

# Non-Invasive Assessment of Microcirculatory Dysfunction in Type 2 Diabetes Patients with and without Ulcers in Western Province of Saudi Arabia

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## Abstract

The present study aimed at comparing the predictive trends of transcutaneous oxygen pressure (PtcO<sub>2</sub>) and microcirculation in arbitrary perfusion unit measured at the tip of big toe along with Toe-Brachial Index (TBI) in normal subjects as well as age and sex matched type 2 diabetes mellitus (T2DM) patients with and without ulcers. Thirty healthy subjects along with 51 T2DM patients without ulcer and 40 T2DM patients with ulcer were included. Measurement site for PtcO<sub>2</sub> and Laser Doppler (LD) was at the tip of big toe. LD and PtcO<sub>2</sub> recordings were made before and after heat provocation and 100% normobaric oxygen challenge respectively. T2DM with ulcer group was subdivided into subgroups according to clinical outcomes: healed, unhealed and/or death cases. TBI was significantly lower (P=0.0001) in T2DM with ulcer compared with either healthy or T2DM without ulcer. All death cases only as a subgroup (n=10) too had significantly lower (P<0.05) TBI compared with healed cases (n=15). A %change from the base value of LD after heat provocation was significantly higher in healthy compared with both T2DM control (P=0.029) and T2DM patients with ulcers (P=0.011) while an opposite trend of significantly lower % change in PtcO<sub>2</sub> was observed in healthy as compared with T2DM control (P=0.025) or with T2DM with ulcer (P=0.038). As compared to absolute values, a %change in LD and PtcO<sub>2</sub> base values, upon heat provocation or oxygen challenge, unambiguously differentiate microcirculatory status of healthy and T2DM groups and that the LD-heat and TBI are good predictors of bad outcome (deaths/unhealing) in T2DM with ulcer patients.

**Keywords:** Laser Doppler Flowmetry, Oximetry, Peripheral Arterial Disease, T2DM Ulcers, Toe-Brachial Pressure Index, Transcutaneous Oxygen Partial Pressure

## 1. Introduction

A very disabling long-term complication of Diabetes Mellitus (DM) is the Diabetic Foot Ulcer (DFU). DFU may lead to minor and major lower extremities amputation. The DFU may result due to infection, foot deformities, neuropathy and macro- or micro Peripheral Arterial Disease (PAD). The macro PAD can be assessed clinically by pulse palpation, Ankle/Brachial Index (ABI) measurement and Toe/Brachial Index (TBI)<sup>1,2</sup>. However,

concerns persist regarding their accuracy. ABI may not be accurate in case of calcifications of large arteries in the leg which make them incompressible. In that situation the TBI is more useful<sup>3</sup>. The micro PAD can be assessed by two most commonly employed noninvasive techniques that evaluate the micro circulatory status, namely, skin blood perfusion measurement in arbitrary Perfusion Unit (PU) using Laser Doppler Flowmetry (LD) and transcutaneous oxygen (PtcO<sub>2</sub>) monitoring using oximetry<sup>4-6</sup>.

Blood perfusion measurements using laser Doppler

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reflect extreme dynamics and large spatial variations<sup>7</sup>. Hemodynamic patterns and capillary flow in different areas of skin affect the measurements values of such techniques that in turn anatomically depend on prevalence and lacking in Arteriovenous Anastomoses (AVAs) or other regulator systems. In this respect foot sole and dorsum, fingertips and nail fold are considered peculiar microcirculatory areas<sup>7</sup>. Under normal conditions, the blood perfusion can differ several thousand percent between a cold and warm fingertip. It also exhibits large spatial variations and may vary up to 100 percent in forearm skin if the measurement site is moved one millimeter. As a consequence of the large normal variations observed in the microcirculatory blood flow, provocations are often used to facilitate data interpretation<sup>8</sup>. Provocations allow the user to look at the response to a certain provocation rather than just a basal value of micorcirculatory flow. Commonly used provocations include, 1. heat that indicates maximum dilation, tissue reserve capacity, 2. occlusion that indicates post-occlusive reactive hyperemia, 3. drugs that indicates patch tests, 4. iontophoresis, 5. injections, and 6. posture that reflects veno-arterial reflex, leg elevation etc<sup>8</sup>.

Transcutaneous oxygen (PtcO<sub>2</sub>) is a non-invasive monitoring of the oxygen tension in the skin<sup>5</sup>. The monitoring is done by placing a Clark-type electrode on the skin so that it heats up the skin and provides PtcO<sub>2</sub> values which is a direct indication of the microvascular function<sup>9,10</sup>. As opposed to pressure and volume assessments, PtcO<sub>2</sub> maps the actual oxygen supply available for the skin tissue cells. PtcO<sub>2</sub> also responds to macrocirculatory events, e.g. change in blood pressure and provocation maneuvers such as 100% normobaric<sup>5</sup> or hyperbaric oxygen challenge<sup>11,12</sup>. There are numerous reports that have found PtcO<sub>2</sub> as better predictors for outcome of T2DM foot ulcers and the best method for selection of amputation level<sup>3,5,13</sup>.

In this study that was carried out in the diabetic foot clinic of King Abdulaziz Hospital (KAUH); PtcO<sub>2</sub> and LD were assessed on the tip of big toe of all participants. Participants included three groups (51 known T2DM without ulcer, 40 T2DM with ulcer and 30 healthy non-T2DM). Assessment parameters also included SPO<sub>2</sub> and TBI in three groups of participants aiming to find out the prevalence of both mico-macro asymptomatic PAD.

## 2. Research Design and Methods

### 2.1 Patients

A cross sectional case-control study was conducted in the

diabetic foot clinic of the KAUH between June 2010 and May 2011 that aimed to investigate the macro- and micro-circulation in the feet of three groups of participants: 1. A healthy non-T2DM control group (n=30); 2. A group of known T2DM patients who were ulcer-free (n=51); 3. A group of known T2DM patients with evidence of current ulcer on enrollment to the study (n=40). The control group was a convenient random sample that consisted of healthy non-T2DM volunteers of the hospital workers who accepted our invitation to participate in the study. In the other two groups, participants aged 35 years and older, who had Diabetes Mellitus (DM) for at least 5 years and had clinical evidences of Peripheral Arterial Disease patients (PAD) were eligible for enrollment in group ii and iii of this study. Clinical PAD was diagnosed in participants if they had vascular intermittent claudication and had absent feet pulses on palpation by an experienced physician in vascular surgery. The mean age of T2DM patients with ulcer was 59.3 ± 12 years and the diabetes duration was 5 to 10 years. There was evidence of peripheral arterial occlusive disease, defined as a toe/arm blood pressure index 0.58 ± 0.27, in most of the T2DM with ulcer group of the patients. Demographics and clinical characteristics of this study population are summarized in Table 1.

**Table 1.** Characteristics of T2DM patients with ulcers along with T2DM and normal controls

	Normal Control n=30	T2DM without ulcer n=51	T2DM with ulcer n=40
Age (years)	45 ± 10.6	56.4 ± 10.4	59.3 ± 12.0
Male/Female	17/13	32/19	26/14
T2DM duration	-	5-10 years	5-10 years
Follow-up duration	-	-	12-18 months

The diagnosis of diabetes mellitus was first self-reported by patients and then confirmed by physicians using medical records. The patients were asked to refrain from smoking and coffee for at least 2 h before the investigations. PtcO<sub>2</sub>, LD as explained in later sections including peripheral blood pressures were measured in the supine position after an acclimatization period of 20 min. The room temperature was kept between 22 and 24°C.

In the follow up period, five out of the 40 patients in group iii dropped out and the remaining (n=35) were followed up for a period of 12-18 months with the aim to find out the predictive potential of macro- and

microcirculatory parameters (LD, PtcO<sub>2</sub> and TBI) within this group of T2DM-ulcer patients. The patients were subdivided into subgroups based on clinical outcomes. The subgroups included the good outcome patients (n=15) who healed and those with bad outcome means those who either died or remained unhealed (n=20). In order to differentiate further the unhealed (n=10) and dead patients (n=10) were also grouped separately to decipher any change in the microcirculatory status in terms of measured parameters. Results of healed subgroup and that of unhealed/death subgroup were also compared with the result of major group i.e., T2DM-control group.

## 2.2 Informed Consent

All participants provided an informed consent for the project and for the data to be used in subsequent medical research. The study was approved by the KAUH Research Ethical Committee. Study methods, benefits and objectives of this study were explained to all participants.

## 2.3 Measurements of LD, PtcO<sub>2</sub> and Toe Pressure

Microcirculation was assessed in both feet using PeriFlux System 5000 (Perimed, Stockholm, Sweden). For the micro-circulation the LDPM unit, for local tissue oxygenation PtcO<sub>2</sub> unit and for toe pressure, the pressure unit were used. All measurements were made in supine position. LD flowmetry based microcirculation was measured both before and after the heat provocation. Similarly PtcO<sub>2</sub> recording was made before and after 100% normobaric oxygen challenge to the patients and controls. Time allowed for baseline readings and heat or oxygen challenge test were at least 20 and 10 minutes respectively<sup>14</sup>.

Macro-circulation status of all participants was assessed by calculating Toe/Brachial Index (TBI) as a ratio of brachial blood pressure and toe blood pressure. A trained nurse recorded the brachial blood pressure of subjects in a supine posture with a mercury sphygmomanometer, after allowing a resting for 20 minutes. In each subject, the mean of left and right brachial blood pressure was used in the study. The TBI reading below 0.7 was considered diagnostic for PAD<sup>1</sup>. One abnormal reading in one foot was considered a sufficient indicator for PAD's diagnosis.

## 2.4 Statistical Analysis

Statistical analysis was performed using a personal computer. We rejected the null hypothesis based on Analysis of Variance (ANOVA) conducted on data from all three groups, normal control, T2DM control and T2DM with ulcer. Wherever the means of the three populations were not all equal, we conducted a t-Test to test all pair of means to find even if one pair of means was unequal and reported in terms of p value. Before t-Test an F test was conducted to choose whether a t-Test for equal or unequal variance between pairs to be used. All tests were two-tailed and a value of  $P < 0.05$  were considered to be statistically significant. All parametric data are expressed as mean  $\pm$  standard deviation.

## 3. Results

### 3.1 Comparison among Major Groups (Normal, T2DM controls and T2DM ulcer)

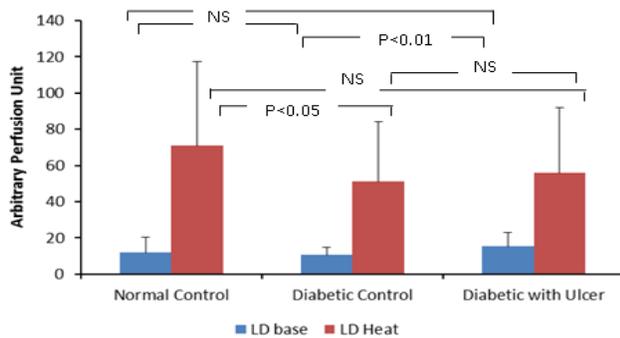
One-way ANOVA determined statistically significant differences between group means for various measured parameters such as LD base, LD heat, TBI and % change in LD and PtcO<sub>2</sub> relative to base values. Absolute PtcO<sub>2</sub> base as well PtcO<sub>2</sub> oxygen values in these three groups were not found to be statistically significant. Table 2. summarizes various measured and calculated parameters in three major groups included in this study.

**Table 2.** Mean  $\pm$  SD and P values (T-test) for three major groups

	Normal Control <i>n</i> =30 Mean (SD)	T2DM without ulcer <i>n</i> =51 Mean (SD)	T2DM with ulcer <i>n</i> =40 Mean (SD)
LD base	11.9 (8.6)	10.6 (4.1)	15.2 (7.7)**
LD heat	71 (46.2)	51.0 (33.0)*	55.9 (36.1)
% increase LD	694.8 (686.6)	398.3 (247.8)*	330.0 (345.6)*
PtcO <sub>2</sub> base	36.5 (21.9)	43 (26.1)	37.1 (19.7)
PtcO <sub>2</sub> Oxygen	73.8 (75.5)	86.9 (46.0)	101.7 (71.4)
% increase in PtcO <sub>2</sub>	58.0 (110.2)	183.3 (362.9)*	272.4 (621.3)*
TBI	0.87 (0.21)	0.80 (0.22)	0.58 (0.27)***
SPO <sub>2</sub>	98.4 (3.9)	98.8 (1.5)	97.3 (7.6)

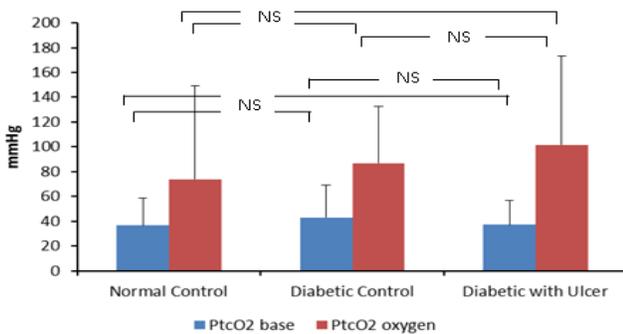
\*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$

Figure 1 illustrates the mean LD base and LD value after heat provocation. LD base value was not significantly different between normal controls and T2DM controls ( $p=0.455$ ) or T2DM with ulcer ( $p=0.057$ ) but it differed significantly between T2DM control and T2DM patients with ulcer ( $p=0.0002$ ). LD heat value was significantly different ( $p=0.047$ ) in normal control as compared to T2DM control but as compared to T2DM with ulcers both the normal control ( $p=0.119$ ) and T2DM control ( $p=0.554$ ) did not show any significant difference in their means for LD heat values.



**Figure 1.** LD heat mean value significantly differed only between normal and T2DM control and LD base mean value differed significantly only between T2DM control and T2DM ulcer group.

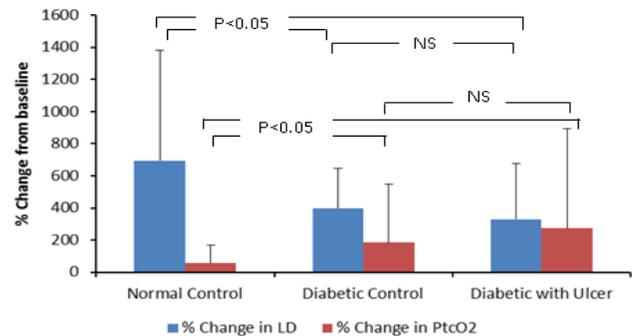
PtcO<sub>2</sub> base value as well as PtcO<sub>2</sub> measured after 100% normobaric oxygen challenge did not differ significantly among all groups as shown in Figure 2.



**Figure 2.** PtcO<sub>2</sub> base mean values as well as PtcO<sub>2</sub> oxygen mean value did not differ significantly among the normal, T2DM control and T2DM ulcer groups.

A % change calculation of perfusion value after heat provocation relative to the base value of LD yielded the results that were significantly different for normal control

when compared either to T2DM control ( $p=0.029$ ) or to T2DM ulcers ( $p=0.011$ ). This % change value from LD base however, did not show any significant difference between T2DM control and T2DM with ulcer groups ( $p=0.275$ ). Similarly a % change in PtcO<sub>2</sub> from base value after breathing 100% oxygen revealed significant difference between normal and T2DM control ( $p=0.025$ ) as well as between normal control and T2DM ulcer group ( $p=0.038$ ). T2DM control and T2DM with ulcer groups did not show any significant % change in PtcO<sub>2</sub> from the base value ( $p=0.424$ ). Both the results of LD % change as well as PtcO<sub>2</sub> % change are presented in Figure 3.

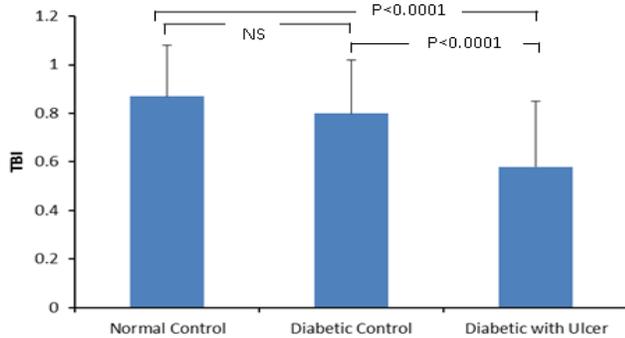


**Figure 3.** A percent change in LD and PtcO<sub>2</sub> values relative to their base values differed significantly in T2DM control as well as in T2DM ulcer groups as compared to normal control group ( $P<0.05$ ).

There was a moderate positive correlations between LD base and LD heat in T2DM control ( $r = 0.55, p < 0.0001$ ) and T2DM with ulcer ( $r = 0.45, p < 0.005$ ) while there was a very weak or no correlation found in normal control for these parameters ( $r = 0.29, p = 0.12$ ). A % change in LD from base value found a strong positive correlation with LD heat values in normal ( $r = 0.71, p < 0.0001$ ) and T2DM control groups ( $r = 0.67, p < 0.0001$ ) as well as in T2DM ulcer group ( $r = 0.78, p < 0.0001$ ). There was a very strong positive relationship between PtcO<sub>2</sub> base and PtcO<sub>2</sub> oxygen in normal control ( $r=0.89, p<0.0001$ ) and also a strong correlation was observed in T2DM control ( $r = 0.67, p < 0.0001$ ) as well as T2DM with ulcer ( $r = 0.69, p < 0.0001$ ). A %change in PtcO<sub>2</sub> from the base value found a very strong positive correlation only in normal controls ( $r = 0.91, p < 0.0001$ ) whereas no correlation was found for these parameters in either T2DM control or T2DM with ulcer groups.

As compared to T2DM with ulcer group TBI was significantly different from normal ( $P<0.0001$ ) and

T2DM control groups ( $P < 0.0001$ ). TBI did not differ significantly between normal and T2DM controls ( $P = 0.314$ ). This result is presented in Figure 4. A moderate positive correlation was found between TBI and PtcO<sub>2</sub> base in T2DM control ( $r = 0.29$ ,  $P = 0.034$ ) and in T2DM ulcer groups ( $r = 0.456$ ,  $P = 0.003$ ).

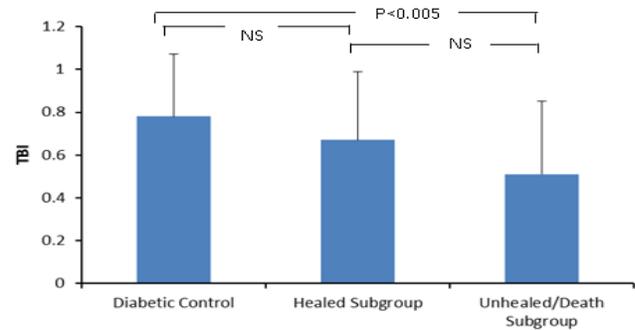


**Figure 4.** TBI mean value in T2DM with ulcer was significantly different as compared to both the normal as well as the T2DM control group ( $P < 0.0001$ ).

A significant relationship ( $r = 0.4$ ,  $P 0.004$ ) was found between PtcO<sub>2</sub> and TBP only in the T2DM patients with ulcers while no significant correlation was seen either in normal or T2DM control groups.

### 3.2 Comparison among Two Subgroups within T2DM Ulcer Group (Healed and Unhealed/Death) and T2DM Control Group

We subdivided the T2DM with ulcer group into two subgroups, the healed subgroup and the unhealed and death cases put together as another subgroup and conducted ANOVA on these two subgroups along with T2DM controls. Except LD base values no other parameters were found to be significantly different and that too only for T2DM control when compared either with healed subgroup ( $P = 0.005$ ) or unhealed/death subgroup ( $P = 0.01$ ). The LD base values could not further significantly differentiate between these two subgroups within T2DM ulcer group ( $P = 0.79$ ). The TBI was still significantly lower in the subgroup comprised of unhealed and death cases ( $P < 0.005$ ) but it was not significantly different in isolated healed cases as compared to T2DM control group as graphically presented in Figure 5.



**Figure 5.** TBI mean value in subgroup of T2DM-ulcer (unhealed and death cases put together) was significantly different as compared to the normal group but did not differ with healed subgroup ( $P < 0.005$ ).

### 3.3 Comparison among Three Subgroups within T2DM Ulcer Group (Healed, Unhealed and Death)

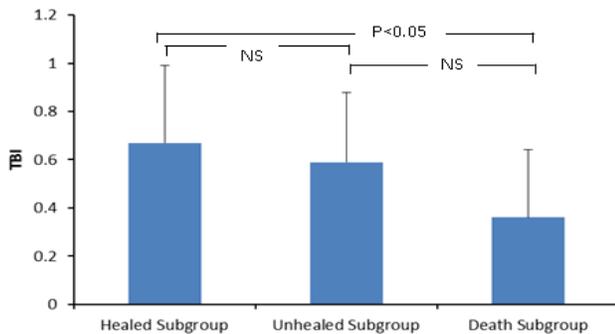
In order to further decipher the effect of ulcer healing or deterioration on measured parameters reflecting macro- and microcirculation we subdivided T2DM ulcer group into three subgroups, namely: 1. Those who died, 2. Those who did not heal and 3. Those who healed. Table 3. Summarizes means and standard deviation values for different parameters.

**Table 3.** Mean  $\pm$  SD and P values (T-test) for three subgroups of T2DM with ulcer groups

	Death Group <i>n</i> =10 Mean (SD)	Healed Group <i>n</i> =15 Mean (SD)	Unhealed Group <i>n</i> =10 Mean (SD)
LD base	14.7 (6.34)	14.27 (7.66)	16.3 (9.04)
LD heat	68.7 (34.81)	37.8 (19.85)*	68.4 (36.34)
% change in LD	403.24 (305.67)	224.40 (265.76)	432.33 (439.93)
TcPO <sub>2</sub> base	40.9 (14.65)	40.4 (19.85)	39.6 (20.86)
TcPO <sub>2</sub>	107.1 (74.05)	105.2 (58.36)	117.2 (88.54)
Oxygen	162.99 (123.42)	171.31 (118.06)	211.75 (203.25)
% change in TcPO <sub>2</sub>	0.36 (0.28)	0.67 (0.32)*	0.59 (0.29)*
TBI	97.8 (1.99)	98.73 (1.71)	99.4 (1.35)
SPO <sub>2</sub>			

\* $P < 0.05$

ANOVA did not show any significant difference among these subgroups for any measured parameters except TBI and LD heat values. All death cases only as a subgroup had significantly lower TBI than subgroup of healed cases ( $P < 0.05$ ) as shown in Figure 6.



**Figure 6.** TBI mean value in T2DM-ulcer subgroup (comprised of only death cases) differed significantly with healed subgroup ( $P < 0.05$ ).

TBI for unhealed group was not significant as compared to both either healed or death sub groups. LD heat was significantly different in healed subgroup as compared to both unhealed and death subgroups ( $P < 0.05$ ). Significantly important LD Flowmetry parameters that differentiate clinically distinct groups are summarized in Table 4.

**Table 4.** Significantly important LD Flowmetry parameters that differentiates clinically distinct groups

Groups of Comparison	LD-base	LD-heat	%change in LD
Major Groups			
T2DM-control vs T2DM-ulcer	√	×	×
T2DM-control vs Normal Control	×	√	√
T2DM-ulcer vs Normal Control	×	×	√
Two Subgroups within T2DM-ulcer			
T2DM-control vs Dead/Unhealed	√	×	×
T2DM-control vs Healed	×	×	√
Three Subgroups within T2DM-ulcer			
Healed vs Dead/Unhealed	×	√	×
Healed vs Dead	×	√	×
Healed vs Unhealed	×	√	×

## 4. Discussion

The % change in LD reading was significantly lower in both the T2DM with ulcer as well as T2DM without ulcer groups that suggest impaired perfusion at the microcirculation level in these groups compared with the normal non-T2DM control group. This effect on perfusion was associated with changes on the tissue oxygenation but in an opposite fashion. The % change observed in  $PtcO_2$  was significantly higher in both the T2DM with ulcer as well as T2DM without ulcer groups compared with the normal non-T2DM control group as shown in Figure 3. This contradictory reciprocation of % change in  $PtcO_2$  and LD results may be attributed to a large number of arterio-venous shunts present in diabetes on the one hand and a loss in vascular reactivity including endothelial functions on the other hand<sup>15</sup>.

Arteriovenous Anastomoses (AVAs) are direct connections like short circuits between arterioles and venules and represent special feature of human skin microcirculation. Our measurement site i.e., the tip of big toe is one of several such apical areas where AVAs are most numerous<sup>16,17</sup>.  $PtcO_2$  values reflect tissue oxygenation or nutritional flow in capillary bed which may be more flooded due to the presence of AVAs. As a result the % change in  $PtcO_2$  in T2DM with or without ulcers is significantly more than normal controls.

The LD values mirrors microcirculation that in turn is affected by microvasculature status including vascular tones and endothelial functions. This method of local heating using LD flowmetry<sup>18-20</sup> actually estimates the vascular reactivity or vasodilator capacity and a part of this response also encompasses the endothelial component. A significantly low % change in LD value in T2DM with or without ulcer as compared to normal controls may be attributed to a compromised vascular reactivity or vasodilator capacity due to hyperglycemia. This is overall impression based on provocative response in terms of % change in LD and  $PtcO_2$  in major groups of patients and subject. Discussions based on absolute values of these parameters and further subdivisions of studied cases are presented below along with an interpretation of estimated TBI values.

### 4.1 $PtcO_2$

While TBI screening confirms PAD in T2DM ulcer groups<sup>1,2</sup> ANOVA shows no difference in  $PtcO_2$  absolute

values (base or after heat provocation) among normal and patients' groups in the present study and therefore seems to rule out the presence of critical limb ischemia or indicate minimal PAD in the study population as per the consensus report<sup>5</sup>. This study records an average  $PtcO_2 > 30$  in all groups of patients and subjects while critical limb ischemia due to rest pain, gangrene or an arterial ulcer measure almost always a  $PtcO_2 < 30$  mm Hg<sup>5,21-24</sup>. One possible reason of this discrepancy could be that, the  $PtcO_2$  recordings in this study were collected from the bottom tip of big toe in order to compare the general microcirculatory status in diseased and normal population and were not from the periwound area of even ulcer patients. This approach was adopted because a low  $PtcO_2$  values especially in periwound tissue may not be exclusively due to critical limb ischemia but may also be caused by local vasoconstriction or lack of angiogenesis, or some other process confined to the wound such as edema, excess consumption caused by inflammation or reversible vasoconstriction caused by pain or dehydration<sup>25,26</sup>. Therefore periwound  $PtcO_2$  measurements cannot be generalized for general microcirculation status of the patients. Moreover, all groups of patients and subjects considered in this study  $SpO_2$  was  $\geq 92\%$ , which also let us assume that arterial hypoxemia was not present<sup>14</sup>. Such an oxygen response indicates that significant macrovascular disease is unlikely<sup>5,27</sup>. The values of  $PtcO_2$  are shown in Table 2. and graphically presented in Figure 2.

The average value for  $PtcO_2$  in healthy subjects measured on the dorsum of the foot while breathing normobaric air is  $>50$  mmHg<sup>27-29</sup> and in general shows an increasing trend from foot to thigh<sup>30</sup>. This trend may account for an average lower value of  $PtcO_2$  recorded on the distal part of foot i.e., tip of the big toe not only in the normal subjects but also in T2DM with or without ulcer in the present study. The absolute  $PtcO_2$  value did not differ significantly between T2DM with or without ulcer and normal groups while breathing both normobaric air or 100% oxygen and therefore is seemingly not an indicative of compromised general tissue oxygenation as per the literature<sup>5</sup>. Only a calculated % increase in the  $PtcO_2$  values from baseline in T2DM control as well as T2DM ulcer cases as compared to normal control were found significant that shows the advantage of a % change calculation in  $PtcO_2$  values over simply the absolute  $PtcO_2$  values after provocative oxygen challenge. No significance was found even in calculated % increase in the  $PtcO_2$

values when it was compared among subgroups of healed, unhealed or death cases within the T2DM ulcer group of patients however.

## 4.2 LD Flowmetry

Assessment of microcirculatory status using laser Doppler Flowmetry among various groups of patients considered in this study was not straight forward. LD-base or LD-heat reading alone was not sufficient to sketch a clear picture among major groups of normal and T2DM controls as well as T2DMs with ulcers. However a % change in perfusion from LD-base value differentiated normal controls from T2DM with or without ulcers. Only LD-base was found to differentiate among T2DM controls with dead/unhealed subgroup of patients, while % change in LD alone indicated a difference between T2DM controls and healed subgroup of patients. Neither LD-base nor % change in LD could differentiate among the three subgroups of T2DM-ulcer viz., dead, unhealed and healed. Based on only LD-heat values these three subgroups could be identified with significant differences. These findings accumulated during the follow-up period suggest that there is significant correlation between the LD-heat and healing of the ulcer. An average decrease in LD-heat by 30 PU was noticed in healed subgroup as compared with dead/unhealed subgroup. This is somewhat unexpected trend observed in healed cases. Though PU and flow is not the same but there is a linear relationship between the two<sup>31,32</sup> and healing microcirculation should mean healthy flow and an increased average LD heat value. However microcirculation domain of the blood flow cannot be oversimplified<sup>33</sup>. The rate of wound closure has been reported to be identical in diabetes and controls subjects despite a significant reduction in maximum hyperemia<sup>34</sup>. Therefore, the development of engineering methodologies enabling noninvasive monitoring of blood vessel activities-such as endothelial function remains a significant and emerging challenge that may explain the observed phenomenon.

## 4.3 Interpretation of TBI

Analogous to Ankle-Brachial Index (ABI), the Toe-Brachial Index (TBI) is calculated by dividing the blood pressure of the great toe by the systolic brachial blood pressure. There are discrepancies in the literature regarding the cut-off values of TBI. Most recent studies

report a cut-off as 0.6<sup>2</sup> and 0.7<sup>1</sup>. Wound healing potential drops as TBI decreases from the normal values. TBI is considered more reliable than ankle pressure in diabetic patients with calcified vessels.

In contrary the TBI readings were lower in the T2DM non-ulcer group compared to the control non-T2DM; however, TBI readings were more significantly lower in the ulcer group compared to the non-ulcer, which may indicate that TBI is more related to the occurrence of ulceration and can be considered as the best predictor of ulceration among the others tests (LD, PtcO<sub>2</sub>, SPO<sub>2</sub>) as it assess the macro-circulation whereas the LD assesses the perfusion at the micro-circulatory level and the PtcO<sub>2</sub> the skin oxygenation.

Table 2. correlates between the various variables and clinical outcomes during the FU period. A significant correlation was observed between the % change in LD and healing of the ulcer. This means that this test may help in predicting a good outcome i.e. ulcer healing. In contrast, TBI predicted the bad outcomes i.e. unhealing or death of patient. This may be explained based on the concept that TBI is assessing the macro-circulation which indicates that patients with low TBI may already have generalized severe atherosclerotic occlusive PAD. TBI of less than 0.36 was correlated to death of patients and to lesser extent to unhealing of ulcers. In contrast, a higher TBI may predict ulcer healing. In view of these results, low TBI recording (less than 0.6) may be considered as a predictor of bad outcomes including unhealing of ulcers or death of patients.

## 5. Conclusion

The results indicate that % change from the baseline of LD as well as PtcO<sub>2</sub> values are equally good indicator of microcirculatory status when it is triggered with heat shock (LD heat) or 100% oxygen tension (PtcO<sub>2</sub> oxygen) in T2DM patients with chronic ulcers and that the TBI provides a trend to better identify a trade off between macro and microcirculation in T2DM patients with ulcer. LD and TcpO<sub>2</sub> measurements may be needed for early screening of skin micro-circulatory disfunctions in patients with diabetes particularly those at high risk of developing a foot ulcer, whereas TBI seems more useful in patients with ulcers to predict healing and support the decision of revascularization in those with low TBI. A TBI of less than 0.36 is associated with higher mortality rates as

observed in this series of patients. This trend may further be established in long-term future studies. It is still not clear if improving the TBI reading by revascularization may reduce the mortality rates in T2DM patients with ulcers.

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