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Identifying Autonomic Neuropathy and Endothelial Dysfunction in Type II Diabetic Patients

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Abstract

Objectives: Previous studies of TM-Oxi and SudoPath systems have presented methods measuring autonomic nervous system and endothelial functions, and how the impairment of those functions were associated with type 2 diabetes Mellitus (T2DM) and Coronary Artery Disease (CAD).

This study aims to complement previous studies performed at the University of Miami, (Florida, USA) and IPC Heart Care Centers in Mumbai (India) with a larger sample population and further analysis of each markers used. Our previous studies using TM-Oxi and SudoPath devices showed high specificity and sensitivity markers comparing a group of healthy people versus a T2DMand CAD people, and if this current study with a cohort of patients confirms the same findings, then the markers of these devices will be needed for the early detection of diabetes and its complications.

Study Design and methods: One thousand ninety six patients were included in this double-blind study, and were grouped by diagnosis of T2DM. Patients in group 1 had been positively diagnosed with T2DM, whereas patients in group 2 had never previously been diagnosed with T2DM. All patients underwent the same experimental protocol. 743 patients were in group 1 (545 male) with a mean age of 54 and undergoing a diabetic treatment. 353 patients were in group 2 (194 male) with a mean age of 41. The 2 groups of patients underwent examination with the TM-Oxi and SudoPath system (LD Technology LLC). The TM-Oxi system provides markers of homeostasis based on:

A. The second derivative of the oximeter waveform (Photoplethysmography or PTG signal)
B. The Fast Fourier Transform harmonic components of the PTG signal
C. RR intervals (Time between each heart beat) at rest using the heart rate variability (HRV) analysis
D. RR intervals and change in blood pressure during Ewing Tests (Valsalva maneuver, Deep breathing and change in Posture).

The SudoPath system uses a galvanic skin response technology to assess sudomotor function. Also, the TM-Oxi and SudoPath systems calculate risk scores for cardiovascular autonomic neuropathy (CAN), sudomotor function, endothelial function and cardio metabolic.

Results: The TM-Oxi and SudoPath markers and scores used to distinguish T2DM groups from control groups returned significantly high sensitivities and specificities in the previous studies, and maintained high sensitivities and specificities from our current and larger sample population.

Conclusion: TM-Oxi and SudoPath markers and score used are very reliable and distinguish diabetic populations from non-diabetic populations.

Keywords: *TM-Oxi; SudoPath; Type 2 Diabetes Mellitus; Coronary Artery Disease*

Introduction

Current Type 2 Diabetes Mellitus (T2DM) diagnostic standards discriminate diabetics at a point that is too late to account for the damage already caused by insulin resistance and progressive beta cell dysfunction. While current treatments demonstrate effectiveness in preventing microcirculatory complications (retinopathy and nephropathy), these treatments do not prevent underlying diabetic conditions that lead to cardiovascular diseases [1].

As of March 2013, about 26 million people in the United States had been diagnosed with diabetes [2]. The diabetic population has grown exponentially since the mid-to-late 1990s, and this trend is expected to continue as an estimated 79 million Americans are pre-diabetic [2]. Currently, estimates of health care and productivity costs of the diabetic population are around $245 billion [2]. Costs will only continue to rise with the growth of the diabetic population. These statistics are not exclusive to the United States either. An estimated 382 million people worldwide are diabetic, and this number is expected to rise to 590 million by 2035 [2].

Estimates of health care and productivity costs in the US alone are $245 billion per year, which is expected to increase as over 70 million Americans are pre-diabetic, roughly 20% of the total American population. Additionally, the American Diabetes Association reports 65% of the diabetic population will die from a heart-related disease [3].

Clearly, these diagnostic standards and treatment strategies have not been able to combat the number of incidences of T2DM as predictive models show T2DM populations will continue to grow, increasing the economic burden on health care systems worldwide.

Given this information, current institutional strategies to approach diagnosis and treatment management or monitoring of diabetes are not effective.

In this study we propose additional pathogenic manifestations in the autonomic nervous system (ANS) and endothelial layers of peripheral vasculature temporally parallel manifestations of insulin resistance and beta cell dysfunction. Non-invasive and cost-effective examinations could identify ANS and endothelial dysfunction prior to diagnosis of T2DM and to monitoring its treatment using the current standards in order to ultimately counteract the T2DM pandemic occurring globally.

Standards such as glycated hemoglobin (HbA1c), fasting plasma glucose (FPG), and Oral glucose tolerance tests (OGTT) aid in identifying irregular blood-glucose content and metabolism and are currently the standard for determining the presence of T2DM [4]. However, not only is reliability of these measurements somewhat questionable, a correct diagnosis of T2DM using these markers would result only in management and palliative treatment of the disease and its symptoms, as the damage is often irreversible [5]. In addition, management of hyperglycemia often requires a combination of pharmaceutical therapies to achieve a target HbA1c, FPG, or other value. Target values are highly variable per patient and can be compromised due to compounding pathogenic expressions. Therefore, there is a clear need for reliable detection of damage prior to the onset of glucose-insulin imbalances observed through blood tests.

In this study we present several methods for identifying autonomic and endothelial dysfunctions in a diabetic population versus control group. These methods are non-invasive, present minimal cost, and take only a few minutes to complete. Our goal is to demonstrate the difference in autonomic and endothelial function between diabetic with or without complications and non-diabetic groups using our methods from a sample population of one-thousand-and-ninety-six patients from the IPC health care centers in Borivali, Parel and Kalyan India participated. While further longitudinal studies are necessary to understand the full capacity of these methods, previous studies showed high specificity and sensitivity between the 2 populations, [5,6], if implemented on a large scale, patient care can be adapted to suit a more individualized treatment regimen and perhaps assuage high mortality rates and economic burdens of the T2DM pandemic.

Methods and Materials

Devices and Methodology

Two marketed medical devices, the TM-Oxi and SudoPath systems (Manufacturer: LD Technology, Miami, FL), were used in the study (see Figures 1-8).

Figure 1: *Photograph of the integrated medical devices (TM-Oxi and Sudo).*

TM-Oxi system

The TM-Oxi uses a pulse oximeter and an automatic oscillometric blood pressure device.

The pulse oximeter waveform or photoplethysmograph (PTG) contour is a simple and non-invasive optical technique that can be used to detect blood volume changes in the micro vascular tissue bed [7].

The PTG waveform is comprised of a pulsatile (AC) physiological waveform attributed to cardiac synchronous changes in the blood volume with each heartbeat and is superimposed on a slowly varying (DC) baseline with various lower frequency components attributed to respiration, sympathetic nervous system activity, and thermoregulation [7].

The TM-Oxi performs PTG algorithms in time domain and spectral domain and provides

a. Accurate beat to beat heart rate (RR intervals) using the first derivative of the PTG contour
b. Markers using the change in amplitude of the PTG contour
c. Markers using the fast Fourier transform.

The RR intervals are using for

1. short time heart rate variability analysis (HRV) related to the autonomic nervous system balance (i.e. HRV Stress Index,) and activity (i.e. HRV Total Power and low frequency/ high frequency ratio) as described by the Task Force of the European Society of Cardiology and the North American Society Task force [8].
2. Ewing autonomic tests [9] related to the parasympathetic response based on the heart rate variation ((i.e. Valsalva ratio, Expiration/ Inspiration ratio and K30:15) and sympathetic response based on the blood pressure svariationas described by the Cardiovascular Autonomic Neuropathy (CAN) Subcommittee of the Toronto Consensus Panel on Diabetic Neuropathy [10].

The change in amplitude analysis of the PTG contour provides arterial stiffness markers such as Reflection Index and negative SDda [11,12].

The Spectral analysis of the PTG contour provides homeostatic markers such as PTG Total Power (PTGTP), PTG index (PTGi), PTG very low frequency (PTGVLF), PTGVLF index and PTG Ratio related to autonomic nervous system and endothelial functions [5,6].

SudoPath system

The sudomotor function is controlled by the post sympathetic cholinergic division of the ANS [13]. The SudoPath is a galvanic skin response (GSR) that uses two large stainless steel electrodes placed on the soles of the feet, where the sweat gland density is very high. The device generates a low voltage signal with weak DC current that is fed to the active electrode. The current is delivered to the contralateral electrode-person circuit in two directions for each pathway. During the patented measurement process, the voltage polarity is alternated at the middle of each measured pathway [14].

Unlike the sympathetic skin response method which can measures the emotions of the patient, the SudoPath provides a quantitative evaluation of the sudomotor function by measuring the induced sweat output following a controlled and constant electrical stimulus of the skin small autonomic unmyelinated nerve (C-fiber) and microcirculation. The sweat output response is expressed in voltage or micro Siemens [14,15].

The microcirculation marker is named Electro Skin Response nitric oxide (ESRNO) and cholinergic fiber (C-Fiber) density markers are the Electro Skin Response latency (ESR L) and the Peak Conductance (Peak C)[14].

Process of measurement

During testing, two surface electrodes are attached to the patient's feet. Additionally, the pulse oximeter is attached to the index finger of the patient and a blood pressure cuff is put on the patient's arm contralateral to that of the pulse oximeter. When the recording is started, the software collects signals from these three devices and saves the recordings for further interpretation.

Initial calibration

The initial calibration ensures the blood pressure measurements are synchronized with the recordings of the PTG waveform.

Baseline recording

Throughout the baseline recording, the patient is sitting. The PTG wave form and GSR are recorded over roughly two minutes. Sudomotor test values as well the RR intervals are displayed in real time.

Valsalva maneuver recording

Immediately before beginning the Valsalva recording, the patient is asked to blow up in a manometer with a pressure of 40 mmHg for 15 seconds. During this time, the PTG waveform is recorded. Shortly after this initial 15 seconds, an oscillometric blood pressure measurement is made. The exhalation causes a pressure increase in the chest and forces blood into the left atrium. This reduces blood flow returning to the heart while also reducing cardiac output. To compensate, the heart rate should increase. Following pressure release, aortic blood volume and cardiac output increases as heart rate returns to more normal values.

Deep breathing recording

Throughout the Deep Breathing recording, the patient is asked to alternate inhaling for 5 seconds and exhaling for 5 seconds over a total of 60 seconds. In this recording, only the PTG measurements are made. From this measurement, respiratory induced control over heart rate can be monitored and taken into consideration when developing markers for autonomic function.

K30/15 Recording

Immediately before beginning the K30/15 recording, the patient is asked to stand up. While standing, the PTG waveform is recorded and an oscillometric blood pressure measurement is made. By standing up, the parasympathetic system would respond by altering the heart rate and the sympathetic system would responding by maintaining or increased the blood pressure. Measurements of this change can be analyzed to make inferences concerning autonomic function in response to posture changes.

Design of the Study

Inclusion criteria

One thousand and ninety six patients were included in this double-blind study and were grouped by diagnosis of T2DM. Patients in group 1 had been positively diagnosed with T2DM, whereas patients in group 2 had never previously been diagnosed with T2DM. All patients underwent the same experimental protocol.

743 patients were in group 1 (545 male) with a mean age of 54 and undergoing a diabetic treatment. 353 patients were in group 2 (194 male) with a mean age of 41.

All the patients have physical exams, medical history and symptoms, reflex tests and clinical context comprising known diagnosis, current treatment and underwent examination with the TM-Oxi and SudoPath systems at IPC health care centers in Borivali, Parel, and Kalyan (India) for assessing autonomic nervous system and endothelial function. Between each examination, all hardware attached to the patient was disinfected according to the manufacturer guidelines. Each patient underwent a TM-Oxi and SudoPath exam performed by trained technician with a good knowledge of the autonomic nervous system tests. The patients were measured at any time of the day without taking the fasting state into consideration. The study was conducted according to the ethical principles of the Declaration of Helsinki. All of the subjects provided written informed consent, and confidentiality was maintained for all subjects.

Exclusion Criteria

Only patients who were older than 18 years of age, could provide written informed consent, and presented no contraindications with the use of the experimental setup were included in this study. Contraindications included: use of an automatic external defibrillator device; had erratic, accelerated, or mechanically-controlled irregular heart rhythms; had arterial fibrillation/flutter; had atrioventricular block; or had any implanted electronic device.

General Demographic Table	Diabetic group N = 757	Healthy subject N = 315	P value
Men/Women	545/212	154/90	NS
Age (years) range	54 (+ 11)	41 (+ 13)	P < 0.0001
BMI (Kgm^2) range	28.2 (+ 5.3)	24.4 (+ 3.8)	P < 0.0001
Systolic pressure (mmHg)	141.8 (+ 20.5)	127.0 (+ 14.8)	P < 0.0001
Diastolic pressure (mmHg)	80.0 (+ 13.6)	75.6 (+ 11.0)	P < 0.0001

Table 1: *Demographic information of sample population.*

Duration of Diabetes and complications	Percent of patients
Duration of diabetes > = 10 years	35 %
Duration of diabetes > 5 years and < 10 years	45%
Duration of diabetes < 5 years	20%
Atherosclerosis associated to the Diabetes	25%
Symptoms of small fiber neuropathy	23%
Symptoms of cardiac autonomic neuropathy	18%
Treatment of diabetic patients	
Metformin	78%
Secretagogue	47%
Insulin	38%
Antihypertensive agents	65%
Antilipidemic agents	65%
Antiplatelet agents	35%

Table 2: *Statistics of the T2DM group.*

Software analysis to obtain markers

Once all recordings are completed, the software can analyze the collected data to compute risk scores, which are based off certain markers and calculations. The markers used in this study will stem from the HRV analysis, PTG contour, spectral analysis of the PTG contour, Ewing tests results, and the GSR.

Marker	Description
PTGi	The sum of the 'very low frequency', 'low frequency', and 'high frequency' peak amplitudes from the FFT of the PPG waveform.
PTGr	The ratio of the 'very low frequency' peak amplitude to PTGi.
PTGVLFi	A marker normalizing the 'very low frequency' peak amplitude to galvanic skin response measurements at the negative electrode.
HRV Total Power	Sum of all the 3 frequencies resulting from the spectral analysis of the RR intervals
Reflection Index (RI)	Amplitude of the dicrotic notch and the maximum high of the wave.
ESRNO	The conductance measured from GSR at the negative electrode.
CMR Score	A score assigned to a patient's examination, evaluating thecardio metabolic risk.
SMR Score	A score assigned to a patient's examination, evaluating thesmall fiber neuropathy risk.
PTG CVD	A score assigned to a patient's examination, evaluating the endothelial dysfunction risk.
CAN Score	A score assigned to a patient's examination, evaluating cardiac autonomic neuropathy.

Table 3: *TM-Oxi and SudoPath analyzed markers.*

Scoring system

CMR (Cardio-metabolic Risk) score: The CMRScore is calculated using the following variables from the TM-Oxi:

1. Systolic
2. Diastolic blood pressure
3. Body mass index (BMI)
4. Arterial stiffness marker (RI)
5. HRV markers (Stress Index, Total Power and LF/HF)
6. PTG spectral analysis marker (PTGTP).

Each variable is scored as 0 = normal, 1 = borderline, or 2 = abnormal to calculate the CMR score.

SMR (SudoMotor Response) Score: The SMRScore is calculated using the following variables from the SudoPath: ESRNO, Peak C and ESR L.

Each variable is scored as 0 = normal, 1 = borderline, or 2 = abnormal to calculate the SMR score.

CAN (Cardiac autonomic neuropathy) Score:

The CAN Score is calculated through the changes detected in the RR intervals during the 3 Ewing tests which provide 3 markers: Valsalva Ratio, E/I Ratio and K30/15.

Each item is scored 0 = normal, 1 = borderline and 2 = abnormal to calculate the CAN score.

PTG CVD (PTG Cardiovascular disease) Score

The PTG spectral analysis provides 3 homeostatic markers: The sum of the amplitudes of all peaks is the PTG Index (PTGi). The adjusted PTGVLF with ESRNO is the PTGVLF Index (PTGVLFi), and the Ratio of the amplitude PTGVLF / PTGi defined as PTG Ratio (PTG R)

Each item is scored 0 = normal, 1 = borderline and 2 = abnormal to calculate the PTG CVD score.

Results of Previous studies [5,6,14].

Lewis., *et al*. Study [5] comparing diabetic and healthy groups (n = 49) using Receiver operating characteristic curves (ROC) showed the following:

The CMRScore had a sensitivity of 92% and specificity of 83% (cut-off score > 4) with an area under the curve = 0.94 (SE = 0.04; 95% CI = 0.87, 1.0) and an asymptotic significance < 0.001.

The PTGVLFi had a sensitivity of 92% and specificity of 87% (cut-off score > 25.5) with the area under the curve = 0.91 (SE = 0.05; 95% CI = 0.81, 1.0) and an asymptotic significance < 0.001.

Gandhi and Rao study [6] comparing atherosclerotic and healthy groups (n = 137) using Receiver operating characteristic curves (ROC) showed the results in table 3 and 4

PTG spectral analysis Markers	Sensitivity %	Specificity %	Cutoff	P value	AUC
PTGi	86.1	87.3	≤ 40.8	0.0001	0.926
PTGVLFi	86.1	93.6	> 27	0.0001	0.963
PTGr	73.8	93.6	>2	0.0001	0.895
PTGCVD Score	82.5	96.8	>2	0.0001	0.967

Table 4: *photoplethysmography (PTG) spectral analysis markers results (data gathered from MedCalc Statistical Software) for detecting atherosclerosis.*

PTG spectral analysis Markers	RI	HRV TP	CAN	Age
PTGi coefficient r	0.87	0.76	0.56	0.54
PTGVLFi coefficient r	0.76	0.51	0.64	0.47
PTGr coefficient r	0.70	0.50	0.41	0.32

Table 5: *Coefficient of Correlation r for photoplethysmography (PTG) spectral analysis markers and endothelial/ autonomic nervous system function markers (data gathered from MedCalc Statistical Software).*

Gandhi and Rao study [6] comparing a diabetic group with diabetic neuropathy symptom (DNS) score, and a diabetic group without such symptoms (n = 133) showed that the Sudomotor response (SMR) Score had a sensitivity of 91.4% and specificity of 79.1% (cutoff number > 3) to detect DNS > =1 (P = 0.0001). Area under the ROC curve (AUC) = 0.893. A correlation analysis of the CAN score and SMR score returned a coefficient of correlation r = 0.68 (P < 0.0001).

Study statistical Methods

Statistical analysis was performed using MedCalc Statistical Software's Receiver Operating Characteristic (ROC) curves with associated area under the curve (AUC) to portray the specificity and sensitivity of each marker and score to correctly identify the presence or absence of T2DM. A comparison of the means of control and T2DM groups for these datasets were performed using t-tests. In addition, the correlation between markers used the coefficient of Correlation r.

Results

Results to identify the presence or absence of T2DM

Fast-Fourier Transform Markers

Marker	Cutoff Values	Sensitivity	Specificity	AUC	P-Value
PTGi	40.6	78.5%	90.2%	0.92	P < 0.0001
PTGr	2.4	72.4%	89.3%	0.77	P < 0.0001
Total Power	803	71.9%	85.2%	0.87	P < 0.0001
PTGVLFi	29	84.1%	89.3%	0.87	P < 0.0001

Table 6: *Statistical information regarding the ability to detect T2DM groups for fast-Fourier transform markers.*

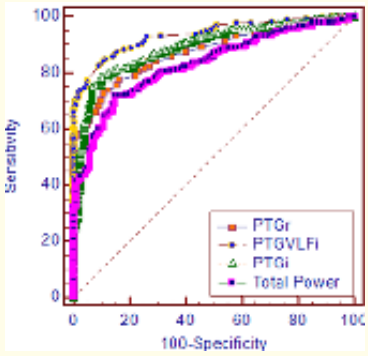

Figure 2: *ROC curves outlining the distribution of data from the markers PTGi, PTGr, Total power, and PTGVLFi.*

Seemingly, within this sample population, these values can distinguish between diabetic and non-diabetic groups. In order to prove differences within these subgroups exist, t-tests comparing the means of each marker were performed.

Significance tests show that with an α-value of 0.01, differences in means between control and T2DM groups for all fast-Fourier transform markers were statistically significant (P < 0.0001). Thus, these markers based on the harmonic components of the PTG signal were able to segregate the T2DM group within the context of our sample population. Furthermore, based on current statistics, a subgroup of the control group in this study could be pre-diabetic without a confirmed diagnosis and subgroups within the T2DM group could have been misdiagnosed with T2DM. These factors would contribute to decreased sensitivity and specificity of our markers.

Photoplethysmograph Reflection Index Marker

Marker	Cutoff Values	Sensitivity	Specificity	AUC	P-Value
RI	> 40	85.4%	73.7%	0.854	P = 0.0001

Table 7: *Statistical information regarding the ability to detect T2DM and control groups for second derivative markers.*

Reflection Index (RI), aimed to detect arterial stiffness, ranged in their ability to detect T2DM.

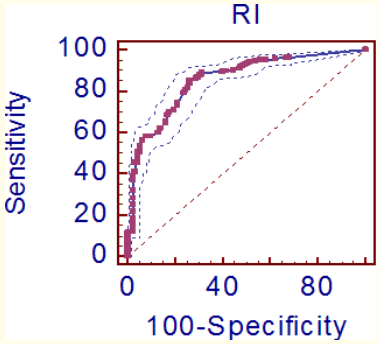

Figure 3: *ROC curves outlining the distribution of data from the markers SDba and SDda.*

Statistical tests were performed to further elucidate the differences between T2DM and control groups.

With an α-value of 0.01, RI presents high discrepancies between the T2DM and control groups ($P < 0.0001$). However, arterial stiffness is not only a symptom of T2DM, but also of atherosclerosis. Additionally, arterial stiffness has been shown to increase in aging populations.

Marker	Cutoff	Sensitivity	Specificity	AUC	P-Value
ESRNO	49	68.7%	84.3%	0.85	P < 0.0001

Table 8: *Statistical informationregarding the ability to detect T2DM and control groups for the ESRNO GSR marker.*

Galvanic skin response markers, aimed to detect microcirculation (ESRNO), produced fair ability to detect T2DM. The t-test comparing data between the T2DM and control groups also returned a high significance level denoting discrepancy between the two datasets ($P < 0.0001$).

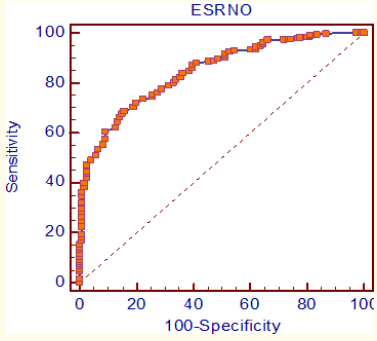

Figure 4: *ROC curve outlining the distribution of data for ESRNOmarker.*

Because ESRNO is a measure of microcirculation, which has been linked to changes in cardiovascular sympathetic tone, ESRNO is used in the calculation of PTGVLFi in order to strongly discriminate patients with both peripheral and cardiovascular neuropathy.

Score	Cutoff Value	Sensitivity	Specificity	AUC	P-Value
CMR	4	81.6%	90.2%	0.92	P < 0.0001
SMR	1	67.2%	81.0%	0.77	P < 0.0001
PTG CVD	2	65.8%	97.5%	0.87	P < 0.0001
CAN	5	74.6%	90.6%	0.87	P < 0.0001

Table 9: *Statistical information regarding the ability to detect T2DM and control groups for the CAN, CMR, SMR, and EndoT scores.*

Risk Scores

Risk scores are assigned to patient examinations and are based off of various markers and demographic information, associated with each facet of pathological complications.

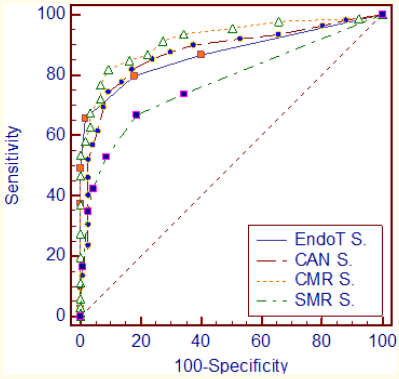

Figure 5: *ROC curves outlining the distribution of data for CAN, CMR, SMR, and EndoT scores.*

Results of markers correlation

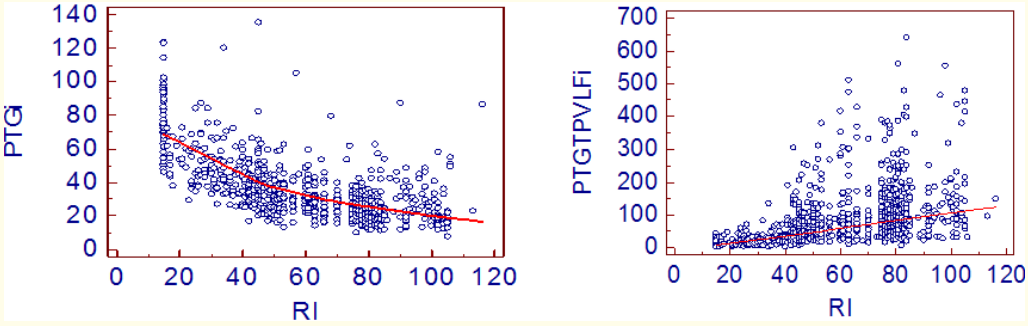

Figure 6: *Correlation between PTGVLFi and RI (left, r = 0.53) and between PTGi and RI (right, r = -0.70).*

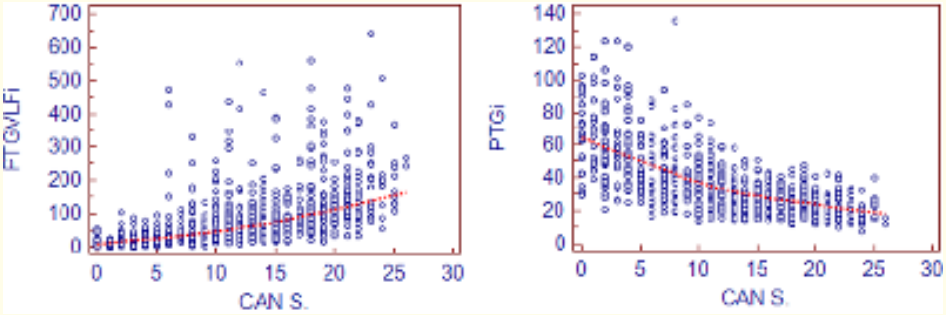

Figure 7: *Correlation between PTGVLFi and CAN score (left, r = 0.691) and between PTGi and CAN score (right, r = -0.582).*

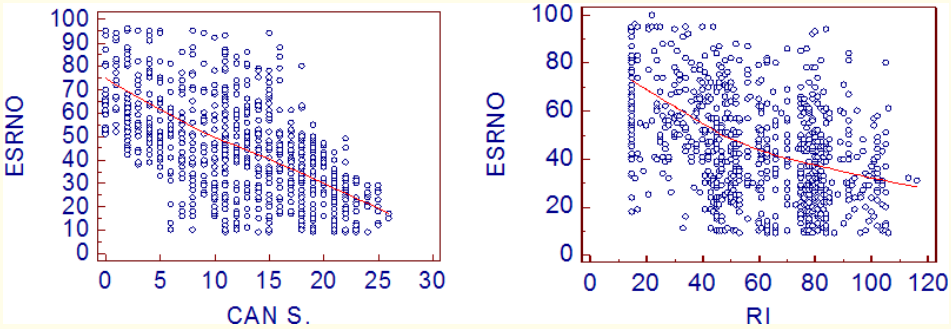

Figure 8: *Correlation between ESRNO and RI (up right, r = -0.51) and between ESRNO and CAN score(down right,r = 0.64).*

Items	CAN score	RI	P-Value
PTGi	r = 0.58	r = 0.69	P < 0.0001
PTGVLFi	r = 0.69	r = 0.51	P < 0.0001
SMR	r = 0.64	r = 0.44	P < 0.0001
ESRNO	r = 0.64	r = 0.52	P < 0.0001

Table 10: *Statistical correlation between CAN score and respectively PTGi, PTGVLFi and ESRNO and between RI and the same markers.*

The correlations between markers of autonomic nervous system and endothelial functions measured by different methods (Galvanic skin response, PTG spectral analysis and Ewing tests) demonstrate that there is a close interrelationship between vascular and autonomic nervous system functions.

These markers are easy to obtain (2 minutes time) and provides a good picture of the vascular and autonomic nervous systems.

Discussion

Statistical analyses of our data from autonomic and vascular markers clearly showed significant differences between the T2DM and control groups, and confirm the results of the previous studies using TM-Oxi and SudoPath systems in diabetic population.

While the sample population only consisted of diagnosed diabetics and non-diabetics, the sensitivity and specificity of markers such as PTGVLFi were high enough to suggest the suspected autonomic damage could be part of the underlying dysfunction causing T2DM pathogenesis.

Additionally, our data shows measurements of endothelial function, such as RI or PTGi also have relatively high specificity and sensitivity when diagnosing T2DM and suggests endothelial dysfunction is prevalent in the diabetic population.

Previous studies showed that endothelial markersare highly correlated with autonomic nervous system function markers [16-19]. It is logical, that there is a close interrelationship between endothelial and autonomic nervous system functions, for example, in hypertension, endothelial dysfunction affects the pathologic process through autonomic nervous pathways and the pathophysiological process of autonomic neuropathy in diabetes mellitus is closely related with vascular function [18].

Moreover, endothelial dysfunction and ANS imbalance often co-exist in the development of various cardiovascular disease processes, suggesting that there are complex interactions between these two systems. Several studies suggested a potential association between heart rate variability and endothelial function [17].

Other groups also suggested a relationship between markers of endothelial function and sympathetic activity in healthy subjects. In a study of 314 healthy subjects, endothelial function in the brachial artery was inversely related to neither plasma nor epinephrine level [19].

In order to clarify how these markers are indicative of autonomic and endothelial function, we must analyze the derivations of these markers developed in this study.

The Fourier-transform of the PTG signal contains components affected by ANS activity. The first 'very low frequency' peak of the Fourier-transformed signal is derived from the average heart rate over the two-minute recording and generally ranges from 0.5 Hz to 1.7 Hz. Heart rate variability would decrease the power of the first peak and increase the power of neighboring frequency bins. The second 'low frequency' peak is derived from the diastolic blood flow phase and ranges from 2.0 Hz to 3.3 Hz. For example, if the diastolic peak was to occur roughly 400 ms (left-ventricular ejection time) following the systolic peak, the characteristic frequency of the second peak in the frequency domain would be around 2.5 Hz. Variability in left-ventricular ejection time would create effects similar to that of heart rate variability in the frequency domain. The third 'high frequency' peak ranges from 3.3 Hz to 5.5 Hz. This peak, along with higher frequency peaks, could be characteristic of several components in the PTG signal. One component could be due to peripheral cutaneous arteries contracting and dilating.

Calculations of PTGVLFi are based on the 'very low frequency' peak amplitude and ESRNO, a measurement of conductance associated with skin microcirculation. Patients with cardiovascular autonomic neuropathy, who often present denervation of fibers in the extremities, can also experience changes in cardiovascular sympathetic tone, suggesting peripheral neuropathy and systemic cardiac neuropathy are linked. Since PTGVLFi could contain information regarding the cardiovascular autonomic function and peripheral fiber density, the autonomic and sympathetic cholinergic functions are distinct in the diabetic population as PTGVLFi was found to be significantly higher and ESRNO was found to be significantly lower in the diabetic group of this study. This would denote the very low frequency amplitude is larger and measurements of conductance lower in the diabetic group when compared to the control group. The larger 'very low frequency' amplitude could be due to limited variability in the heart rate, which may point to lack of autonomic control. Additionally, lower conductance measurements could point to c-fiber denervation from the sweat glands. Together, the limited heart rate variability and c-fiber denervation could be indicative of diabetic neuropathy. As neuropathic conditions worsen through different stages of diabetes, PTGVLFi could portray the diabetic/pre-diabetic state of the patient and if treatments are improving the patient's health, though these conceivable associations were not tested in this study.

Additional calculation from the Fourier-transform of the PTG waveform also presented significant differences between the T2DM and control groups. PTGi, the sum of peak amplitudes from the three frequency bands, was found to be significantly lower in the T2DM

group. Because PTGr and PTGVLFi were significantly higher in the T2DM group, 'very low frequency' amplitudes cannot be contributing as much to the lower PTGi measurements in the diabetic population. Therefore, 'low frequency' and 'high frequency' peaks must be much lower in the T2DM groups. This may be because the PTG variability exists in higher frequency components such as left-ventricular ejection time and arterial dilation. A correlation analysis between PTGi and RI indicates a high inversely proportional correlation between the two markers (r = -0.6906). So, variability in the higher frequency components could be associated with arterial stiffness. PTGr, the ratio of the 'very low frequency' peak amplitude to PTGi, was found to be significantly higher in the T2DM group. Similar to the reasoning behind higher PTGVLFi measurements, higher PTGr measurements may be due to higher 'very low frequency' peak amplitudes of patients in the T2DM group in comparison to that of the 'low frequency' and 'high frequency' peak amplitudes. Like PTGi, lower peak amplitudes from the 'low frequency' and 'high frequency' bands would point to higher variability in left-ventricular ejection time and arterial dilation.

The RI marker is calculated from averages of many PTG waveforms throughout the two minute recording. This gives us a sense of the typical blood movement patterns through the cutaneous arteries. RI represents the ratio of the dicrotic notch amplitude and the maximum amplitude of the waveform and is derived from the fluctuations in blood volume from systolic and diastolic blood flow, affected by arterial dilation and contraction. If elasticity of the blood vessel is reduced, the blood volume between systolic and diastolic phases will exhibit a delay in the temporal profile. The marker RI aim to measure the change in the temporal blood volume profile. RI was found to be significantly higher in diabetic populations, which could point to arterial stiffness. Arterial stiffness could stem from sympathetic denervation or by hyperglycemic and hypoglycemic conditions. Since RI had a high correlation with PTGVLFi, suggesting arterial stiffness could be at least partially caused by decreased sympathetic tone, either systemically or peripherally. While vasodilation can be achieved through several pathways, studies have acknowledged acetylcholine release from peripheral c-fibers and subsequent mechanisms account for an estimated one-third of these pathways. [20-24]. conceivably denervation of the c-fibers could lead to a decreased ability for the vasculature to dilate.

These markers from the Fourier-transform, original waveform and GSR are used in the determination of risk scores in the TM-Oxi and SudoPath software. Risk scores are used to convey the degree in which signs and symptoms are detected, while taking into consideration a patient's demographic information. All of these scores were able to significantly distinguish between T2DM and control groups. Since these scores are derived from methods used to assess cardio metabolic risk, endothelial function or cardiovascular risk, cardiovascular autonomic neuropathy, and sudomotor response, diabetic complications must be prevalent in each of these facets.

As traditional tests for cardiovascular autonomic neuropathy are difficult to perform, time-consuming and therefore rarely performed in daily practice [25]. Independent markers with high specificity and sensitivity would be extremely useful to allow for better management of patients with T2DM and help in avoiding the development and progression of life-threatening complications. Previous studies have shown hyperglycemic medication can cause hypoglycemic unawareness, and increase mortality because of this [26]. Markers, potentially stemming from a two minute Fourier-transformed PTG recording (e.g.PTGi, PTGVLFi, PTGr), could indicate whether a diabetic patient has cardiovascular autonomic neuropathy risk or cardiovascular risk and serve as a cautionary measure when considering treatment options for hyperglycemia.

Additional Remarks

In this study, we aimed to reaffirm data collected from a separate, smaller sample population. When comparing the data collected in this study and the data from the previous study, we see no drastic changes in metrics used to measure the strength of correlation of some markers or score and marker to detect T2DM. While, in some instances, the optimal sensitivity and/or specificity and coefficient of correlation were slightly different (except for the correlation of the spectral analysis markers and RI),some variability in these metrics was expected due to larger sample population sizes. However, all the markers and scores maintained high specificity and sensitivity.

Score	Cutoff Value		Sensitivity		Specificity		AUC		P-Value	
	Pr	Current	Pr	Current	Pr	Current	Pr	Current	Pr	Current
CMR	4	4	91.2%	81.6%	90.0%	90.2%	0.96	0.92	< 0.0001	< 0.0001
SMR	3	3	91.4%	67.2%	79.1%	81.0%	0.89	0.77	< 0.0001	< 0.0001
EndoT	2	2	88.2%	65.8%	88.6%	97.5%	0.90	0.87	< 0.0001	< 0.0001
CAN	4	5	79.0%	74.6%	88.5%	90.6%	0.90	0.87	< 0.0001	< 0.0001
PTGi	44.9	44.6	91.0%	78.5%	80.9%	90.2%	0.93	0.92	< 0.0001	< 0.0001
PTGVLFi	26	29	96.0%	84.1%	93.6%	89.3%	0.99	0.87	< 0.0001	< 0.0001
ESRNO	49	49	68.6%	68.7%	87.1%	84.4.3%	0.86	0.85	< 0.0001	< 0.0001

Table 11: *Comparison of marker and score metrics between previous (Pr) and current studies.*

Items	Coefficient of correlation		P values	
	Pr	Current	Pr	Current
PTGi /CAN score	0.56	0.58	< 0.0001	< 0.0001
PTGi/ RI	0.87	0.70	< 0.0001	< 0.0001
PTGVLFi / CAN score	0.64	0.69	< 0.0001	< 0.0001
PTGVLFi / RI	0.76	0.52	< 0.0001	< 0.0001
SMR/CAN score	0.68	0.64	< 0.0001	< 0.0001
SMR/RI	NA	0.44	< 0.0001	< 0.0001
ESRNO/ CAN score	NA	0.64	< 0.0001	< 0.0001
ESRNO/ RI	NA	0.51	< 0.0001	< 0.0001

Table 12: *Comparison of correlations between previous (Pr) and current studies.*

Conclusion

Markers and scores used to identify autonomic, sudomotor and endothelial dysfunction reliably distinguish diabetic populations from non-diabetic populations. The markers proposed could be used to produce highly reliable risk scores to reinforce diagnoses of T2DM from hyperglycemic markers. If these neuropathic or vascular conditions also started to manifest in early-stage diabetic pathogenesis, paralleling insulin resistance and beta-cell dysfunction, these markers could indicate pre-diabetic states. Additionally, with a full understanding of the state of a patient's neural, vascular, hepatic, and pancreatic systems, treatment strategies could be individualized and take into account the totality of the pathological conditions. Granted, the relationship between insulin resistance, autonomic function and T2DM pathogenesis cannot be entirely elucidated with the knowledge base we have today. Additional longitudinal studies need to be performed alongside current standards for detecting insulin resistance and glucose metabolism.

Limitations

When assessing the ability of these variables to distinguish between the two groups in this dataset, we should note that there may have been patients who were misplaced into the groups. No preliminary screening was performed prior to group assignment. Therefore, the assignment was based completely on previous diagnoses and could present bias in the data. Patients who have not been diagnosed with diabetes but present data that is aligned with that of a patient with T2DM could have presented bias in our control group. Conversely, patients who were incorrectly diagnosed with T2DM could have presented bias in our T2DM group.

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