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Assessment of cardiac autonomic function tests and pro-inflammatory marker in normotensive overweight individuals

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Abstract

Introduction: Obesity, resulting from the imbalance in energy metabolism has abundance of complications. Autonomic dysfunction has dual relation with obesity in the causation and in the complications. Chronic subclinical inflammation even in early overweight state results in adverse adaptation of various systems. Perplexing interaction between autonomic nervous system and inflammation mediates the outcome of the disease. The role of the inflammatory chemokine, CCL-19 and cardiac autonomic function in overweight population is poorly studied.

Aim: This study aims to assess the serum CCL-19 level and cardiac autonomic function tests in normotensive overweight individuals and to compare them with age and gender matched control group.

Materials and Methods: In this cross-sectional comparative study, 30 overweight individuals (BMI 24.51 ± 0.58 Kg/m^2) and 30 age, gender matched healthy controls (BMI 20.62 ± 1.25 Kg/m^2) participated. Heart rate variability at rest, autonomic reactivity tests (lying to standing, deep breathing and iso-metric hand grip tests) were done using task force guidelines. Serum CCL-19 was measured by ELISA. Unpaired t test and Mann-Whitney U test were used for the comparison in the mean differences based on the type of data distribution.

Conclusion: Systolic blood pressure is significantly increased and the proinflammatory cytokine, serum CCL-19 level is significantly reduced in overweight individuals.

Keywords: CCL19, Heart rate variability, Overweight

Introduction

Autonomic nervous system (ANS) plays an important role in the regulation of metabolism and weight gain

[1]. Inflammatory chemokines are also involved in the control of metabolism in normal and diseased conditions [2]. Autonomic dysfunction and altered inflammatory status are pivotal in the development of many chronic diseases like hypertension and diabetes mellitus [3&4].

An Indian study from National Family Health Survey has reported that the prevalence of overweight in men and women are 38.4% and 36.2% respectively [5]. Adverse outcomes in the pre-obese condition are mainly linked with alteration in autonomic functions and inflammatory dysregulation [6&7].

CCL-19 is a pro-inflammatory cytokine released from lymph nodes and various organs like gastro intestinal tract and adipocytes [8]. It is involved in the immune-surveillance in healthy individuals [9] and takes part in the early atherogenesis and cancer development [10&11]. Assessment of autonomic function together with homeostatic chemokine, CCL-19 will give better insight into the regulation of metabolism and autonomic cardiovascular responses in overweight. To best of our knowledge, this is the first attempt to assess the serum CCL-19 level and cardiac autonomic function tests in normotensive overweight individuals and to compare them with age and gender matched control group.

Materials and methods

The study was conducted in the Department of Physiology JIPMER, Puducherry. Post-graduate research monitoring committee (PGRMC) and the Institute Ethical Committee approvals were obtained before the commencement of the study. In this cross sectional, comparative study, the subjects were recruited based on the following inclusion and exclusion criteria.

Inclusion and exclusion criteria

20-35 years old healthy volunteers were included in this study. Based on the body mass index (BMI), participants were divided into two overweight (BMI= 23-24.9 kg/m^2) and control (BMI= 18-22.9 kg/m^2) groups as per the WHO criteria for Asian population [12]. Female participants with regular menstrual cycle participated in the study. Participants with regular physical activities like exercises, yoga and people on chronic medications were excluded from the study. They were also instructed to avoid coffee, tea, smoking for at least 12 hours prior to the recording.

Anthropometric measurements

Height and weight were taken using mounted stadiometer (Bioplus) and weighing scale (Kaups) respectively. Body Mass Index was calculated using the Quetelet's formula. BMI = Weight (kg)/ Height (m^2). Body fat (%) was calculated by bioelectrical impedance analysis (BIA) technique (Bodystat Quadscan 4000).

Heart rate variability (HRV) analysis

ECG recordings were taken for five minutes with spontaneous breathing using the BIOPAC MP150 data acquisition system (BIOPAC Inc., USA), after 15 minutes of complete rest in the supine position, in a room with the temperature maintained at 26°C between 10 am to 11.30 am. The data were saved in American Standard Code for Information Interchange (ASCII) format and analyzed for power spectral analysis of HRV using the software Kubios HRV Version 2.1 (Kubios, Kuopio, Finland). HRV analysis was done by the standard procedure as recommended by Task force [13] of the European Society of Cardiology, 1996.

Heart rate response in supine to standing

After 15 minutes of supine rest, the subjects were instructed to lean against a support to minimize muscular

effort during standing, and their heart rate were recorded continuously with Lead II ECG tracings for 5 minutes. RR intervals were retrieved and artifacts were removed manually following which 30:15 ratio (ratio of RR interval around 30th second and 15th second after standing) were calculated.

E:I ratio during deep breathing

Lead II ECG readings were taken continuously form the subjects when they were instructed to take deep breaths at the rate of 6 per minute. E:I ratio was calculated from the averages of longest RR interval during expiration and the shortest RR interval during inspiration, in 6 cycles.

BP response to sustained hand grip

The subjects were asked to sit comfortably in a chair in erect posture. The Basal BP and HR were recorded from the subjects. They were asked to grip the dynamometer with maximal force with the dominant hand and the Maximum voluntary contraction (MVC) values for the subjects were recorded. Then they were asked to maintain 30% of maximum voluntary contraction in handgrip. The difference between the baseline diastolic BP and diastolic BP that was obtained during isometric handgrip was taken as ΔDBP_{IHG}.

Measurement of serum CCL-19

5 ml of venous blood was collected using aseptic technique for measuring the serum CCL-19 level by ELISA method as per the manufacturer (FineTest, Hubei; Cat# EH0002) guidelines.

Statistical Analysis

Data were collected and analyzed using MS Office, 2019 and JASP 0.16.3,2022 softwares. Comparisons between the overweight and control groups were done using Mann-Whitney test and t-test, based on the type of data distribution. p value less than 0.05 was considered statistically significant

Results

Table 1: Demographic details of the participants (Mean ± SD)

	Overweight (n=30)	Control (n=30)	p-Value
Age (years)	25.57 ± 2.34	24.73 ± 2.24	0.165
Gender			
Male (n)	22	22	1
Female (n)	8	8	
BMI (Kg/m^2)	24.51 ± 0.58	20.62 ± 1.25	<0.0001 *
Body Fat (%)	17.45 ± 3.55	12.90 ± 2.06	<0.0001 *

Values were given in mean ± SD. BMI: body mass index. Comparisons between the overweight and control group for age, and BMI were done using unpaired t-test. Chi-square test was used to compare the gender difference between the groups. P-value <0.05 was considered as the level of significance.

Table 2: Comparison of blood pressure and heart rate variability (HRV) analysis between overweight and control group (Median (IQR))

	Overweight (n=30)	Control (n=30)	p-Value
SBP (mmHg)	116.5 (40)	110 (37)	0.002 *
DBP (mmHg)	72 (39)	68 (31)	0.055

Mean HR (bpm)	68.68 (37.33)	75.6 (34.03)	0.359
RMSSD (ms)	47.2 (149.8)	37.25 (352.9)	0.267
NN50 (count)	77.5 (217)	48 (146)	0.193
LF Power (ms2/Hz)	704.5 (2154)	661.5 (4672)	0.668
HF Power (ms2/Hz)	719 (9693)	541 (5239)	0.579
TP Power (ms2/Hz)	2412 (13157)	2233.5 (9709)	0.734
LF (nu)	50.98(61.26)	53.71 (55.29)	0.28
HF (nu)	49.0 (61.21)	46.26 (55.29)	0.261
LF/HF	1.04 (3.8)	1.15 (4.37)	0.284

Data were presented as median (interquartile range). Data were analyzed using the Mann-Whitney U test. *p-value <0.05 was considered statistically significant.

SBP (mmHg): Systolic blood pressure in mm of mercury; DBP (mmHg): Diastolic blood pressure in mm of mercury; Mean HR: mean heart rate in beats per minute; RMSSD: root mean square of successive differences between normal heartbeats; NN50: Number of normal inter beat intervals more than 50 ms; LF: low frequency; HF: high frequency; LF (nu): low frequency power in the normalized unit; HF (nu): high frequency power in the normalized unit.

Table 3: Comparison of autonomic reactive tests between overweight and control groups (Median (IQR))

	Overweight (n=30)	Control (n=30)	p-Value
30:15 Ratio	1.47 (1.08)	1.45 (0.62)	0.297
E: I Ratio	1.4 (0.59)	1.41(0.57)	0.304
Δ DBP in IHG	14.5 (35)	13 (26)	0.445

Data were presented as median (interquartile range). Data were analyzed using the Mann-Whitney U test. *p-value <0.05 was considered statistically significant.

30:15 Ratio: Ratio of RR interval around 30th second and 15th second after active standing; E:I ratio: ratio of the longest RR interval during expiration and the shortest RR interval during inspiration from the average of 6 cycles; Δ DBP in IHG. Delta change in the diastolic blood pressure in Iso-metric Hand Grip test.

Table 4: Comparison of serum CCL-19 levels between overweight and control groups (Mean ± SD)

	Overweight (n=30)	Control (n=30)	p-Value
serum CCL-19 (pg/dL)	5.24 (13.67)	10.0 (20.75)	< 0.001 *

Data were presented as median (interquartile range). Data were analyzed using the Mann-Whitney U test. *p-value <0.05 was considered statistically significant.

Systolic blood pressure was significantly higher in overweight group. RMSSD, NN50, HF band in absolute power and normalized unit were higher in overweight group. Blood pressure responses in isometric hand grip test were similar in overweight and control groups. Vagal modulation of heart rate in the autonomic reactivity tests were also normal in both groups. These differences in the autonomic function test parameters at rest and reactivity maneuvers were

not statistically significant. However, significant decrease in serum CCL-19 level was observed among the overweight individuals.

Discussion

There is no significant difference in the age of the subjects between the overweight and control groups (Table 1). Systolic blood pressure is significantly increased in overweight (p value = 0.002) (Table 2). This observation is in accordance with previous studies [14–16]. Basal heart rate is lower in overweight participants compared to the control group.

Though the time domain & frequency domain analysis (Table 2) between overweight and control groups are not statistically significant, relative decrease in the resting heart rate and increase in the RMSSD, HF power (ms^2/Hz) and HF power (nu) are observed in the overweight group. This is suggestive of higher vagal tone on the sinus node. FFT spectrum of the HRV analysis also shows reduction in the sympathetic activity on heart which is evident form the reduced LF power (normalized units) & LF/HF ratio in overweight. Conversely, many studies have reported reduced parasympathetic and increased sympathetic activities in overweight and obese groups [17&18].

Our observation could be due to the following reasons. BMI and body fat (%) of the participants in the above-mentioned studies are higher than the average BMI (24.51 ± 0.58 Kg/m^2) and body fat percentage (17.45 ± 3.55 %) of our study population. Since, the overweight group has relatively lower BMI in our observation compared to other studies, the autonomic dysfunction is not prominent as expected to have sympathetic activation and vagal attenuation. However, increased systolic blood pressure is observed in the overweight individuals of our study (Table 2). It shows the significance of local inflammatory factors in atherosclerosis compared to ANS regulation of blood pressure in early stages of obesity as suggested by other reports [19]. Raised blood pressure (within physiological range; SBP: 116.5 (40) mm Hg) in the overweight individuals can bring out the sympathetic inhibition and more vagal activation on cardiac nodal tissues through baroreceptor reflex mechanism which is also reflected in the HRV indices with reduced LF-nu, LF/HF values and increased HF (nu), RMSSD values in HRV analysis.

Autonomic reactivity tests (E:I ratio in deep breathing, 30:15 ratio in postural challenge and BP changes in isometric hand grip tests) are also normal in the overweight group (Table 3). It also indicates that the autonomic dysfunction is not prominent in this stage of obesity.

Nevertheless, serum CCL-19 level was lower in overweight group compared to control with statistical significance (Table 4). Increased pro inflammatory markers and low level of anti-inflammatory markers are observed in obesity [7,20&21]. Few studies have also reported with increased level of anti-inflammatory cytokines in obesity [22&23]. Change in the level of serum CCL-19 could be due to the neural or humoral mechanism. Normally, proinflammatory effects of the sympathetic nervous system in the early phase chronic inflammatory conditions are reported [24]. Reduced sympathetic tone as noted in cardiac autonomic function tests in our study, could have been the reason for the reduced level of pro-inflammatory chemokine, CCL-19. Vagal mediated suppression of inflammation by the neuro-humoral feedback [25&26] can be another possible mechanism behind our observation (reduced serum CCL-19 level), since relative increase

in the vagal tone is noticed in the overweight group of our study. It is evident from this study that homeostatic chemokine, CCL-19 level is significantly altered in the overweight group even before the changes that could be observed in the cardiac autonomic function tests.

Limitations of the study

Baroreflex sensitivity (BRS) measurement and estimation of CCL-19 from adipocyte, will help in deeper understanding on autonomic and inflammatory regulation of metabolism and cardiovascular responses in overweight individuals.

Conclusion

In the young overweight individuals, resting heart rate and vagal modulation of heart rate in the autonomic reactivity tests are apparently normal. Within the normal range, systolic blood pressure is higher in the overweight group compared to the control group. Serum CCL-19 level is significantly reduced in the overweight group.

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