Association of hypertension status and cardiovascular risks with sympathovagal imbalance in first degree relatives of type 2 diabetics

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ABSTRACT
Aims/Introduction: As reports show cardiovascular (CV) risks in first-degree relatives (FDR) of type 2 diabetics, and autonomic imbalance predisposing to CV risks, in the present study we have assessed the contribution of sympathovagal imbalance (SVI) to CV risks in these subjects.

Materials and Methods: Body mass index (BMI), waist-to-hip ratio (WHR), basal heart rate (BHR), blood pressure (BP), rate pressure product (RPP), and spectral indices of heart rate variability (HRV) were reordered and analyzed in FDR of type 2 diabetics (study group, n = 293) and in subjects with no family history of diabetes (control group, n = 405).

Results: The ratio of low-frequency (LF) to high-frequency (HF) power of HRV (LF–HF), a sensitive marker of SVI, was significantly increased (P < 0.001) in the study group compared with the control group. The SVI in the study group was due to concomitant sympathetic activation (increased LF) and vagal inhibition (decreased HF). In the study group, the LF–HF ratio was significantly correlated with BMI, WHR, BHR, BP and RPP. Multiple regression analysis showed an independent contribution of LF–HF to hypertension status (P = 0.000), and bivariate logistic regression showed significant prediction (odds ratio 2.16, confidence interval 1.130–5.115) of LF–HF to increased RPP, the marker of CV risk, in the study group.

Conclusions: Sympathovagal imbalance in the form of increased sympathetic and decreased parasympathetic activity is present in FDR of type 2 diabetics. Increased resting heart rate, elevated hypertension status, decreased HRV and increased RPP in these subjects make them vulnerable to CV risks. SVI in these subjects contributes to CV risks independent of the degree of adiposity.

INTRODUCTION
According to the World Health Organization, the total number of people with diabetes was 171 million in 2000, and it is projected to increase up to 366 million in 2030. An important cause for the rise in the prevalence of diabetes is the steady increase in bodyweight and obesity in many parts of the world, in addition to the contribution by environmental factors, social trends toward higher energy intake and reduced energy expenditure. India has been declared as a global leader in diabetes, currently with the second largest pool of diabetes in the world. The Asian Indian phenotype is uniquely predisposed to develop type 2 diabetes because of strong familial aggregation, and lifestyle factors of imprudent diet and sedentary physical habits.

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Diabetes has recently been seen to be more prevalent in the younger population, in whom there is a tendency towards premature development of complications that not only have an impact on the quality of life, but also have an unfavorable influence on the long-term outcome, raising the possibility of a serious public health challenge in the next few decades. Recently, it has been observed that the burden of cardiovascular disease (CVD) and diabetes in developing countries is more in the productive younger age group, which has serious economic implications.

Diabetes shares many characteristics and risk factors with CVD; and thus the risk for CVD escalates with the increase in prevalence of diabetes. Both genetic and environmental factors play major roles in the causation of diabetes and CVD. Therefore, early detection of diabetes and CVD, and prevention of their complications, especially in the younger age group, is a major concern worldwide. One of the strategies to achieve this goal is the screening for diabetes and CVD in high-risk populations. The first-degree relatives (FDR) of diabetic patients have been reported to have a high risk of developing diabetes compared with the general population. Although one recent report suggests that FDR of diabetics are prone to develop diabetes, but not CVD,

Several reports of earlier studies show altered autonomic balance with sympathetic hyperactivity in FDR of diabetic patients. However, to the best of our knowledge, no study has been carried out to date to assess the link of sympathovagal imbalance with CV risks in FDR with a family history of type 2 diabetes mellitus. Recently, spectral analysis of HRV has been established as a sensitive tool for assessment of autonomic functions in health and diseases.

Therefore, in the present study, we have assessed the association of sympathovagal imbalance with CV risks in FDR of diabetic patients by analyzing the spectral indices of HRV.

MATERIALS AND METHODS

After obtaining the approval of the Research Council and Institutional Ethics Committee of Jawaharlal Institute of Post-graduate Medical Education and Research (JIPMER), Puducherry, India, 698 participants were recruited from the first year MBBS, M.Sc. (Medical Biochemistry), B.Sc. (Medical Laboratory Technology) and B. Sc. (Nursing) courses of JIPMER of the years 2008, 2009, 2010 and 2011, for the present cross-sectional study. They were classified into two groups: (i) the control group (n = 405), comprising healthy subjects without family history of diabetes; and (ii) the study group (n = 293), comprising normal healthy FDR with a family history of type 2 diabetes mellitus. Participants in the study group (FDR with history of type 2 diabetics) were defined as having either of their parents or siblings diagnosed with type 2 diabetes mellitus for at least 1 year and receiving treatment for the same. This was done as part of a hypertension research project, in which a family history of diabetes was one of the questionnaires in the data sheet. The participants were also interviewed to verify the presence of diabetes in their family. Written informed consent was obtained from all participants before commencement of the clinical and laboratory investigations.

All participants were examined clinically by a physician to rule out the presence of any acute or chronic illness. Healthy participants (participants without illness) were included in the study. Participants receiving any medication, participants with a history of diabetes, smoking or hypertension, and hypertensive patients receiving medication were excluded from the study. As the level of physical fitness is a major determinant of vagal tone, participants carrying out regular athletics and body building exercises were excluded from the study.

Recording of Anthropometric and Basal CV Parameters

Participants were asked to report to the autonomic function testing (AFT) laboratory of the Physiology Department at approximately 09.00 hours after a light breakfast, without tea or coffee. After obtaining informed consent, their age, height, bodyweight, body mass index (BMI) and waist-to-hip ratio (WHR) were recorded. Blood pressure (BP) of all the participants was recorded in the AFT laboratory. The temperature of AFT laboratory was maintained at 25°C for all the recordings. Omron (SEM 1 Model), the automatic blood pressure monitor (Omron Healthcare Co. Ltd, Kyoto, Japan) was used for BP recording. For BP recording, the participant was asked to sit upright with their back straight on a wooden armed chair keeping one forearm on a wooden table kept in front and close to the participant. The BP cuff was tied just tight (neither too tight nor loose) on the arm approximately 2 cm above the cubital fossa. It was ensured that the BP cuff remained at the level of the heart. After 5 min of rest in the same sitting posture, the “Start” button of Omron was pressed, which automatically inflated and deflated the cuff, and systolic blood pressure (SBP), diastolic blood pressure (DBP) and basal heart rate (BHR) were noted from the display screen of the equipment. The mean arterial pressure was calculated from the SBP and DBP values. For each parameter, the mean of the four recordings was considered. Rate pressure product (RPP) was calculated using the formula. RPP = systolic pressure × heart rate × 10^{-2}.

Recording of HRV

After 15 min of supine rest on a couch in the AFT laboratory, an electrocardiogram (ECG) was recorded for 5 min for short-term HRV analysis following the standard procedure as described earlier. For recording of HRV, the recommendation of the Task Force on HRV was followed. For this purpose, ECG electrodes were connected and a Lead II ECG was acquired at a rate of 1000 samples/s during supine rest using...
the BIOPAC MP 100 data acquisition system (BIOPAC Inc., Goleta, CA, USA). The data was transferred from BIOPAC to a windows-based PC with AcQKnowledge software version 3.8.2 (BIOPAC Inc., Goleta, CA, USA). Ectopics and artefacts were removed from the recorded ECG. HRV analysis was carried out using the HRV analysis software version 1.1 (Bio-signal Analysis group, Kuopio, Finland). Frequency domain indices, such as total power (TP) of HRV, low-frequency (LF) and high-frequency (HF) powers, normalized LF power (LFnu), normalized HF power (HFnu), ratio of low-frequency to high-frequency power (LF–HF ratio) and time-domain indices, such as mean heart rate (Mean RR), square root of the mean squared differences of successive normal to normal intervals (RMSSD), standard deviation of normal to normal interval (SDNN), the number of interval differences of successive NN intervals >50 ms (NN50) and the proportion derived by dividing NN50 by the total number of NN intervals (pNN50), were calculated.

Statistical Analysis
SPSS version 13 (SPSS Software Inc., Chicago, IL, USA) and GraphPad InStat softwares (GraphPad Software Inc., San Diego, CA, USA) were used for statistical analysis. All the data were presented as mean ± standard deviation. Normality of data was tested by Kolmogorov–Smirnov test. For parametric data, the level of significance between the groups was tested by Student's unpaired t-test and for nonparametric data, the Welch's corrected t-test was used. The association between LF–HF ratio with BMI, WHR, BHR, blood pressure and RPP was assessed by Pearson's partial correlation analysis. The independent relationship of various factors, such as BMI, WHR, BHR and hypertension status with LF–HF ratio was assessed by multiple regression analysis. Bivariate logistic regression was carried out for adjusted odds ratio (OR) to assess the prediction of CV risk (increased RPP) by sympathovagal imbalance (LF–HF ratio), adjusted for obesity indices (BMI and WHR) and BP status. A P-value <0.05 was considered statistically significant.

RESULTS
There was no significant difference in age (P = 0.1118) between the participants of the control group and the study group (Table 1). The BMI, WHR, BHR, SBP, DBP, MAP and RPP of the study group participants were significantly more (P < 0.0001) compared with that of control group participants (Table 1). Among the frequency domain indices of HRV (Table 2), TP, HF and HFnu were significantly reduced (P < 0.0001), and LF, LFnu and LF–HF ratio were significantly increased (P < 0.0001) in the study group participants compared with the control group participants. All the time domain indices (mean RR, RMSSD, SDNN, NN50, pNN50) were significantly less (P < 0.0001) in the study group participants compared with that of control group participants (Table 2). Although there was no significant correlation of the LF–HF ratio with any of the parameters in the control group, the correlation was significant for all the parameters in the study group (Table 3).

Multiple regression analysis showed a significant individual contribution of BMI, WHR, BHR and hypertension status to the LF–HF ratio in the study group (Table 4). Bivariate logistic regression (Table 5) showed a significant prediction of LF–HF ratio to RPP in the study group (OR 2.16, 95% confidence interval 1.130–5.115; P = 0.005) compared with that of the control group (OR 0.82, 95% confidence interval 0.750–2.545; P = 1.036).

DISCUSSION
In the present study, the prevalence of type 2 diabetes in the FDR of medical and paramedical Indian students was 41.97% (293/698). As there was no significant difference in age between the study group and control group (Table 1), the alteration in cardiovascular and autonomic functions between the groups is not attributed to the effect of age. In FDR of type 2 diabetics, the LF–HF ratio was significantly increased compared with the control participants (Table 2), indicating a considerable enhancement in sympathetic activity in these participants, as the increase in LF–HF ratio indicates accentuation of sympathetic activity, and a decrease in this ratio represents facilitation of parasympathetic activity21,26. As, the LF-HF ratio is a sensitive measure of sympathovagal balance21,26, an increase in this ratio confirms the presence of a sympathovagal imbalance in FDR of type 2 diabetics. Sympathovagal imbalance in these subjects is due to alterations in both sympathetic and parasympathetic activities. An increase in sympathetic activity in the study group participants was shown by an increase in both LF and LFnu (P < 0.0001), as an increase in these two HRV indices reflects increased sympathetic drive to the heart21,26. A decrease in parasympathetic activity in participants of the study group was reflected by a decrease in both HF and

| Parameters | Control group | Study group | P-values |
|------------|---------------|-------------|----------|
| Age (years) | 20.26 ± 2.65 | 20.60 ± 2.96 | <0.0118 |
| BMI (kg/m²) | 21.25 ± 3.18 | 24.86 ± 4.35 | <0.0001 |
| WHR | 0.765 ± 0.08 | 0.878 ± 0.09 | <0.0001 |
| BHR (per min) | 69.38 ± 7.20 | 80.42 ± 8.62 | <0.0001 |
| SBP (mmHg) | 105.80 ± 5.78 | 116.18 ± 6.70 | <0.0001 |
| DBP (mmHg) | 68.50 ± 4.45 | 79.25 ± 5.30 | <0.0001 |
| MAP (mmHg) | 80.66 ± 5.90 | 91.56 ± 6.32 | <0.0001 |
| RPP (mmHg/min) | 73.28 ± 6.84 | 93.54 ± 7.56 | <0.0001 |

Data presented are mean ± standard deviation. A P-value <0.05 was considered statistically significant. Control group: participants with no family history of diabetes. Study group: first-degree relatives of type 2 diabetics. BHR, basal heart rate; BMI, body mass index; DBP, diastolic blood pressure; MAP, mean arterial pressure; RPP, rate-pressure product; SBP, systolic blood pressure; WHR, waist-hip ratio.
Table 2 | Frequency domain indices and time-domain indices of heart rate variability recorded in the supine position of the control group and study group participants

| Parameters       | Control group (n = 405) | Study group (n = 293) | P-values |
|------------------|-------------------------|-----------------------|----------|
| FDI              |                         |                       |          |
| TP (ms²)         | 1087.90 ± 408.70        | 875.20 ± 354.15       | <0.0001  |
| LF (ms²)         | 385.78 ± 120.32         | 428.10 ± 150.45       | <0.0001  |
| HF (ms²)         | 635.42 ± 212.80         | 310.50 ± 105.56       | <0.0001  |
| LFnu             | 39.40 ± 18.15           | 54.66 ± 21.60         | <0.0001  |
| HFnu             | 60.12 ± 24.75           | 45.20 ± 20.27         | <0.0001  |
| LF/HF ratio      | 0.62 ± 0.30             | 1.30 ± 0.56           | <0.0001  |
| Mean RR (s)      | 0.864 ± 0.132           | 0.746 ± 0.141         | <0.0001  |
| RMSSD (ms)       | 62.80 ± 24.50           | 45.34 ± 17.30         | <0.0001  |
| SDNN             | 47.40 ± 16.30           | 25.80 ± 11.17         | <0.0001  |
| NNS5            | 42.40 ± 15.36           | 30.72 ± 12.78         | <0.0001  |
| pNN50            | 28.25 ± 10.55           | 15.12 ± 6.15          | <0.0001  |

Data presented are mean ± standard deviation. A P-value <0.05 was considered statistically significant. Control group: participants with no family history of diabetes. Study group: first-degree relatives of type 2 diabetics. FDI, frequency domain indices; HF, high-frequency power; LF, low-frequency power; LFnu, normalized LF power; LF/HF ratio, ratio of low-frequency to high-frequency power; Mean RR, mean heart rate; NNS5, the number of interval differences of successive NN intervals >50; pNN50, the proportion derived by dividing NNS5 by the total number of NN intervals; RMSSD, the square root of the mean of the sum of squares of the differences between adjacent NN intervals; SDNN, standard deviation of normal to normal interval; TDI, time-domain indices.

Table 3 | Correlation of ratio of low-frequency to high-frequency power with body mass index, waist-to-hip ratio, basal heart rate, blood pressure and rate pressure product of control group and study group participants

| Parameters      | Control group | Study group | P-value |
|-----------------|---------------|-------------|---------|
| BMI             | 0.090         | 0.398       | 0.005   |
| WHR             | 0.165         | 0.547       | 0.000   |
| BHR             | 0.120         | 0.310       | 0.012   |
| SBP             | 0.196         | 0.725       | 0.000   |
| DBP             | 0.145         | 0.450       | 0.001   |
| MAP             | 0.098         | 0.364       | 0.012   |
| RPP             | 0.130         | 0.502       | 0.000   |

P-values <0.05 were considered significant. Control group: participants with no family history of diabetes. Study group: first-degree relatives of type 2 diabetics. BHR, basal heart rate; BMI, body mass index; DBP, diastolic blood pressure; MAP, mean arterial pressure; RPP, rate pressure product; SBP, systolic blood pressure; WHR, waist-to-hip ratio.

HFnu (P < 0.0001), as a decrease in these two parameters represents decreased vagal modulation of cardiac drive21,26. In the present study, the findings of increased sympathetic activity in FDR of type 2 diabetics corroborate with the reports of earlier studies18,19. However, to date there is no convincing report on the alteration in parasympathetic tone in these subjects.

In the present study, the quantum of heart rate variability was found to be considerably decreased in FDR of type 2 diabetics, as TP of HRV spectrum was significantly decreased (P < 0.0001) in these participants compared with that of control participants (Table 2). The TP not only represents the magnitude of HRV, but also the vagal drive of cardiac modulation21,26. Decreased power of HRV has recently been observed to be associated with sudden cardiac death and cardiac morbidities16,17,27–29. Thus, decreased HRV in FDR of diabetic patients predisposes these high-risk subjects to adverse CV events. This was further supported by higher BHR in these participants (Table 1). Resting heart rate is an index of vagal tone30, and increased heart rate has recently been reported to be associated with increased CV risks31–33. It has also been reported that BHR more than 70 b.p.m. increases the risk of major CV events34. As BHR was significantly high (P < 0.0001) in the study group participants compared with the control group participants (Table 1), the FDR of diabetics could be at an increased risk of adverse CV events.
The time domain indices (TDIs) of HRV (RMSSD, SDNN, NN50 and pNN50) were significantly reduced ($P < 0.0001$) in study group participants compared with that of control group participants (Table 2). This further confirms the decreased vagal tone in FDR of diabetics, as TDIs represent parasympathetic modulation of cardiac activity$^{21,26}$. In short-term HRV recording, RMSSD exclusively reflects vagal modulation of heart rate on a short-term basis, and therefore, RMSSD is considered as an important indicator of parasympathetic tone$^{21}$. The RMSSD was significantly less ($P < 0.0001$) in the study group compared with that of the control group (Table 2), which indicates poor cardiac vagal control in FDR of diabetic patients. Thus, the findings of present study show that the sympathovagal imbalance (alteration in LF–HF ratio) in FDR of type 2 diabetics is due to concomitant increased sympathetic activity and decreased vagal activity.

Although the exact mechanism of sympathovagal imbalance cannot be ascertained from the present study, obesity appears to contribute to it, as BMI and WHR were significantly more increased ($P < 0.0001$) in the study group participants compared with the control group participants (Table 1), and the correlation of BMI ($P = 0.005$) and WHR ($P = 0.000$) with LF–HF ratio was significant in the study group participants (Table 3). Furthermore, multiple regression analysis showed the independent contribution of BMI ($P = 0.025$) and WHR ($P = 0.009$) to the LF–HF ratio. Obesity has been reported to be more prevalent in individuals with a family history of diabetes, which contributes to metabolic dysfunctions in this high-risk population$^{35,36}$. Thus, increased adiposity in FDR of diabetics could be among the potential contributors to sympathovagal imbalance in these subjects, as obesity has been reported to cause autonomic imbalance$^{37,38}$. However, as obesity indices (BMI and WHR) were not significantly correlated with LF–HF ratio in the control group (Table 3), it is unlikely that the BMI and WHR contribute to sympathovagal balance in subjects without family history of diabetes.

Rate pressure product (RPP) is an indirect measure of myocardial load and oxygen consumption$^{24}$. Increased RPP has been documented as an established CV risk$^{34}$. In the present study, RPP was not only significantly increased in the study group compared with the control group (Table 1), but was also significantly correlated with LF–HF ratio in the study group (Table 3). Therefore, it is proposed that the sympathovagal imbalance in FDR of type 2 diabetics is linked to the increased myocardial energy load and expenditure, which could be a potential CV risk. Obesity per se can increase BP, and excess adiposity is a known CV risk$^{39,40}$. Therefore, we assessed the independent association of RPP with LF–HF ratio in the study group participants by logistic regression analysis adjusted for BMI, WHR and BP status (Table 5), and we found a significant prediction of LF–HF ratio to RPP (OR 2.16, 95% confidence interval 1.130–5.115). Thus, it appears that the sympathovagal imbalance in FDR of type 2 diabetics contributes to CV risk independent of adiposity. SBP, DBP and MAP were significantly high ($P < 0.0001$) in the study group participants compared with the control participants. Furthermore, LF–HF ratio had an independent contribution to hypertension status ($P = 0.005$), as shown by multiple regression analysis (Table 4), further confirming the increased risk of CV morbidity in these participants. In this regression model, SBP, DBP and MAP were not included to avoid multicolinearity.

In the present study, FDR of type 2 diabetics were young adults with the mean age of approximately 20 years. As such prediabetes, prehypertension, and insulin resistance in adolescents and young adults remain for a longer duration, exposing them to premature cardiovascular risks before clinically manifesting as full blown diabetes and hypertension during their adulthood$^{41,42}$. Furthermore, FDR of type 2 diabetics are more prone to developing insulin resistance, diabetes and cardiovascular morbidities$^{10–15}$. The limitation of the present study was that we did not assess the association of sympathovagal imbalance with levels of blood glucose, insulin, lipid profile and inflammatory markers in FDR of type 2 diabetics. Notwithstanding the limitations of not assessing insulin resistance, dyslipidemia and low-grade inflammation in these subjects, this preliminary study shows the link of sympathovagal imbalance to hypertension status and CV risks in FDR of type 2 diabetics. Thus, the findings of the present study suggest that the FDR of type 2 diabetics are susceptible to potential risk of CV morbidities contributed by sympathovagal imbalance. Therefore, future studies should analyze the association of sympathovagal imbalance with cardiac dysfunctions in FDR of type 2 diabetics.

Also, further research warrants the evaluation of the link between sympathovagal imbalance and metabolic derangements, such as insulin resistance, dyslipidemia and low-grade inflammation in FDR of type 2 diabetics. Furthermore, studies should be carried out to assess the effect of sympathovagal homeostasis achieved by various non-pharmacological means on reduction of hypertension status and CV risks in these high-risk young individuals, as practice of yoga and slow breathing exercises have been reported to decrease sympathetic discharge and increase vagal tone$^{35,44}$.

In summary, the results of the present study show the presence of sympathovagal imbalance in the form of increased sympathetic and decreased parasympathetic activity in young FDR of type 2 diabetics. Increased resting heart rate, elevated hypertension status, decreased HRV and increased RPP in this unique category of subjects make them vulnerable to CV risks. Sympathovagal imbalance in these subjects contributes to CV risk independent of the degree of adiposity. Despite its limitations of not assessing metabolic biomarkers, this preliminary study suggests carrying out future research to assess if restoration of sympathovagal homeostasis can reduce the CV risks in FDR of type 2 diabetics.

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