consent was obtained from each study participant. Approval for the study protocol was granted by the local Ethics Board of Ankara Ataturk Education and Research hospital.

RESULTS

Demographic characteristics

The study included a total of 118 participants, comprising 67 patients with IBD and 51 healthy control subjects. The baseline characteristics of the study population are presented in Table 3. No significant differences were found between the patients and the control group regarding demographic features ($p>0.05$). There were 43 male (51.7%) and 24 female patients in the IBD group. The mean age was 42.67 ± 12.8 years in the patient group, and 43.2 ± 15.3 years in the control group. No statistically significant difference was determined between the groups in respect of age, gender, body mass index (BMI), fasting blood glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, hemoglobin (Hgb), white blood cell (WBC) or platelets (PLT). There were 6 hypertensive patients and 6 smokers in both groups. Triglyceride levels were significantly higher in the patient group, but no difference was seen between the groups in terms of LDL cholesterol or HDL cholesterol. In the evaluation of inflammatory markers, erythrocyte sedimentation rate (ESR) was higher in the IBD group, but did not reach a statistically significant level. The Hs-CRP level was determined to be significantly higher in the IBD group than in the control group ($p = 0.01$).

Disease duration was 4.57 ± 3.43 years. Active disease was determined in 42.1% of the patient group. Of the patients with IBD, 20.9% had CD, 9% had proctitis, 37.3% had left-side colitis, and 31.3% had pancolitis. All patients used medications for IBD. The majority (94%) of the patients were using 5-aminosalicylic acid (5-ASA), and a few patients were taking immunosuppressive drugs such as azathiopurine (11.9%) and steroids (10.4%)

Heart Rate Variability parameters

The comparisons of HRV parameters between the IBD patients and the healthy control group are shown in Table 4. No differences were determined between the groups in respect of time domain measures and frequency domain measures. No differences were determined between the groups in respect of the basic parameters of minimum/maximum/mean heart rate, supraventricular extrasystole (SVES) and ventricular extrasystole (VES) count.

To analyse the effects of disease activity on the HRV parameters, the study population was separated into 3 groups of active disease, remission and control. The baseline characteristics of the 3 groups are presented in Table 5. No significant difference was determined between the groups in terms of age, gender, BMI, smoking status, and HT. The LDL, HDL, total cholesterol, and creatinine levels were observed to be similar in all the groups. The hs-CRP, ESR, and PLT values were significantly higher in the active disease group compared to the other two groups (Table 5). WBC was significantly higher and the hemoglobin level was lower in patients with active disease compared with the control group and patients with remission, respectively. Triglyceride and ESR levels were higher in IBD patients in remission than in

the control group. No significant difference was observed in respect of hs-CRP, WBC, PLT, and Hgb between IBD patients in remission and the control group.

Comparison of HRV measures according to disease activity status are shown in Table 6. SDANN5, RMSSD, SDTF, SDHF and LF values were lower in the active disease group than in the control and remission groups, with no statistically significant difference observed. No significant differences were determined between the groups in terms of maximum/minimum/mean heart rate (HR), SVES and VES count. The active IBD patients differed significantly from both the control and remission groups in terms of SDNN5, triangular index, AVG, SDVLF, and SDdef. Significant differences were determined between active patients and the control group in respect of SDNN and PNN50. No significant differences were found between IBD patients in remission and the control group in respect of any HRV parameters.

Correlation analyses between inflammatory markers and HRV parameters are shown in Table 7. In all the study groups, CRP was positively correlated with mean HR and minimum HR, and negatively correlated with SDTF, SDANN5, AVG, SDNN and SDVLF. There was a significantly negative correlation of ESR levels with SDVLF, SD, AVG and a positive correlation with minimum/mean HR. Age was determined to be negatively correlated with maximum HR, SDNN and SDANN5. There was a positive correlation between SDNN, SDANN5, SDNN5, AVG, SDVLF and albumin levels. Hemoglobin levels were negatively correlated with HR, and positively correlated with SDANN5, AVG, SDTF, and SDVLF.

In the evaluation of the effects of disease activity scores, such as the Mayo score, on HRV parameters, minimum HR and mean HR were positively correlated and SDNN5, AVG were negatively correlated. Correlation analysis with CDAI could not be performed since the number of patients in the Crohn's disease group was insufficient for analysis. When the Crohn's and UC active status patients were analysed together in the active group, CRP was determined to be positively correlated with minimum/mean/maximum HR and negatively correlated with SDVLF, AVG and SDTF. In the active disease group, the same interaction was observed with ESR level. In the remission and control groups, CRP and ESR levels were not significantly correlated with most of the HRV parameters (Table 8).

DISCUSSION

IBD, including UC and CD is estimated to affect 2.2 million individuals in Europe. Although the etiology of IBD is not fully known, the process is thought to be triggered by a combination of environmental, genetic, and immunological factors in genetically predisposed individuals, leading to a normal endothelial cell immune response causing intestinal microvascular damage resulting in chronic intestinal inflammation [16].

In some studies, it has been suggested that patients with IBD are at increased risk of cardiovascular events and atherosclerosis [17], but the cause of increased cardiovascular risk has not yet been fully explained. A chronic inflammatory process may lead to atherosclerosis. During the course of IBD, the typical elevation of proinflammatory cytokines, such as CRP, tumor necrosis factor alpha, and interleukin, has been shown to be associated with subclinical atherosclerosis [18]. Inflammation is known to have a role in all stages of atherosclerosis, from initiation to eventual plaque rupture and thrombosis.

IBD is typically seen with periods of exacerbations in which the disease is active and asymptomatic remission periods. Inflammation plays a critical role in the pathogenesis of IBD and proinflammatory cytokines have been shown to contribute to the process of the disease [19]. However, there is conflicting evidence in respect of a significantly increased risk of MI, stroke, and cardiovascular mortality for IBD patients. Two registry-based studies of approximately 40,000 IBD patients, and a meta-analysis of 11 studies with a total of almost 14,000 patients have reported increased risk of MI and cardiovascular mortality in IBD compared to matched control subjects without IBD[20,21].

In the last decade new evidence has emerged that the risk of MI, stroke, and cardiovascular mortality is significantly increased in IBD patients, especially during periods of IBD activity. Kristensen *et al*. recently published very important data of a Danish registry study of approximately 20,000 patients. A statistically significant increased risk of MI, stroke, and cardiovascular death was reported for patients with active IBD periods when the relative risk of MI increased nearly two-fold (1.49 (CI: 1.16 -1.93) for flare and 2.05-fold (CI: 1.58 - 2.65) for persistent IBD activity[2]. The same study group also demonstrated that IBD was associated with a greater risk of hospitalization for heart failure and that this risk was strongly related to periods of active disease[3]. In a study of 86,790 Danish patients with first-time MI between 2002 and 2011, the effect of active IBD was evaluated on major adverse cardiovascular outcomes after MI. It was determined that IBD was associated with hazard ratios of 1.21 (95%CI: 0.99-1.49) for recurrent MI, 1.14 (95%CI: 1.01-1.28) for all-cause mortality, and 1.17 (95%CI: 1.03-1.34) for the composite end point. In comparison with the non-IBD group, IBD flare-ups were strongly associated with an increased risk of recurrent MI and all-cause mortality, whereas during remission, no increased risk was detected [22]. The latest and most comprehensive study by Panhwar *et al* evaluated over 250,000 patients with IBD from a database and compared these with well-matched patients without IBD. Both CD and UC were reported to have an increased risk of MI, and highest risk was seen in younger patients. It was concluded that aggressive risk factor modification for MI is essential for patients with IBD[23].

In the current study group, inflammatory markers such as HsCRP and ESR were higher, especially in the active disease group, which were expected results. In accordance with recently published data, it was demonstrated that HRV deteriorated only during active IBD compared to both the control group and the remission group, which suggested a role of shared pathophysiological inflammatory mechanisms. Clinical interpretation of these findings can be summarized as: HRV parameters, which are predominantly modified by parasympathetic ANS, were decreased with higher levels of positive phase reactants, a higher Mayo score and active disease status, and increased with higher levels of albumin and hemoglobin. The opposite of this statement is also true.

There seems to be considerable potential for HRV in the assessment of the role of ANS fluctuations in normal healthy individuals and in patients with various cardiovascular and non-cardiovascular disorders. The HRV Taskforce guidelines recommend four measures for time domain HRV assessment (1) SDNN (estimate of overall HRV), (2) HRV triangular index (estimate of overall HRV), (3) SDANN (estimate of long-term components of HRV), and (4) RMSSD (estimate of short-term components of HRV)[15].

In addition, the marked relationship between ANS, IBD and cardiovascular disease over inflammatory pathways, was seen with similar but less elusive interaction–between IBD and HRV through

the enteric nervous system (ENS). There is an increasing body of evidence suggesting that the ANS and the immune system have a complex relationship in the pathogenesis of IBD[24] . ANS have several roles in the–gastrointestinal tract, including the modulation of motility and secretion functions, and regulation of mucosal immune and inflammatory responses [25]. In IBD patients, functional changes in colonic mucosa result in abnormal colonic motility and transit functions. These motor disturbances are suggestive of alterations in colonic neuromuscular components including enteric neurons [26]. Focal destruction of ANS axons is also present in inflamed and non-inflamed CD patients' small bowel tissue. However, it is not very clear if the inflammation of ENS triggers the etiopathogenetic cascade or if ENS plays a minor role, which is only affected by the process.

It has been thought that ANS dysfunction might have a role in the pathogenesis of IBD[27]. Some studies have suggested that increased sympathetic activity may be responsible for the augmentation of bowel inflammation[28,29]. Both hypofunction (autonomic neuropathy) and hyperfunction (autonomic hyperreflexia) have been described in IBD. In previous studies, autonomic neuropathy has been detected at a high prevalence in IBD, ranging from 40% to 50%, although in further studies, cardiovascular autonomic neuropathy has been found to be rare in IBD, with prevalence rates of approximately 5%[30].

The association of altered HRV and IBD has been examined in several studies. Although some observational studies have focused on impaired HRV in patients with IBD, conflicting results have emerged from studies of the autonomic functions in patients with both UC and CD. This could be attributed to the use of different protocols in the recording of HRV, small sample sizes, and different disease activity status of the patients. Using time domain and spectral HRV from a 24-h ambulatory ECG, Mouzas *et al* showed an increase in vagal functions in IBD patients with no distinction between UC and CD subgroups [31]. With the use of another technique (20-min HRV record), Coruzzi *et al* concluded that compared to both CD patients and the control group, UC patients had decreased parasympathetic tone and increased sympathetic tone[32]. Sharma *et al*[33] reported lower cardiovascular autonomic functions in patients with IBD in clinical remission. It was also indicated that UC patients in particular, had significantly lower parasympathetic function compared to those with CD and healthy control subjects. In a recent study by Sarli *et al*, it was demonstrated that IBD was associated with an abnormal heart rate reduction following a treadmill exercise test, but the parameters were not compared between patients with active disease and those in remission. Unlike other studies, the heart rate recovery (HRR) index was used to investigate autonomic function rather than HRV[34].

Although there are conflicting results from studies that have not taken disease activity into consideration, there seems to be increasing evidence that active and persistent periods of disease are a risk factor for cardiovascular disease and arrhythmia. In contrast to previous studies, the current study shows that the HRV parameters were not affected in IBD patients with clinical remission. It was also shown for the first time with the use of 24-h ambulatory ECG, that most of the HRV indices were impaired in active IBD patients.

Several limitations need to be mentioned. This study was cross-sectional in nature and therefore, the findings cannot be generalized. Another important limitation of the study was the low number of patients, which may also prevent the generalization of these results to all IBD patients. Therefore, large

scale studies are needed to confirm these results. As inflammation was successfully suppressed with anti-inflammatory therapy in the patients, the majority of IBD patients in the study were in remission, and the number of patients with active IBD was low. Another important limitation was the significant difference between the patient group (active and remission) and the control group in respect of the triglyceride, hematological and inflammatory marker levels. Moreover, that the patients could not be followed up prospectively for future major adverse cardiac events is the most important limitation of the study.

CONCLUSION

The results of this study provided clear evidence that the active phase of IBD is associated with ANS abnormalities, with significant impairment of cardiac vagal modulation compared to both the control group and IBD patients in remission. It can be speculated that in active periods of disease, “an enhanced inflammatory load” is likely to worsen HRV. These results indicate that a treatment strategy aiming to reduce the duration and number of flare-ups in patients with IBD may be warranted to be able to decrease the cardiovascular risk, especially for patients with prolonged or recurrent disease activity. Further studies are needed to explore the predictive role of diminished HRV in the development of future cardiovascular complications in patients with active IBD.

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Table 1: Selected Time Domain Measures of Heart rate variability for the study

Variable	Units	Description
Statistical Measures		
SDNN	ms	Standard deviation of all NN intervals Clinical importance: It consists of parts from the sympathetic and parasympathetic nervous systems. The SDNN can be described as an overall variability or total power
SDANN5	ms	Standard deviation of the mean values of the NN intervals in the 5-minute intervals within the defined period Clinical importance: Higher values indicate increased parasympathetic activity
RMSSD (root mean of squared successive differences)	ms	The root of the average sum of squares of two differences of successive RR intervals within the defined period Clinical importance: Higher values indicate increased parasympathetic activity
SDNN5	ms	Mean of the standard deviations of all NN intervals for all 5-minute segments of the entire recording
pNN50	%	Percentage of the NN intervals within the defined period that differ from the previous NN interval by more than 50 mS Clinical importance: Higher values indicate increased parasympathetic activity
HRV triangular index		Total number of all NN intervals divided by the height of the histogram of all NN intervals measured on a discrete scale with bins of 7.8125 ms (1/128 seconds)

NN: Interval between two heartbeats (emphasis on "normal" heartbeats)

RR: Interval between two heartbeats (R spikes in the QRS complex / ECG).

Table 2: Selected Frequency Domain Measures of Heart rate variability for the study

Variable	Units	Description
AVG		Average value of all RR intervals
SDTF	ms^2	Standard deviation of TF over 24 hours
SDVLF	ms^2	Standard deviation of VLF over 24 hours. Clinical importance:The energy in LF and VLF are due to both sympathetic and parasympathetic systems
SDHF	ms^2	Standard deviation of HF over 24 hours. Clinical importance: The energy in HF is vagal mediated
SDdef	ms^2	Standard deviation in the predefined frequency range
LF%	%	Percentage of LF in TF

TF:Total frequency 0.01-1.00 Hz

VLF-Very low frequency band 0.003 – 0.04 Hz

HF–High frequency band 0.15 – 0.4 Hz

LF -Low frequency band 0.04 – 0.15 Hz

Table 3: Baseline Characteristics of the Study Population

Variables	IBD (n:67)	Controls (n:51)	P
Age, yrs	42.67 ± 12.8	43.2 ± 15.3	0.83
Male (%)	64.2	54.9	0.31
Smoking (%)	6	7.8	0.28
Hypertension (%)	6	7.8	0.28
BMI, kg/m2	23.8 ± 2.1	24.5 ± 3.0	0.10
Fasting blood glucose (mg/dL)	93.2 ± 9.9	95.4 ± 11.1	0.26
HDL Cholesterol (mg/dL)	46.8 ± 19.1	52.08 ± 13.1	0.31
LDL Cholesterol (mg/dL)	100.6 ± 34.8	89.8 ± 32.2	0.13
Triglyceride (mg/dL)	125 ± 73.6 102 (71.25 - 163.75)	83.1 ± 41.2 75(58 - 102)	0.02
AST	19.5 ± 8.1	20.1 ± 5.7	0.77
ALT	18.9 ± 10.6	17.4 ± 7.1	0.54
ESR (mm/h)	20.8 ± 24.3 11 (5 - 26)	12.9 ± 5.06 12 (10 - 15.75)	0.61
C-reactive protein (mg/L)	1.35 ± 2.49 0.42 (0.29 - 1.12)	0.6 ± 1.14 0.3 (0.06 - 0.47)	0.01
Hgb, g/dL	13.4 ± 1.7	13.1 ± 1.6	0.37
PLT, mL/mm3	299.4 ± 93.5	258.04 ± 58.05	0.59
WBC, mL/mm3	7.53 ± 2.01	6.51 ± 1.36	0.38
Creatinine, mg/dL	0.73 ± 0.16	0.81 ± 0.15	0.53
Medications, % Steroid Azathiopurine 5 ASA	 10.4 11.9 94		
Active disease, %	42.1		
Disease duration, yr	4.57 ± 3.43		
ALT: Alanine aminotransferase AST: Aspartate aminotransferase, 5-ASA:5 aminosalicylic acid, BMI: body mass index, ESR: erythrocyte sedimentation rate, Hgb: hemoglobin, PLT : platelets, WBC; white blood cells.			

Table 4: Differences in heart rate variability measures between the study population

Variables	IBD(n:67)	Controls(n:51)	P
Time Domain Measures			
SDNN (ms)	137 ± 43.1	156.8 ± 58	0.06
SDANN5 (ms)	125.2 ± 39.7	125.6 ± 45.6	0.99
RMSSD (ms)	47.7 (30.7 - 68.9)	56.2 (36.3 - 89.7)	0.21
SDNN5 (ms)	63.8 (51.3 - 77.5)	71.4 (53 - 93.4)	0.09
pNN50 (%)	9.55 (5.07 - 15.8)	13.2 (5.2 - 25.3)	0.06
HRV triangular index	35.1 (27.8 - 43.9)	38 (30.6 - 52.1)	0.24
Frequency Domain Measures			
AVG	1599.2 ± 275	1686 ± 266	0.27
SDTF	45.3 (40.6 - 49.02)	45.7 (42.2 - 48.7)	0.78
SDVLF	212.9 ± 38.4	220.5 ± 33.7	0.43
SDHF	0.76 (0.4 - 1.33)	0.78 (0.48 - 1.32)	0.74
SDdef LF%	0.39 (0.2 - 0.76)	0.45 (0.29 - 0.74)	0.052
LF%	75.6 (65.2 - 84.4)	72.7 (61.6 - 81.3)	0.4
Basic parameters			
Mean HR	75.8 ± 9.3	73.7 ± 11.2	0.3
Max HR	126.6 ± 16.9	129.6 ± 18.1	0.36
Min HR	55.7 ± 9.1	54.6 ± 11.1	0.56
SVES (n)	4.5 (2 - 14)	5 (1 - 11)	0.95
VES (n)	1 (0 - 10)	2 (0 - 8)	0.66
The values are presented as median (interquartile range), SVES: Supraventricular extrasystole, VES: Ventricular extrasystole, HR: Heart rate,			

Table 5: Comparison of the baseline characteristics according to disease activity status

Variables	Active disease (n:24)	Remission (n:43)	Controls(n:51)	P
Age, yrs	45.08 ± 13.5	41.3 ± 12.4	43.2 ±15.3	0.55
Male (%)	70.8	60.5	54.9	0.42
Smoking (%)	4.2	7	7.8	0.78
Hypertension (%)	12.5	9.3	7.8	0.22
BMI, kg/m2	24.9 ± 5.46	25.3 ± 3.6	24.5 ± 3.0	0.84
Fasting blood glucose (mg/dL)			95.4 ± 11.1	
HDL Cholesterol (mg/dL)	44.7 ± 18.4	48.02 ± 19.6	52.08 ± 13.1	0.27
LDL Cholesterol (mg/dL)	91.6 ± 37.2	105.7 ± 32.7	89.8 ± 32.2	0.09
Triglyceride (mg/dL)	87 (72 - 135)	109 (70.5 - 170) [c]	83.1 ± 41.2 75 (58 - 102)	0.005
AST	17.1 ± 7.5	20.9 ± 8.2	20.1 ± 5.7	0.14
ALT	16.7 ± 12.4	20.2 ± 9.4	17.4 ± 7.1	0.31
ESR (mm/h)	33 (10.2 - 76.2)[a,b]	9 (3 - 14)[c]	12.9 ± 5.06 12 (10 - 15.75)	0.0001
C-reactive protein (mg/L)	1.44 (0.37 - 3.2)[a,b]	0.35 (0.2 - 0.65)	0.6 ±1.14 0.3 (0.06 - 0.47)	0.0001
Hgb, g/dL	12.6 ± 1.76 [b]	13.9 ± 1.6	13.1 ± 1.6	0.008
PLT, mL/mm3	342.4 ± 98.5 [a,b]	275.3 ± 82.3	258.04 ± 58.05	0.001
WBC, mL/mm3	8.15 ± 2.59 [a]	7.18 ± 1.52	6.51 ± 1.36	0.015
Creatinine, mg/dL	0.74 ± 0.2	0.72 ± 0.1	0.81 ± 0.15	0.14

a was significant between active disease and control groups

b was significant between active disease and remission groups

c was significant between remission and control groups

significance $p<0.05$

Table 6: Comparison of heart rate variability measures according to disease activity status

Variables	Active disease (n: 24)	Remission (n: 43)	Control (n: 51)	p
Time Domain Measures				
SDNN (ms)	127.5 ± 9.3 [a]	146.03 ± 6.7	156.8 ± 58	0.02
SDANN5 (ms)	115.5 ± 8.94	131.5 ± 6.03	125.6 ± 45.6	0.36
RMSSD (ms)	45 (27.2 - 58.5)	51.7 (34.5 - 77.7)	56.2 (36.3 - 89.7)	0.11
SDNN5 (ms)	55 (33.8 - 75.7) [a,b]	64.8 (53.3 - 80.7)	71.4 (53 - 93.4)	0.013
pNN50 (%)	9.1 (1.2 - 18) [a]	10.2 (6.2 - 18.3)	13.2 (5.2 - 25.3)	0.027
HRV triangular index	32.3 (21.7 - 40.8)[a,b]	39.9 (32.3 - 47)	38 (30.6 - 52.1)	0.009
Frequency Domain Measures				
AVG ($ms^{2)}$	1545.4 ± 39.1 [a,b]	1684.7 ± 41.2	1686 ± 37	0.001
SDTF ($ms^{2)}$	42.9 (37.2 - 47.6)	45.6 (41.4 - 51)	45.7 (42.2 - 48.7)	0.12
SDVLF ($ms^{2)}$	202.7 ± 5 [a,b]	222.6 ± 6.4	220.5 ± 33.7	0.01
SDHF ($ms^{2)}$	0.47 (0.26 - 1.36)	0.8 (0.51 - 1.33)	0.78 (0.48 - 1.32)	0.23
SDdef ($ms^{2)}$	0.24 (0.07 - 0.76)[a,b]	0.47 (0.23 - 0.81)	0.45 (0.29 - 0.74)	0.02
LF (%)	76.1 (64.5 - 87.2)	75.6 (65.9 - 82.08)	72.7 (61.6 - 81.3)	0.39
Mean HR	78.6 ± 2.16	74.1 ± 1.33	73.7 ± 11.2	0.13
Max HR	126.9 ± 3.03	126.4 ± 2.9	129.6 ± 18.1	0.65
Min HR	58.9 ± 2.2	53.8 ± 1.18	54.6 ± 11.1	0.14
SVES(n)	4 (2 - 14)	5 (1 - 15)	5 (1 - 11)	0.9
VES(n)	2 (0 - 13)	1 (0 - 10)	2 (0 - 8)	0.25
The values are presented as median (interquartile range), SVES: Supraventricular extrasystole, VES: Ventricular extrasystole, HR: Heart rate,				
a was significant between active disease and control groups **b** was significant between active disease and remission groups **c** was significant between remission and control groups significance $p<0.05$				

Table 7: Correlation between HRV parameters and laboratory and clinical parameters in all study group (Pearson's test r values and their significance).

	Age	Mayo score	Albumin	Leukocyte	Hemoglobin	ESR	CRP
Max HR	**-0.456$^{p<0.001}$**	NS	NS	NS	**-0.231$^{p:0.024}$**	NS	NS
MinHR	NS	**0.362$^{p:0.011}$**	**-0.283$^{P:0.026}$**	NS	**-0.264$^{p:0.009}$**	**0.268$^{p:0.006}$**	**0.387$^{p<0.001}$**
Mean HR	NS	**0.374$^{p:0.008}$**	**-0.312$^{P:0.014}$**	NS	**-0.319$^{p:0.002}$**	**0.269$^{p:0.006}$**	**0.353$^{p<0.001}$**
SDNN	**-0.193$^{p:0.047}$**	NS	**0.312$^{p:0.014}$**	NS	NS	**-0.213$^{p:0.031}$**	**-0.263$^{p:0.019}$**
SDANN5	**-0.369$^{P<0.001}$**	NS	**0.272$^{P:0.032}$**	NS	**0.287$^{p:0.005}$**	NS	**-0.302$^{p:0.007}$**
SDNN5	NS	**-0.369$^{P:0.009}$**	**0.314$^{P:0.013}$**	NS	NS	NS	NS
AVG	NS	**-0.291$^{P:0.042}$**	**0.283$^{p:0.026}$**	NS	**0.313$^{p:0.002}$**	**-0.229$^{p:0.021}$**	**-0.291$^{p:0.009}$**
SDTF	NS	NS	NS	NS	**0.296$^{p:0.003}$**	NS	**-0.242$^{p:0.031}$**
SDVLF	NS	NS	**0.259$^{p:0.042}$**	NS	**0.310$^{p:0.002}$**	**-0.213$^{p:0.032}$**	**-0.267$^{p:0.016}$**

r: correlation coefficient, NS: Non-significant p>0.05, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate

Table 8: Correlation analysis of inflammatory parameters and heart rate variability measures in Active, Remission and control groups

Group	Lab. parameters	MinHR	Mean HR	MaxHR	SDNN	SDANN5	SDVLF	SDTF	AVG	Triangular index
Active	**CRP**	**0.44**$^{0.03}$	**0.47**$^{0.02}$	**0.58**$^{0.003}$	NS	NS	**-0.47**$^{0.02}$	**-0.47**$^{0.02}$	**-0.46**$^{0.02}$	NS
	ESR	NS	**0.44**$^{0.032}$	**0.48**$^{0.019}$	NS	NS	**-0.45**$^{0.03}$	**-0.53**$^{0.01}$	**-0.45**$^{0.02}$	NS
Remission	**CRP**	**0.34**$^{0.03}$	**0.37**$^{0.02}$	NS	NS	NS	NS	NS	**-0.32**$^{0.04}$	NS
	ESR	NS	NS	NS	NS	NS	NS	NS	NS	NS
Control	**CRP**	**0.54**$^{0.02}$	NS	NS	**-0.57**$^{0.01}$	NS	**-0.47**$^{0.04}$	**-0.49**$^{0.03}$	NS	NS
	ESR	NS	NS	NS	NS	NS	NS	NS	NS	NS

r: correlation coefficient, NS: Non-significant p>0.05, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate