Clinical Research

Association of Impaired Glucose Tolerance with Increased Heart Rate and Subclinical Inflammation

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Introduction: This study was designed to assess possible alterations in heart rate (HR), heart rate variability (HRV) and circulating serum levels of proinflammatory cytokines in patients with impaired glucose tolerance (IGT).

Methods: Forty-five patients, aged 34-68 years, with IGT were compared with 28 age-matched healthy controls. Using a 24-hour ambulatory electrocardiogram, we calculated mean HR during daytime (HR-D), nighttime (HR-N) and the entire 24-hour period (HR-24h), as well as time domain HRV parameters. From blood samples interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-a) and its soluble receptor (sTNFRII) were also calculated by immunoassay.

Results: Patients showed higher mean HR compared to controls and significantly elevated circulating levels of TNF-a, sTNFRII, and IL-6. Pearson correlation analysis showed that TNF-a was positively correlated with mean HR-D (r: 0.304, p=0.042). IL-6 was also positively correlated with mean HR-24h (r: 0.299, p=0.046) and with mean HR-D (r: 0.410, p=0.005).

Conclusion: IGT patients have an increased HR and elevated cytokine levels. These changes could serve as an index of the primary atherosclerotic process.

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Patients with diabetes mellitus (DM) have an increased risk for cardiovascular disease.[1] There is also an increased risk for patients in a pre-diabetic state, such as those with impaired glucose tolerance (IGT).[2,3] The early identification of diabetes is thus very important as a way of reducing the risk of cardiovascular disease. The prominent aetiology of increased cardiovascular disease incidence in patients with IGT appears to be strongly related to insulin resistance syndrome.[4-6]

It is known that inflammation plays a significant role in the pathogenesis and the progression of atherosclerosis and the concentration of inflammation markers has been shown to be significantly correlated with cardiovascular mortality and morbidity.[7,8] It has been suggested that for patients with IGT, inflammation is involved in an early metabolic defect, while the hypothesis that pro-inflammatory cytokines play an active role in this process has not always been supported.[9]

Heart rate variability (HRV) describes variations of both instantaneous heart rate (HR) and RR intervals, and is commonly performed utilising 24-hour electrocardiographic recording. HR and HRV represent two of the most promising quantitative markers of autonomic nervous activity.[10,11] Abnormalities of the autonomic nervous system, reflected by increased HR and reduced HRV, are associated with cardiovascular

mortality and morbidity and have been proven to be strong predictors of the progression of focal coronary atherosclerosis.[11] However, the association between the inflammation process and autonomic nervous system abnormalities in patients with IGT has never been well studied.

The aims of this study were as follows:

1. To assess the mean 24-hour HR, time domain HRV parameters and serum levels of inflammation markers in patients with IGT, in comparison to healthy controls,
2. To evaluate the possible relationship between HR, HRV parameters and serum inflammation markers in these patients.

Methods

Study population

Over a period of 36 months, we enrolled 45 patients (27 men, 18 women), mean age 50.3 ± 6.6 years (range 34-68), with a diagnosis of IGT in our study.[12] Patients were selected from our university-based outpatient vascular disease prevention clinic. They all had fasting serum glucose between 100 mg/dl and 126 mg/dl and their serum glucose levels, 2 hours after glucose loading, (oral glucose tolerance test - OGTT), were between 140 mg/dl and 200 mg/dl. Thirty-six of them had a family case history of DM.

Twenty-eight healthy volunteers from the hospital staff (16 men and 12 women), mean age 51.4 ± 6.1 years (range 39-62), with normal fasting serum glucose (<100 mg/dl), made up the control group.

All of the above 73 subjects were asymptomatic, without a history of hypertension (mean systolic blood pressure <140 mmHg, mean diastolic blood pressure <90 mmHg). At the start of the study their serum low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels were below 170 mg/dl and 160 mg/dl, respectively, and their high-density lipoprotein cholesterol (HDL-C) levels were above 35 mg/dl. None of them was taking any medication.

At the start of the investigation all patients and controls underwent a physical examination, electrocardiographic and 2-D transthoracic echocardiographic examinations and an exercise test (Bruce protocol). No indications of ischaemia, arrhythmias, systolic-diastolic dysfunction, or heart valve disease were detected.

None of the subjects was in the habit of drinking more than 2 cups of coffee or more than 2 glasses of an alcoholic drink per day. We excluded patients with clinical covariates that might affect autonomic function such as hypertension, obesity, dyslipidaemia, smoking, coffee drinking and alcohol consumption. Patients with chronic inflammatory disease, acute infection and neoplasm were also excluded.

All subjects gave their consent to participate in the study. The University of Crete ethics committee approved the protocol.

24 hour Holter monitoring and HRV

All patients and controls underwent continuous ambulatory Holter monitoring, commencing at 11 a.m. on the day of the study. All recordings were obtained outside the hospital in an attempt to achieve conditions as close to the subjects' daily life as possible. Holter monitoring was performed using a 3-channel bipolar recorder and was automatically evaluated after digitization by an Elatec analyser $V_{3,}$ (Ela Medical, Paris, France).

During the analysis, only cycles in which beats had normal morphologic characteristics and were within 25% of the preceding cycle length were selected for the calculation of HRV. The QRS filter window excluded the coupling time of all ectopic beats and their compensatory pause. The beat classification was verified manually and corrected appropriately by an experienced cardiologist.

We measured mean 24 hour HR (HR-24h), mean daytime HR (HR-D), from 12 noon to 6 p.m. and nighttime HR (HR-N), from 12 to 6 a.m. Time domain HRV components were also measured, and the following parameters were identified:

- The standard deviation of all normal to normal (NN) intervals: SDNN (ms).
- The standard deviation of the averages of NN intervals in all 5-minute segments of the 24-hour recording: SDANN (ms).
- The root mean square of the differences between adjacent NN intervals: RMSSD (ms).
- The percentage of successive R-R intervals that deviated more than 50 ms from the prior R-R intervals: pNN50(%).

Blood sampling

Concentrations of trimeric bioactivity of tumour necrosis factor alpha (TNF-a) (detection limit 0.18 pg/ml), interleukin (IL)-6 (detection limit 0.1 pg/ml) were determined by a high-sensitivity, enzyme-linked immunoassay according to the instructions of the manufacturer (R & D Systems, Abingdon, UK). Serum concentration

Table 1. Clinical characteristics of the study population.

	Patients (N=45)	Controls (N=28)	p
Age (years)	50.3 ± 6.6	51.4 ± 6.1	0.47
BMI (kg/m^2)	29.8 ± 1.8	24.7 ± 1.3	0.001
SBP (mmHg)	124.2 ± 8.7	122.7 ± 7.9	<0.001
DBP (mmHg)	82.1 ± 3.2	73.6 ± 5.6	<0.001
GLU (mg/dl)	116.2 ± 4.4	92.0 ± 2.9	0.009
TG (mg/dl)	140.6 ± 7.2	136.5 ± 4.6	<0.001
HDL-C (mg/dl)	37.0 ± 2.0	43.8 ± 3.4	<0.001
LDL-C (mg/dl)	141.1 ± 7.6	133.6 ± 6.7	<0.001
Men	60 %	57%	0.81
Family history of DM	80 %	25%	<0.001

BMI – body mass index; DBP – diastolic blood pressure; DM – diabetes mellitus; GLU – fasting serum glucose; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; SBP – systolic blood pressure; TG – triglycerides.
Continuous variables are expressed as mean ± standard deviation.

of soluble TNF receptor p75 (sTNFRII) (detection limit 1.0 pg/ml) was measured by assays employing the sandwich enzyme immunoassay technique as instructed by the manufacturer (R&D Systems). Intra- and inter-assay coefficients of variation for all enzyme immunoassays were <5% and <10%, respectively.

Serum concentrations of total cholesterol, HDL-C, and TG were measured using an automated chemistry analyser (Olympus AU-600). LDL-C was calculated according to the Friedewald formula. Serum glucose concentrations were determined by standard methods in routine use.

Statistical analysis

The association between HR, HRV parameters and cytokine levels in patients and controls was evaluated using Student's t-test. Correlations between variables were tested using Pearson correlation analyses. A p-value <0.05 was considered as the criterion for significance.

Results

The clinical characteristics of our study population can be seen in table 1. The classical risk factors for coronary artery disease, such as blood pressure, serum LDL-C, HDL-C, TG and body mass index, were significantly higher in patients than in controls, although they were within normal limits in both groups. The vast majority of the patients had a family history of DM. The two groups were similar as regards age and gender.

As can be seen from table 2, patients with IGT also had significantly elevated circulating levels of TNF-a, IL-6, and sTNFRII compared to controls (Figure 1). In addition, as shown in table 3, we calculated HR and HRV time domain parameters pNN50, RMSSD, SDANN, and SDNN. The 24-hour mean HR was significantly higher in patients than controls (77.73 versus 71.69 beats per minute, p=0.01) (Figure 2). Time domain parameters did not differ significantly between the two groups.

Using Pearson correlation analyses we found that TNF-a levels were positively correlated with HR-D (r: 0.304, p=0.042) (Figure 3). IL-6 levels were positively correlated with HR-24h (r: 0.299, p=0.046) (Figure 4) and with HR-D (r: 0.410, p=0.005) (Figure 5).

Discussion

Our study shows that there is an increased HR and an elevation of proinflammatory cytokines in patients with IGT compared to healthy individuals. HR and HRV

Table 2. Proinflammatory cytokines in patients and controls.

	Group	Mean value	SD	p
IL-6 (pg /ml)	P	2.0856	0.72368	0.03
	C	1.7393	0.50705	
TNF-a (pg /ml)	P	2.4667	0.44107	<0.001
	C	1.6054	0.65791	
sTNFR II (ng /ml)	P	2.0480	0.53030	<0.001
	C	1.5907	0.40857	

C – controls; IL-6 – interleukin-6; P – patients; sTNFRII – soluble TNF receptor II; TNF-a – tumour necrosis factor-alpha

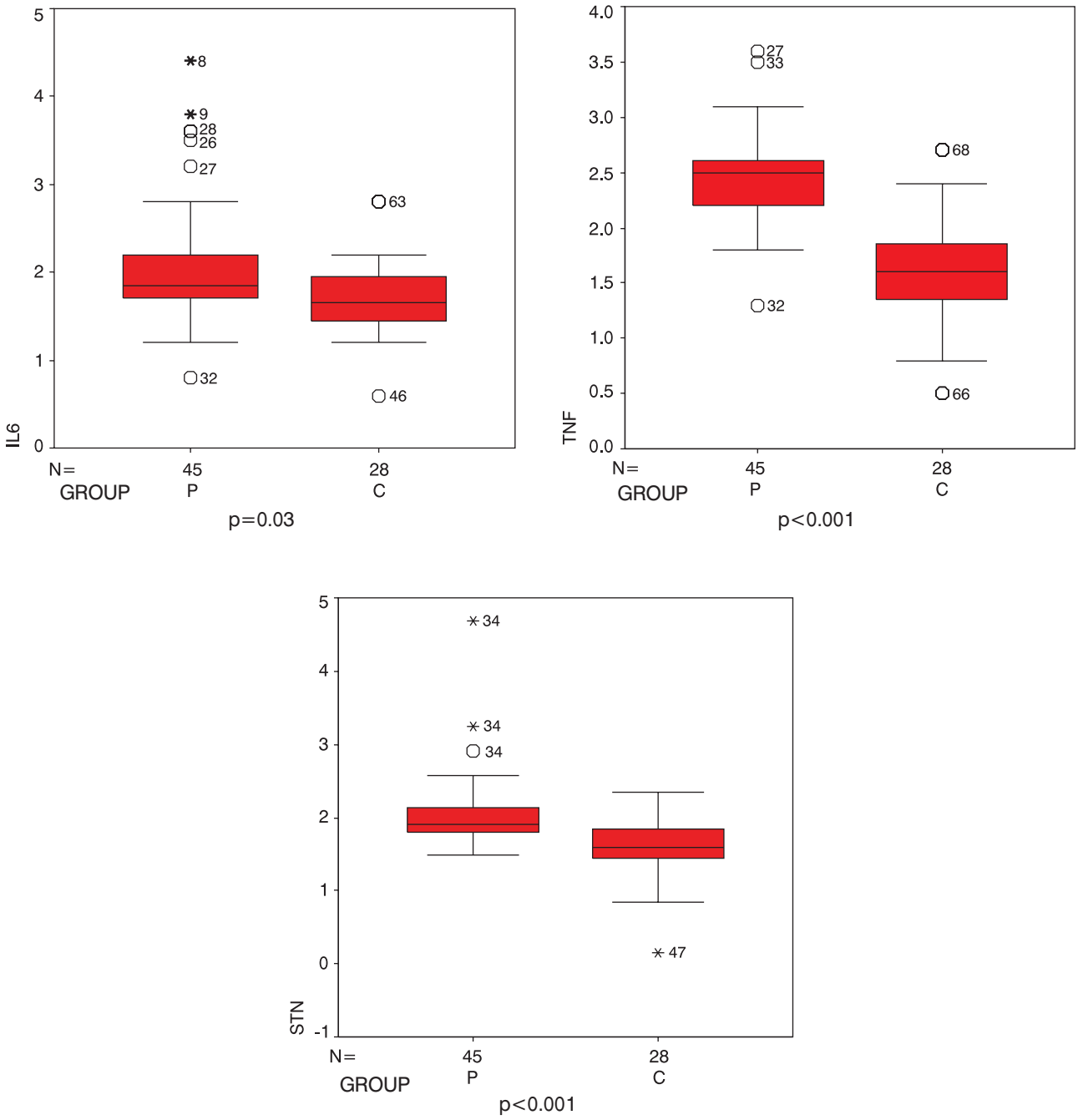

Figure 1. Levels of proinflammatory cytokines interleukin-6 (IL6), tumour necrosis factor-alpha (TNF) and soluble TNF receptor II (STN) in patients (P) and controls (C).

are both under sympathovagal influence and are considered to be significant indexes of the autonomic nervous system. They may be altered in several conditions, such as DM, hypertension, after acute myocardial infarction, in hyperglycaemia and mental depression.[10,11]

Patients with DM show an increased incidence of silent myocardial infarction, cardiac arrest and sudden death.[13,14] It has been also proved that elevated HR consists a major risk factor for cardiovascular disease.[15]

HR, being regulated by the balance between sympathetic and parasympathetic pathways, is not just a simple index of sympathetic activation. and the analyses of HR and HRV parameters have been proven useful in detecting diabetic neuropathy.[10,15] HRV may be

Table 3. Heart rate (HR) and time domain parameters of heart rate variability (HRV) in patients and controls.

	Group	Mean value	SD	P
HR-24h (bpm)	P	77.7289	9.99864	0.01
	C	71.6857	8.55387	
HR-day (bpm)	P	82.2911	10.06772	0.032
	C	75.4296	16.61102	
HR-night (bpm)	P	71.4956	11.96875	0.006
	C	64.6964	8.44716	
pNN50-24h (%)	P	10.7507	16.10956	0.24
	C	15.5582	18.05397	
RMSSD-24h (ms)	P	43.7307	56.64693	0.26
	C	59.4604	58.26190	
SDANN-24h (ms)	P	56.6460	37.27757	0.20
	C	68.3754	38.50157	
SDNN-24h (ms)	P	94.7102	40.41554	0.18
	C	107.8986	39.97945	

C – controls; P – patients;

evaluated by time domain and frequency domain methods. We measured HRV time domain parameters, as they are considered to be ideal for the analysis of long-term recordings. Among them, SDANN and SDNN reflect both sympathetic and parasympathetic modulation of HR, whereas pNN50 and RMSSD are markers of parasympathetic activity.[10,11]

In our study we found that patients with IGT show an increase in both daytime and nighttime HR, in 24 hour HR, as well as a trend towards a reduction in HRV time domain parameters. While a previous study has shown that TNF-a may not be detected in IGT patients, we found significant elevation not only of IL-6, but also of TNF-a and sTNFRII levels.[16]

IGT belongs to the insulin resistance syndrome. Several studies have suggested that inflammation may be associated with hyperinsulinaemia and insulin resistance. Insulin resistance syndrome has been associated with a number of risk factors such as elevated TG, LDL-C and plasminogen activator inhibitor-1.[17]

The only traditional cardiovascular disease risk factor that was significant for the patients was a positive family history of DM. This could be due to the fact that we tried to enroll subjects who were as free as possible from clinical covariates that might affect autonomic function, such as hypertension and obesity. However, IGT patients had increased inflammation marker levels in conjunction with an early subclinical autonomic dysfunction.

A major finding of this study was the correlation between HR and inflammation markers. It seems that mean HR is positively associated with proinflammatory cytokines. In fact, several lines of evidence suggest that proinflammatory cytokines, through a primary stimulatory effect on the hepatic synthesis of acute phase proteins, may play a key role in inducing inflammation. It is known that circulating TNF-a has several functions in inflammation, promoting leukocyte adhesion, migration, regulating macrophage activation and lymphocyte development. In atherogenesis, TNF-a promotes T-cell activation, foam cell formation, and induces macrophage colony stimulating factor.[18]

IL-6 also derives from activated leukocytes, fibroblasts, endothelial cells and adipose tissue. It is involved in endothelial dysfunction, the expression of adhesion molecules and in platelet activation.[19] The brain controls immune functions through certain pathways, and IL-6, which has also been found in the brain, seems to have a major role in all these procedures. These pathways include the regulation of the body temperature, the immune status regulation through endocrine pathways – namely the hypothalamic pituitary adrenal axis – and the innervation of the organs of the immune system (spleen, lymph nodes) via the autonomic nervous system.[20] The lymph reticular system, like the bone marrow, is innervated by autonomic nerves and is under the influence of the autonomic system. An imbalance in the autonomic nervous system in favor of the sympathetic system (reduced parasympathetic or increased sympathetic activity) could trigger an inflammatory reaction.[21-23] Other inflammatory substances

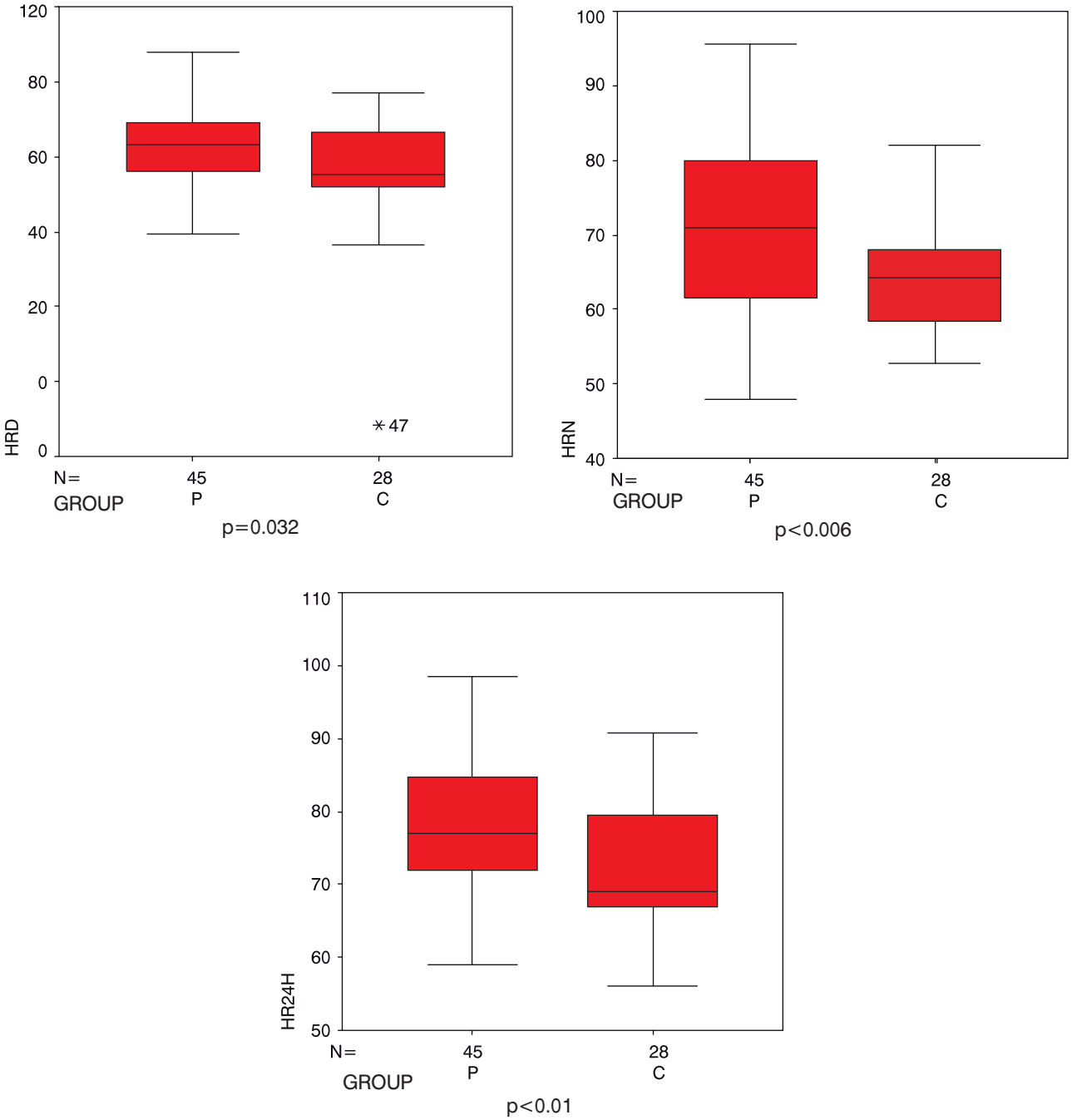

Figure 2. Mean HR daytime (HRD), nighttime (HRN) and 24-hour (HR24H) in patients (P) and controls (C).

could theoretically have similar activity. The autonomic imbalance and inflammation may play a primary role in affecting each other.[24]

The atherogenicity of the patient in a pre-diabetic state may be expressed by increased HR and even non-high grade inflammation. In this case, the bioavailability of endogenous NO is decreased, and cytokines, such as TNF-a and IL-6, may constitute important keys to the whole process.[25,26]

Increased HR, in concordance with inflammation markers in IGT patients, can be used as an early warning sign of diabetic neuropathy. They may constitute an index of a primary endothelial dysfunction and atherosclerotic process.[27,28]

The results of the present study should be interpreted within the limitations of the small number of patients studied and the fact that we did not measure high sensitivity C-reactive protein.

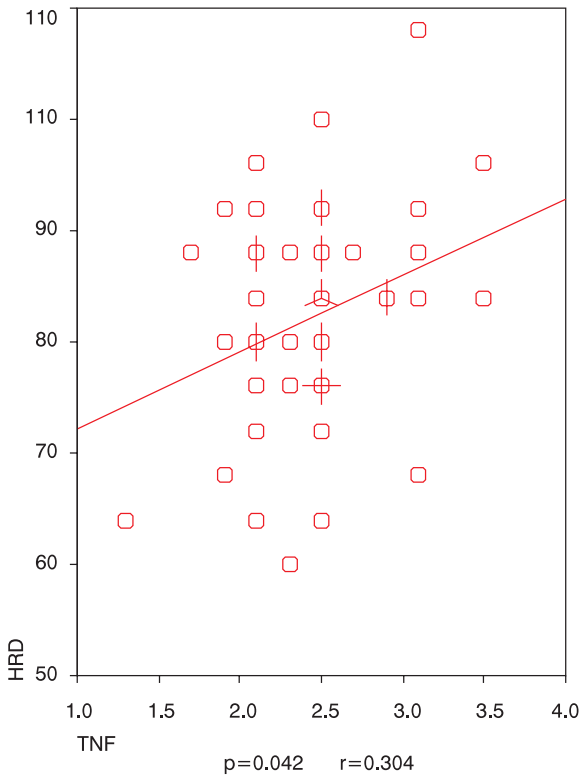

Figure 3. Correlation of daytime HR (HRD) with tumour necrosis factor-alpha (TNF) in patients.

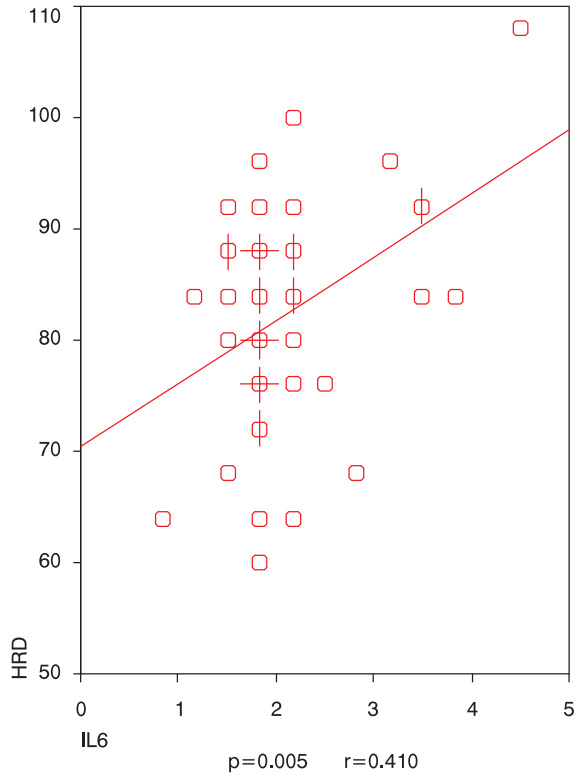

Figure 4: Correlation of daytime HR (HRD) with interleukin-6 (IL6) in patients.

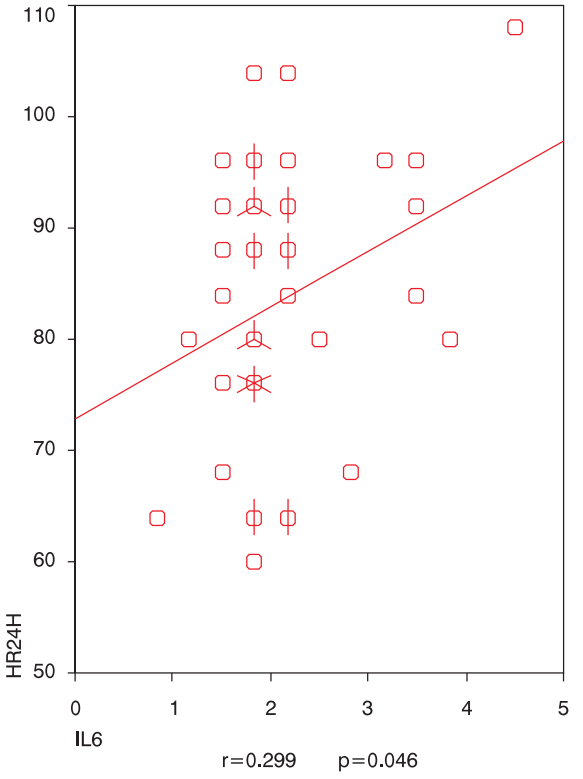

Figure 5. Correlation of 24-hour HR (HR24H) with interleukin-6 in patients.

Conclusion

Patients with IGT seem to have increased HR and elevated proinflammatory cytokine levels. This could be interpreted as a degree of interaction between inflammation and autonomic nerve alterations. The detection of early autonomic dysfunction among patients with IGT is significant for risk stratification, further evaluation and possible pharmacological recommendations and lifestyle modification.

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