Original Article

Association of heart rate variability indices with inflammatory markers in non-diabetic obese dyslipidemic middle aged Saudi population

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Abstract

Background and Aim: It has been documented that obesity is associated with diabetes, hypertension and dyslipidemia that are known cardiovascular (CV) risks. However, there are few obese individuals, who do not develop diabetes early. In the present study, we have assessed the association of inflammatory markers in non-diabetic dyslipidemic obese subjects, as metabolic biomarkers have not been adequately studied in obesity without diabetes, especially in the assessment of CV risks.

Methods: Twenty non-diabetic obese dyslipidemic subjects (study group) and 20 healthy non-obese subjects (control group) were included in this study. They were assessed for their body mass index (BMI) and heart rate variability (HRV) indices. Glucose, insulin, lipid profile and inflammatory markers were estimated from the fasting serum sample. Association of HRV with various parameters was determined by Pearson's correlation analysis.

Results: The basal CV parameters, HRV, lipid profile, glucose, insulin and the inflammatory markers were significantly altered and correlated with ratio of low frequency to high frequency (LF: HF ratio), a marker of sympathovagal imbalance (SVI) in the study group when compared with the control group.

Conclusion: SVI in the form of increased sympathetic and decreased parasymapathetic activity occurs in non-diabetic obese dyslipidemic subjects. Association of BMI, metabolic parameters and chronic low grade inflammation with LF: HF ratio may partly explain their augmented risks to future cardiac morbidities.

Key words: Autonomic imbalance, dyslipidemic, heart rate variability, inflammatory markers, non-diabetic obese

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INTRODUCTION

Obesity along with dyslipidemia, pro-inflammatory state and impaired glucose metabolism is considered as a group of atherosclerotic risk factors that tend to gather and increase the development of diabetes and the risk of cardiovascular (CV) disease,[1] like atrial fibrillation, acute coronary syndrome, cardiac death and overall mortality.[2]

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Studies have reported autonomic dysregulation as an important mediator in the development of obesity and its co-existing morbidities.[3,4] Because of the increasing obesity prevalence, understanding the mechanism connecting obesity and autonomic nervous system (ANS) function is of importance.[5] Heart rate variability (HRV) is a non-invasive technique that can be used to measure sympathovagal balance and its influence on CV functions in a variety of clinical conditions.[6-9] Obesity and its risk factors are independently reported to be linked with lower HRV.[10-13,14]

Although there are many studies reporting decreased HRV, increased inflammatory mediators and the correlation between HRV and inflammatory markers in obesity, to the best of our knowledge, there are scarcity of reports about HRV and inflammatory mediators in non-diabetic obese hyperlipidemic subjects. Therefore,

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the present study was designed to assess the relationship of HRV indices with inflammatory markers in obese subjects with dyslipidemia and without diabetes mellitus.

MATERIALS AND METHODS

Subjects

Group I: Control group

Twenty (15 healthy male and 5 female) volunteers in the age group of mean ± SD (52.15 ± 5.87 years) were recruited as controls for this study.

Group II: non-diabetic obese dyslipidemic group

The subjects were recruited from patients attending the Internal Medicine Clinics for medical investigations in different hospitals in Taif, KSA as King Adulaziz and King Faisal hospitals. This group included 20 non-diabetic patients, 10 males and 10 females with mean ± SD age (51.20 ± 4.40 years). The criteria for non-diabetic obese dyslipidemic subjects were based on the National Cholesterol Education Program Adult Treatment Panel III (2002) (NCEP ATP III) guidelines.[15]

- Abdominal obesity was defined as waist circumference (WC) exceeding 102 cm and 88 cm in men and women, respectively
- Fasting serum triglycerides (TG) more than 150 mg/dL (1.7 mmol/L)
- Fasting serum high density lipoprotein cholesterol (HDL) less than 40 mg/dL (1.0 mmol/L) and 50 mg/dL (1.3 mmol/L) in men and women, respectively
- Fasting blood sugar (FBS) <110 mg/dL (6.1 mmol/L)

Exclusion criteria

A medical history of any diseases known to affect the autonomic cardiac function such CV dysfunctions, neurological diseases, endocrine disorders and taking medication were excluded from the study.

All individuals included in this study were subjected to full history taking, focusing on family history of type 2 diabetes mellitus, hypertension, smoking and physical activity. Thorough clinical examination was done for all participants who gave informed consent and the study followed the rules of the Medical National Committee for Medical and Bio-ethics. Body weight and height were measured in subjects clothed in a light gown without shoes; these measurements were used to calculate the body mass index (BMI; kg/m^2). WC and blood pressure (BP) measurements were done for the subjects in both the groups. BP measurements were performed with a standard manual sphygmomanometer while the participants were in sitting position. Mean arterial pressure (MAP) was calculated.[16] Laboratory investigations included FBS, post-prandial blood sugar (PPBS), fasting serum insulin (FSI) and lipid profile. Homeostasis Model Assessment-Insulin Resistance Index (HOMA-IR) and the atherogenic index (AI) were calculated. The inflammatory markers such as high-sensitive C reactive protein (HsCRP), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α) were also estimated.

Sampling

Total of 5 ml of ante-cubital venous blood was collected after 12-14 h fasting under complete aseptic precautions in plain test tubes without anticoagulant. After coagulation, samples were centrifuged (at 1500 × g for 15 min). The separated serum was divided into three aliquots. One was selected for the immediate assay of fasting glucose and lipid profile. The other two aliquots were stored at −20 °C for subsequent assay of insulin. Repeated freezing and thawing were avoided. Hemolyzed samples were discarded.

Analytical methods

Serum glucose level, total cholesterol, TG and HDL were analyzed using Synchron CX-9 (Instruments Inc.; Scienfitic Instruments Division, Fillertron, CA 92634, 3100, USA) system autoanalyzer applying enzymatic colorimetric method.[17-20] Low density lipoprotein cholesterol (LDL) was calculated according to "Friedewald equation" provided that the serum TG level is <400 mg/dL.[21]

LDL = Total cholesterol −(HDL +TG/5)

FSI was assayed by Micro-particle Enzyme Immunoassay (MEIA) on the AxSYM (Abbott Ireland, Diagnostic Division-Lisnamuck, Longford Co. Longford, Ireland) for the quantitative determination of insulin in human serum or plasma. The AI considered as log (TG/HDL) has been reported as an significant predictor of atherosclerosis.[22] HOMA-IR was calculated using the equation:

HOMA-IR = Fasting glucose(m/dl) × fasting insulin (μU/ml)/405

The cutoff point to define insulin resistance was HOMA-IR ≥ 3.8.[23]

Inflammatory markers

Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) were measured using a sandwich ELISA (Human TNF-α and IL-6, Quantikine, R&D Systems, Instrumentation Laboratory, Milano, Italy). hs-CRP was measured in a central laboratory on plasma frozen at −80°C with an immunonephelometric assay on a BNII analyzer (Siemens Healthcare Diagnostics, Deerfield, Illinois) according to the manufacturer's protocol.

Heart rate variability

All subjects fasted and finished their health check-up prior to the assessment of their HRV. Routine electrocardiogram (ECG) was performed between 9 and 11 AM in the Physiology Laboratory, College of Applied Medical Sciences Taif, KSA under standardized conditions.[24,25] Fifteen minutes resting Lead II ECG was

obtained (AD Instruments). An artifact-free 5-min segment of the ECG was analyzed offline using LabChart software that permits visual inspection of the raw ECG, so as to obtain the HRV parameters in time-domain and frequency-domain. The recorded ECG signals were conveyed through analog digital converter FE132 Bio Amp (using Power Lab, 8/35 model PL3508 8 channel data acquisition system, AD Instruments, Australia) with a sampling rate of 20 Hz.

Time-domain analysis

This involves comparing 2 different signals and data were analyzed using descriptive statistical measures. The heart rate fluctuations were measured using various variables including, (a) standard deviation of RR intervals sensitive to all sources of variation (SDNN); (b) Standard deviation of the averages of NN intervals in all 5 min segments of the entire recording (SDANN); (c) root mean square successive difference of RR intervals (RMSSD).[24,25]

Frequency-domain analysis

The non-parametric Fast Fourier Technique (FFT) was performed for frequency-domain parameters. Different components of FFT with their specific frequency ranges were: (a) Total power (TP) (0-0.4 Hz) which reflects sympathetic and parasympathetic tone; (b) high frequency (HF) (0.15-0.4 Hz) which is indicative of parasympathetic tone and respiration; (c) low frequency (LF) (0.04-0.15 Hz) which indicates sympathetic as well as parasympathetic tone, (d) very low frequency (VLF) (0.003-0.04 Hz) which indicates thermoregulation, and can be used to calculate LF normalized unit (LFnu) and HF normalized unit (HFnu) that represent the relative value of each component in proportion to the TP minus the VLF component; and (e) LF/HF which reflects sympathovagal balance and the sympathetic modulation.[24,25] To limit the influence of diurnal and environmental variations, the HRV measurements were taken in the subjects in a sitting position after resting for 20 minutes. The measurements were taken in the morning and at the same room by one trained research assistant according to a standardized method. The HRV measurement was taken twice for each subject with a short-term interval in-between. Premature beats (i.e. >20 % shortening) were excluded manually and replaced with interpolated values and accounted for <1 % of each participants' dataset. RR intervals were interpolated at 4 Hz and detrending was performed using the smoothness priors method described by Tarvainen *et al.*[26] The same duration (5 min) of data were analyzed as established by the Task force system.[8] Recordings with non-sinus beats that were more than 1% of the total number of beats were also excluded. Premature beats and artifacts were carefully eliminated automatically and manually by visual inspection of all RR intervals.

Statistical analysis of data

The data was analyzed using Statistical Package for the Social Sciences software (SPSS version 22, SPSS Software Inc., Chicago, IL, USA). After log transformation independent sample '*t*' test was used to look for significant differences in the study parameters between patients and controls. Significance was assessed at the 5% level. Results of continuous measurements of HRV and clinical features were presented as mean ± standard deviation (SD). Pearson's correlation test was performed to determine the association between HRV indices and the study variables.

RESULTS

Using independent *t* test the mean values of the biochemical parameters, anthropometric and HRV indices between the 2 study groups were compared. The study group was associated with significantly higher ($P < 0.001$) weight, BMI, WC, SBP, DBP, FBS, PPBS, all lipid profile (except HDL), fasting serum insulin HOMA-IR, AI, hs-CRP, IL6 and TNF-α when compared to controls [Table 1].

Among the HRV indices LFnu and LF/HF ratio were significantly higher ($P < 0.001$, $P < 0.05$, respectively) in obese group than in the control group. Moreover, HFnu, mean NN interval and RMSSD were significantly lower ($P < 0.001$, $P < 0.05$, respectively) in obese group than in the control group. SDANN and SDNN was also found to be lower in obese subjects but the differences was not significant ($P > 0.05$) [Table 2].

Bivariate correlation analysis revealed a significant positive correlation ($P < 0.05$) between LF/HF ratio and WC [Table 3]. Moreover, in study group [Table 4], LF/HF

Table 1: The physical characteristics of the control and study groups

Parameters	Control group (*n*=20)	Study group (*n*=20)	*P value*
Age (year)	52.15±5.87	49.25±3.99	NS
Weight (kg)	66.25±2.22	78.00±4.17	<0.001
BMI (kg/m^2)	25.41±1.22	29.53±2.04	<0.001
WC (cm)	81.25±6.21	118.1±3.99	<0.001
SBP (mm Hg)	120.80±5.82	137.40±2.93	<0.001
DBP (mm Hg)	72.00±4.78	90.20±2.42	<0.001
FBS (mg/dL)	76.30±4.22	117.00±4.69	<0.001
PPBS (mg/dL)	112.80±6.78	126.40±3.95	<0.001
FSI (μU/mL)	4.70±0.74	11.60±1.31	<0.001
HOMA –IR	2.23±0.43	3.74±0.68	<0.001
CHOL (mg/dL)	181.65±11.20	229.55±15.14	<0.001
LDL (mg/dL)	106.82±12.17	161.85±15.52	<0.001
HDL (mg/dL)	52.75±3.88	30.50±2.06	<0.001
TG (mg/dL)	110.30±5.16	186.00±4.83	<0.001
AI	7.46±2.98	13.25±1.21	<0.001
hs-CRP (ng/ml)	0.06±0.02	0.22±0.06	<0.001
IL6 (ng/ml)	1.26±0.25	1.99±0.41	<0.001
TNF-α (pg/L)	2.77±0.41	4.45±1.07	<0.001

Data expressed as mean ± SD. *P* value, 0.05 was considered significant. NS: Not-significant, BMI: Body mass index, WC: Waist circumference, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBS: Fasting blood sugar, PPBS: Post prandial blood sugar, FSI: Fasting serum insulin, HOMA –IR: Homeostasis model assessment-insulin resistance index, CHOL: Total cholesterol, LDL: Low density lipoprotein, HDL: High density lipoprotein, TG: Triglycerides, AI: Atherogenic index, hs-CRP: Highly specific-c-reactive protein, IL6: Interleukin- 6, TNF-α: Tumor necrosis factor- alpha

ratio positively correlated with BMI, WC, SBP, FBS, HOMA-IR and AI. As well, HFnu negatively correlated with BMI and HOMA–IR. Higher SBP, WC, FBS and AIP were associated with lower LFnu and HFnu. Table 5 depicts that LF/HF ratio was correlated to inflammatory markers, hs-CRP and IL6 in obese group when compared to control group.

DISCUSSION

In agreement with previous reports,[27,28] we observed a swing in sympathovagal balance in the direction of increased sympathetic activation expressed as increased LF/HF ratio in non-diabetic obese dyslipidemic patients. This increased LF:HF ratio represents prominence of sympathetic activity and decrease in the vagal tone.[8,29] Since there was decrease in HFnu in obese subjects with dyslipidemia, sympathovagal imbalance (SVI) in these subjects may be due to decreased parasympathetic activity as decrease in HFnu represents decreased vagal modulation of cardiac drive.[8,29]

Table 2: HRV indices of control and study groups

Parameters	Mean±SD		*P value*
	Control group (*n*=20)	Study group (*n*=20)	
Frequency-domain indices			
LFnu	38.78±19.50	56.56±20.00*	0.004
HFnu	57.66±17.76	28.94±10.14*	0.014
LF/HF	1.44±1.16	2.83±0.50*	0.025
Time-domain indices (ms)			
Mean RR	909.59±249.49	696.72±66.60*	0.001
RMSSD	147.82±162.94	58.58±21.35*	0.020
SDANN	148.33±163.25	79.14±68.94	0.089
SDNN	133.11±116.32	81.94±39.47	0.070

Data expressed as mean±SD. *P<0.05 was considered significant. LFnu: Normalized low-frequency power, HFnu: Normalized high-frequency power, LF-HF ratio: Ratio of low-frequency to high frequency power, Mean RR: Mean heart rate, Mean RR: Mean RR intervals, RMSSD: The square root of the mean of the sum of the squares of the differences between adjacent NN intervals, SDNN: Standard deviation of normal to normal interval, SDANN: Standard deviation of the averages of NN intervals

SVI in study group subjects could be due to increase in sympathetic activity as there was increased LFnu, which reflects more sympathetic and less parasympathetic drive to the heart.[8,29] However, our study does not support a previous report[30] that revealed significantly lower values of overall HRV expressed as lower SDNN and SDANN. This may be attributed to the small number of subjects in the present study.

The FBS, FSI and HOMA-IR were significantly high in study group, although the precise cause of SVI cannot be ascertained from the present study; insulin resistance could be a reasonable mechanism. The cause of increased sympathetic activity in obese subjects could be linked to the chronic hyperinsulinemia that may lead to enhanced sympathetic tone and CV risks due to increased adrenergic activity.[31] Additionally, HOMA-IR was significantly correlated with LF-HF ratio in study group. As insulin resistance has been reported to cause autonomic imbalance,[32] insulin resistance could add to SVI in obesity. For further explanation of our results,

Table 3: Correlation of the studied parameters in control group

	Age	BMI	WC	SBP	FBS	HOMA-IR	AI
LFnu	−0.113	−0.188	−0.155	0.071	0.313	0.282	−0.068
HFnu	0.155	−0.235	−0.160	−0.014	−0.044	−0.031	0.109
LF/HF	−0.095	−0.254	0.722**	−0.390	−0.028	0.241	−0.165
RMSSD	−0.063	0.398	−0.110	0.163	0.033	−0.016	−0.030
SDANN	−0.064	0.397	−0.110	0.163	0.032	−0.017	−0.031
SDNN	−0.129	0.370	−0.101	0.103	0.064	0.027	−0.090
Mean NN interval	−0.302	0.149	0.128	−0.121	−0.006	−0.006	−0.255

*Correlation is significant at the 0.05 level (2-tailed) **Correlation is significant at the 0.01 level (2-tailed). LFnu: normalized low-frequency power, HFnu: normalized high-frequency power, LF-HF ratio: Ratio of low-frequency to high frequency power, Mean RR: Mean heart rate, Mean RR: Mean RR intervals, RMSSD: The square root of the mean of the sum of the squares of the differences between adjacent NN intervals, SDNN: standard deviation of normal to normal interval, SDANN: Standard deviation of the averages of NN intervals, BMI: Body mass index, WC: Waist circumference, SBP: Systolic blood pressure, FBS: Fasting blood sugar, HOMA –IR: Homeostasis model assessment-insulin resistance index, AI: Atherogenic index

Table 4: Correlation of the studied parameters in study group

	Age	BMI	WC	SBP	FBS	HOMA-IR	AI
LFnu	0.291	−0.320	−0.504*	−0.537*	−0.519*	−0.213	−0.512−*
HFnu	0.107	−0.811−**	−0.960**	−0.939**	−0.911**	−0.805−**	−0.941−**
LF/HF	0.144	0.662**	0.675**	0.620**	0.606**	0.750**	0.645**
RMSSD	0.099	0.148	0.301	0.359	0.315	0.048	0.273
SDANN	−0.316	0.015	−0.062	−0.080	0.002	0.060	−0.036
SDNN	0.085	0.224	0.089	0.021	0.093	0.366	0.077
Mean NN interval	0.081	0.258	0.288	0.331	0.225	0.231	0.272

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed). LFnu: Normalized low-frequency powe, HFnu: Normalized high-frequency power, LF-HF ratio: Ratio of low-frequency to high frequency power, Mean RR: Mean heart rate, Mean RR: Mean RR intervals, RMSSD: The square root of the mean of the sum of the squares of the differences between adjacent NN intervals, SDNN: Standard deviation of normal to normal interval, SDANN: Standard deviation of the averages of NN intervals, BMI: Body mass index, WC: Waist circumference, SBP: Systolic blood pressure, FBS: Fasting blood sugar, HOMA –IR: Homeostasis model assessment-insulin resistance index, AI: Atherogenic index

Table 5: Correlation of LF/HF ratio with inflammatory markers in control and study group

	Normal group		Study group	
	Pearson correlation (*r*)	Sig. (2-tailed) *P* value	Pearson correlation (*r*)	Sig. (2-tailed) *P* value
hs-CRP	0.023	0.923	0.649**	0.002
IL6	0.339	0.143	0.890**	0.000
TNF-α	−0.055	0.817	0.297	0.204

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed). hs-CRP: Highly specific-C- reactive protein, IL6: Interleukin- 6, TNF-α: Tumor necrosis factor- alpha, LF:HF: Ratio of low-frequency to high-frequency power

we studied the interaction and possible link between inflammatory markers and autonomic systems.[33] The LF-HF ratio was correlated with hsCRP and IL6, which is in agreement with the other studies.[34,35]

Low-grade inflammation has been reported in obesity,[30] and inflammatory markers such as hs-CRP, IL-6 and TNF-α have been reported to induce SVI in various stress related disorders.[36] In the present study, not only IL6, hs-CRP and TNF-α were significantly high in study group, but also were significantly correlated with LF-HF ratio, especially IL6 and hs-CRP. It is proposed that the activation of the vagus nerve may suppress inflammatory cytokines and possibly even slow the development of atherosclerosis.[37] The mechanism for this vagal inhibition involves the reaction of acetylcholine with its acetylcholine-receptor (AChR) on macrophages. The administration of nicotinic AchR (α7 subunit) agonists, results in a reduced production of TNF-α, interleukin-1 (IL-1), IL-6 and interleukin-8 (IL-8).[14] This mechanism might, to a certain extent, explain higher CV risk associated with the obesity. It has been suggested that retrograde inflammation could be the pathophysiologic link, as the increased sympathetic activity induces a pro-inflammatory state by IL-6 production, which in turn results in an acute phase response.[38] In the present study, all lipid profile parameters (except HDL) and lipid risk factors were significantly high in study group. AI was significantly correlated with LF-HF ratio indicating its contribution to SVI in obesity. Predominance of the sympathetic ANS favors an elevation in the serum TG.[39] Increased TG is reported to produce oxidative stress,[40] and oxidative stress is known to induce SVI.[41] This pathway could be the possible link between the dyslipidemia and SVI in obese patients.

Many studies have observed that obesity affects HRV.[42,43] However, others[44,45] have not detected any relationship between HRV and BMI. Various clinical studies have documented decreased parasympathetic activity in obesity.[27,46] Our results appear to corroborate with afore-mentioned studies as there were significantly lower HFnu, SDNN and RMSSD in obese than in control subjects. Additionally, we verified a negative correlation between anthropometric measurements (especially BMI and WC) and the HFnu parameter that reflects parasympathetic activity.

Thus, increased BMI is likely to be a potential contributor to SVI in these subjects. Salamin *et al.,*[47] have reported that although BMI and subcutaneous adiposity are not associated with cardiac parasympathetic indices of HRV, visceral adiposity contributes to decreased HRV.[47] Also, our results agree with report of Koskinen *et al*.,[48] who found that higher BP was associated with higher LF/HF ratio in both sexes.

In summary, the results of the present study indicate the presence of SVI in the form increased sympathetic and decreased parasympathetic activity in middle-aged non-diabetic dyslipidemic obese subjects.

Limitations of the study

As diabetes was excluded in obese patients, the sample size was less due to less availability of obesity without prediabetes or diabetes in Saudi population. The sample size was only 20, which is very small for such type of highly variable data, especially for HRV indices. Also, due to the less sample size regression analysis could not be done to assess the cause-effect relationship between the changes in HRV parameters and inflammatory markers.

CONCLUSION

Decreased HRV, atherogenic lipid profile, and low-grade inflammation in these subjects make them vulnerable to amplified CV risks, and SVI acts as the physiological basis for progress of these CV risks. SVI in the form of lower vagal modulation and increased sympathetic predominance was observed in obese dyslipidemic non-diabetic subjects when compared to controls. This may partly explain the higher CV risks and the development of other obesity related co-morbidities such as diabetes and hypertension in future.

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