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Comparison of Ultrasound Blood Flow Characteristics of the Lower Limb in Diabetics with Early-Stage Peripheral Artery Disease and Non-Diabetic Controls

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Abstract

The aim of this study was to compare lower limb blood flow in asymptomatic diabetic patients with early-stage peripheral artery disease (PAD) and non-diabetic controls using duplex ultrasound parameters. This was a comparative cohort study of lower limb blood flow in 35 Black-African diabetic patients (*25 females and 10 males* with early-stage PAD *median age 54* [*IQR*, *47 - 61*] *years*; *median* HbA_{1c} *6.3* [*IQR*, *5.7 - 8.0*] *%*; *BMI 29.2 ± 6.7*; *ABI 1.1 ± 0.1*) and 36 non-diabetic controls (28 females and 8 males; median age 54 [IQR, 47 - 61] years; median HbA_{1c} 6.3 [IQR, 5.7 - 8.0] %, BMI 29.2 ± 6.7; ABI 1.1 ± 0.1). Peak systolic velocity (PSV), pulsatility index (PI) and resistive index (RI), were utilised to compare blood flow in the popliteal arteries (PA), anterior tibial arteries (ATA) and posterior tibial arteries (PTA) in addition to ankle brachial index. All the ultrasound parameters showed good ($ICC \geq 0.7$; *0.50 - 0.85*, *95% CI*) to excellent ($ICC = 1.0$; *1.0 - 1.0*, *95% CI*) reliability within groups as well as acceptable variability (<*10% CV*) other than pulsatility index of the anterior tibial artery within diabetic patients (*11.1% CV*). PSV, RI and PI were significantly and meaningfully higher ($P < 0.001$; $d \geq 0.33$), in diabetic patients compared to non-diabetic controls except for PI - PTA ($P = 0.72$; $d = 0.11$). Differences in PSV and RI highlighted the effects of early-stage PAD on lower limb blood flow of diabetic patients. In contrast, the effects of early-stage PAD on blood flow were not demonstrated in the PTA and ATA of diabetic patients by PI.

Keywords

Atherosclerosis, Peak Systolic Velocity, Pulsatility Index, Resistive Index

1. Introduction

The results of prior studies on peripheral arterial disease (PAD) have shown a high prevalence of asymptomatic PAD in the primary health care set up [1] [2] [3]. Therefore, enhanced early detection of PAD in patients at risk for PAD and cardiovascular diseases such as diabetics is essential to enable earlier initiation of treatment to delay the patients' from progressing to late-stage PAD symptoms such as critical limb ischaemia and gangrene.

Prior studies on PAD have shown a high prevalence of asymptomatic PAD in the primary health care set up [1] [3]. Therefore, enhanced early detection of PAD in patients at risk for PAD and cardiovascular diseases such as diabetics is essential to enable earlier initiation of treatment and lifestyle modification to delay the patients' from sliding into late-stage PAD symptoms such as critical limb ischaemia and gangrene.

Current guidelines [4] [5] [6] have recommended ankle brachial index for the screening and quantification of asymptomatic PAD even though this recommendation was for office based or vascular laboratory diagnostic use and not intended to serve as a population screening tool. However, the 2005 guideline recommendation stated the usefulness of Doppler waveform analysis or a combination of ankle brachial index with duplex ultrasound to document the presence and location of PAD in the lower extremity [4]. This investigation therefore aimed to utilise duplex ultrasound parameters to compare blood flow in the lower limbs of Black/African diabetic patients with early stage/asymptomatic PAD with non-diabetic controls while ankle brachial index was used as a parallel test. Any significant difference in blood flow between the two groups was to be interpreted as the detected effects of early stage PAD on the lower limb blood flow of the diabetic patients. The findings of this study could provide an evidence base which could justify the utilisation of duplex ultrasound parameters as a screening tool for early stage PAD through blood flow assessment augmenting the findings of ankle brachial index in the lower limb arteries of Black/African diabetic patients.

The results of previous studies have shown that ultrasound parameters demonstrate high sensitivity (80% - 98%) and specificity (89% - 99%) in detecting late-stage PAD which causes ≥ 50% arterial lumen stenosis in diabetic patients [7] [8] [9]. However, no prior study was done showing utilisation of duplex ultrasound parameters including Peak systolic velocity (PSV), Pulsatility Index (PI), Resistive Index (RI) and Vessel Diameter Inner to inner (VDI) to demonstrate effects of early stage PAD which causes less than 50% luminal stenosis on blood during the writing up of this investigation.

The aim of this investigation was therefore to utilise duplex ultrasound parameters which include peak systolic velocity, pulsatility index and resistive index in comparing blood flow between asymptomatic Black/African Zimbabwean diabetic patients with early stage PAD with non-diabetic controls. The justification for the undertaking of this investigation is that no prior study was done

which showed evidence on the utilisation of duplex ultrasound in the screening or quantification of asymptomatic early stage PAD where ankle brachial index was so far the only recommended diagnostic pathway in the current guidelines [4] [5] [6]. This evidence could be utilised in the formation of a new diagnostic pathway for augmenting the screening and quantification of early-stage PAD in diabetic patients using duplex ultrasound alongside ankle brachial pressure.

A homogenous Black/African sample with early stage PAD was selected for this investigation only as a way to control effect of ethnic variabilities which could have introduced measurement error in the findings. [10] noted that the epidemiology of PAD in various ethnic groups, various lifestyles and diets is not uniform, thus for this investigation, it was important to recruit the required sample from a homogenous population.

2. Methods Experimental Design

A comparative cohort design was used to compare ultrasound blood flow parameters in diabetic patients with early-stage PAD and non-diabetic controls. Demographic data collection and stratification of participants with anonymous codes was undertaken with blinding in-order to minimise selection bias [11] through blinding of the rater during this process.

The ultrasound parameters measurements were performed while blinded to the archived findings of each coded patient and PSV, PI and RI were utilised to compare lower limb arterial blood flow measurements between the two groups in this investigation. Data was gathered over 36 days spanning from the end of May to June 2017, and two patients were booked per day for a scan to allow uncompromised patient care during the gathering of data. The ultrasound measurements were performed thrice per each participant and the mean value was recorded for each parameter in one measurement session and this minimised measurement error.

2.1. Participants

The sample size for this investigation was determined through power calculation for the reliability justification of a diagnostic tool and Schuman's two-sided *t*-test procedure was used to calculate it (Equation (1a) and Equation (1b)).

$$n = \frac{2CV^2 \times \left(Z_\alpha + Z_\beta\right)^2}{d^2} \tag{1a}$$

where;

- Coefficient of variation (CV) = 50%, the median intra-individual variability when ultrasound parameters are measured as reported in the literature.
- $Z_\beta = 0.84$, the standard value for normal distribution at power (β) = 80%.
- $Z_\alpha = 1.96$, the standard value for normal distribution at the level of significance (α) = 5% *i.e.*
- $d = 25\%$, the significant difference in the mean value of ultrasound parameters that we expect between health and diseased subjects.

- Hence $n = \frac{2CV^2 \times \left(Z_\alpha + Z_\beta\right)^2}{d^2} = \frac{2 \times 0.5^2 \times \left(1.96 + 0.84\right)^2}{0.25^2} = 62$ (1b)

A priori sample size was then adjusted for a study dropout of 10% and made the required sample size to be 68 participants (*i.e.*, 34 in the diabetic lower limb arteries group and 34 in the controls) and finally the recruited sample size for the study was 71 and all the hypothesis tests were evaluated at 5% level of significance ($P \leq 0.05$).

The participants were 35 Black-African diabetic patients (*25 females and 10 males*) with early-stage PAD *median age 54* (*IQR*, *47 - 61*) *years*; *median* HbA_{1c} *6.3* (*IQR*, *5.7 - 8.0*) *%*; *mean BMI 29.2* (*±6.7*); *mean ABI 1.1* (*±0.1*) and the controls were 36 non-diabetic participants (28 females and 8 males); median age 54 (IQR, 47 - 61) years; median HbA_{1c} 6.3 (IQR, 5.7 - 8.0) %, mean BMI 29.2 (±6.7); mean ABI 1.1 (±0.1).

Both groups of participants for this were drawn from the same Zimbabwean Black/African population which was easily accessible during the gathering of data and there is a noted higher incidence of diabetes and its complications in this type of population [12]. A homogenous Black/African population enabled the eradication of the potential counteracting variables which could have emanated from a sample of participants from different ethnic groups. The sample for diabetic patients was drawn from Black/African diabetic patients attending the diabetic clinic at Mpilo Central Hospital while the control group was recruited from the staff and students of the National University of Science and Technology in the city of Bulawayo, Zimbabwe through convenience sampling as well. The control for the age limit for the recruited adult participants was put at 18 - 70 years, since the consenting age for adults in Zimbabwe is 18 years and also the fact that type 2 diabetes usually starts manifesting from adolescence onwards [13] [14]. However, the age of the recruited participants was limited up to 70 years since there is evidence that there is an increased risk of late-stage PAD in subjects of 70 years and above [15] [16] [17] such that the probability of getting a sample of participants with early-stage PAD in this category is low.

A measure was put in place to exclude pregnant individuals were excluded because prior evidence has shown that blood pressure decreases while systemic blood flow increases as a result of systemic vasodilation in pregnancy [18]. Therefore, all the participants of childbearing age who were unsure of their last menstrual dates were excluded from this investigation.

Smokers and ex-smokers were also excluded as there is a strong correlation between tobacco smoking and PAD [15] [16] [17]. Therefore, it would not have been possible to get a representative sample of diabetic subjects with early-stage PAD amongst smokers and ex-smokers [15] [16] [17]. This was done to reduce bias from the misclassification of exposure and outcomes which would have emanated if participants of different stages of PAD were to be recruited.

As each of the asymptomatic diabetic patients entered the physician's room for their usual consultation, they had reactive hyperaemic test taken and all their

forms were indicated if they were eligible to be categorised as having early-stage PAD. All participants with indicated forms had their right leg recruited into the sample for the sake of consistency thus thirty-five legs were recruited.

Accordingly, the sample for the non-diabetic control group was feasibly recruited from the National University of Science and Technology volunteering staff and students through a mass advert which was sent to the mass email for the University staff and students. Volunteering staff and students who responded to the advert were recruited until a predetermined sample size of 36-non-diabetic controls was achieved.

The Qdiabetes risk calculator was utilised to screen for the non-diabetic control group participants. Recruitment for participation in this control group was similarly voluntary just as was outlined for the diabetic participants and this was stressed in the advert which was a flight on the mass students' and staff members' website.

Good contact was maintained with participants through text messages and telephoning reminding them of their appointment booking as well as their dietary preparations before blood flow measurements.

The information sheets highlighted that all the diabetic patients who did not qualify to be categorised as having early-stage PAD were no longer eligible to participate in the investigation but were left to continue their treatment with the physician in the diabetic clinic. All the participants in both groups provided written informed consent for participation while the Medical Research Council of Zimbabwe and the Salford University Ethics board approved the study.

2.2. Participants Preparation

Demographic data such as the socio-medical history of participants was blindly gathered using a validated Qdiabetes risk calculator in a bid to minimise recall bias. All the participants were instructed to adopt a low nitrate vegetable diet and no meat or fish for three days before testing and they were told to fast six to twelve hours before the examination.

The participants were again instructed to avoid alcohol at least forty-eight hours before the examination which was booked at eight o'clock in the morning and they were advised not to take their prescribed diabetic and high blood pressure medications but to bring them on their appointment day. The justification for abscorndment of morning medication was because the patients had been made to fast 6 hours before undertaking blood flow and blood pressure measurements, thus they had a high chance of sliding into hypoglycaemia.

These preparation measures were put in place in-order to minimise the effects of a nitrate-rich diet, a recent meal, alcohol and medication on the basal blood flow of the participants before the undertaking of blood flow measurements to reduce measurement error. To check on compliance to prior preparation instructions the participants were instructed to diarise all the foods they had eaten three days before the undertaking of measurements and this enabled the re-

booking of participants who had failed to comply with prior dietary preparations. All the listed control settings enabled minimisation of measurement error since the participants needed to have a constant basal blood flow which was not influenced by the external factors which were controlled above. These measures then allowed the effects of early-stage PAD on blood flow to be assessed with the reduced measurement error.

The participants were given a refreshment of 100% fruit juice and a low sugar biscuit after completing the measurements and then they were allowed to take their prescribed diabetic and blood pressure reducing medications. The participants were observed for about 20 minutes before being dismissed to go home.

2.3. Data Collection Procedures

Ankle brachial index measurements were done on day 5 together with the ultrasound measurements soon after the undertaking of blood tests in the laboratory which was located upstairs from the ultrasound private rooms. Blood tests to determine glycaemic control and renal function were important to establish the general health of the participants and were utilised as demographic markers confirming early-stage PAD which had already been confirmed through prior reactive hyperaemic testing. All the recruited participants were escorted from the laboratory to the ultrasound room downstairs on day five. In the ultrasound room, the participants were assessed in quiet, calm conditions at standard room temperature of about 23˚C - 25˚C by a thermometer.

In the Ultrasound room, Body Mass Index (BMI) for the participants was calculated to enable documentation of their health status by measuring the ratio between their mass and height (Kg/m^2). Weight and height of the participants was measured and then stratified with the participants' anonymised codes on Microsoft Excel sheets. Body mass index was calculated for the recorded weight and height of each patient and stratified with the anonymised codes using Equation (2) as follows;

$$\text{Body Mass Index} = \frac{\text{weight}}{\text{height}^2} \tag{2}$$

Ankle brachial index was performed as a parallel test to the Doppler ultrasound parameters measurements and the upper arms and ankles blood pressure measurements were taken at a similar site on each participant. The automated blood pressure machine (*CareVue*, *Shenzhen*, *China*) was calibrated before being utilised in the measurements for ankle brachial Index and blood pressure.

Ankle brachial index was performed on the participants after a supine rest of about 10 minutes and the highest ipsilateral ankle pressure was recorded and was subsequently divided by the highest ipsilateral upper arm pressure. Ankle Brachial Index were calculated for each participant and then collated in Microsoft excel sheets with anonymised identification codes.

Reactive hyperaemic testing was performed on the right leg of each of the patients for the sake of consistency. In this study the classification for PAD grade

zero for the asymptomatic subjects [19] [20] was utilised in recruiting early stage PAD patients from a large central hospital's diabetic clinic and these included participants who did not illicit a decrease/or elicited a small decrease in ankle blood pressure after a period of about 4 - 5 minutes' thigh blood flow occlusion with a pneumatic cuff (reactive hyperaemia) from the basal ankle blood pressure at rest, since small plaque lesions of early stage PAD will not yet be showing significant haemodynamic effects [19] [20]. Accordingly, Several prior studies have noted that with moderate exercise, normal subjects maintain a stable ankle pressure or show a slight increase [21] [22] and this was partly owed due to the presence of normal bioavailability levels of nitric oxide and other vasodilatory metabolites in the endothelium of their arteries which affords adequate vasodilation to enable compensatory increase in blood flow that occurs after this short period of tissue ischaemia, [21] [22], while this response is blunted in patients with cardiovascular risk factors like hypertension and diabetes, partly owed due to the presence of endothelial dysfunction in their arteries which may result in reduced bioavailability of nitric oxide and other vasodilatory metabolites [21] [22]. The classification for asymptomatic grade zero or early-stage PAD [19] [20] was utilised during the recruitment of diabetic patients. The patients who did not illicit a decrease or who elicited a small decrease in ankle blood pressure at rest following reactive hyperaemia tests were included. All the diabetic patients who did not qualify to be categorised as having early-stage/asymptomatic grade zero PAD no longer eligible for this investigation but were left to continue with their care with the physician in the diabetic clinic.

Duplex Ultrasound Measurements

The quality control tests which were undertaken for the ultrasound scanner included internal grid assessment for testing lateral and axial resolution for the 7.5 - 10.0 MHz probe of an ultrasound machine (Mindray model Z5, Shenzhen, China). Ultrasound B-mode imaging was performed in the measurements of vessel diameter inner to inner followed by Doppler ultrasound parameters measurements for peak systolic velocity (PSV) and end diastolic velocity (EDV) which enabled automatic calculation of resistive index (RI) and pulsatility index (PI).

The linear array probe utilised has short wavelengths and high frequency which enabled high-resolution images during the scanning of superficial structures in this case blood vessels [23] [24]. This justification was deducted from the wave equation [23] [24] which relates wavelength to the speed and frequency of the ultrasound wave as shown in Equation (3);

$$\text{ultrasound beam wavelength} = \frac{\text{Speed of sound in soft tissue}}{\text{ultrasound beam frequency}} \tag{3}$$

Deducting from the equation it can be seen that the wavelength of the ultrasound beam is directly proportional to the speed of sound in soft tissue but inversely proportional to the frequency of the ultrasound beam.

To ensure consistency the ultrasound parameters measurements were taken by a rater holding more than 5 years of experience in vascular ultrasound scanning in a bid to minimise performance bias. The ultrasound parameters which included PSV, EDV, RI and PI were measured from the still image of the spectral Doppler waveforms while the B-mode parameter which included vessel diameter inner to inner was measured from the still image of the longitudinal section of the popliteal arteries, anterior tibial arteries and posterior tibial arteries [25].

In-order to minimise measurement error these measurements were performed three times for each participant and the mean value was recorded.

Ultrasound gel was placed over the linear probe and over each artery for transverse scanning and then rotated 90˚ for the longitudinal scanning to enable the undertaking of Doppler and B-mode measurements [24] [26].

Blood flow was sampled for the popliteal arteries, anterior tibial arteries, the posterior tibial arteries and the dorsalis pedis arteries with B-mode imaging, colour and then Doppler in the longitudinal section. The longitudinal section enabled the manipulation of the ultrasound beam from the probe to be parallel to the blood flowing in the arteries thus enabling manoeuvring for a Doppler angle of less or equal to 60˚ which gives maximum Doppler shifts interpreted as the blood velocity on the Doppler spectral display [23] [24].

The Doppler equation was utilised in the scanning technique since it identified all factors which affected the magnitude of the Doppler shift as follows in Equation (4);

$$FD = \frac{2ftv(\cos\theta)nx}{c} \tag{4}$$

From Equation (3), *FD* refers to Doppler shift frequency (positive in arteries and negative in veins),

1) *2* is a constant and can be ignored,

2) Transmitted frequency (*FT*) is directly proportional to Doppler shift frequency (*FD*).

3) velocity of blood (*v*) is directly proportional Doppler shift frequency (*FD*) and (*C*) speed of sound which is 1.540 m/s and a constant [23] [24].

Deducting the Doppler equation above the Doppler angle (θ) was maintained at less or equal to 60 to enable a cosine value that was high and which was directly proportional to high Doppler shifts which were also directly proportional to high blood velocity.

The colour box was made as small as possible and the sample volume cursor was placed within an arterial lumen to enable recording of more accurate and maximum Doppler shift frequencies [27] [28]. The blood flow velocities were then displayed in the *y-axis* in cm/s against time in seconds in the *x-axis* on the Doppler spectrum.

The pulsed spectral Doppler parameters including PI and RI were calculated automatically after the measurements for PSV and EDV were done on the displayed spectral Doppler waveform in each arterial segment for each participant.

Participants were given some refreshments before being dismissed to go home.

3. Statistical Analyses

Within sessions reliability was determined using intraclass correlation coefficient (ICC) and the associated 95% confidence interval (CI). The ICC values were classified as follows; 1) good = 0.60 - 0.74; 2) very good = 0.75 - 0.89 and 3) excellent ≥ 90 based on the lower-bound 95% confidence intervals [29]. Additionally, to establish the variability of the ultrasound parameters the percentage coefficient of variation (% CV) was calculated, and the acceptable variability was set at an upper limit of less than 10% CV, thus in comparison to previously established % CV values reporting good reliability [29] [30] [31].

Normality of data was determined using Shapiro-Wilk's test. Comparisons between groups for all normally distributed demographic data were conducted using two samples *t*-test and the data was reported as mean (standard deviation [SD]) (Table 1). Additionally, Cohen's *d* effect sizes were calculated to determine the magnitude of any differences between the demographic data for these two groups and it was categorised as *d < 0.20 trivial*; *d = 0.20 - 0.49 small*; *d = 0.50 - 0.80 medium and d > 0.80 large* respectively [32] [33]. Comparison of all non-normal demographic data was done using the two-sample Wilcoxon's rank-sum test and the data was reported as median (interquartile range [IQR]) (Table 1).

High blood pressure was documented as the confounding variable between the two samples data to establish its variability amongst the sample exposed to diabetes and the sample not exposed to diabetes using the Chi-square test (Table 2).

Table 1. Comparison of subject characteristics between 35 (49%) diabetic patients and 36 (51%) non-diabetic patients where Body Mass Index (BMI) and Ankle Brachial Index (ABI) and Estimated Glomerular Filtration Rate eGFR did not show a significant or meaningful difference between groups while a significant difference was observed between groups in age and glycated haemoglobin levels (HbA_{1c}).

Non-normal demographic data				
Parameter	**Control Median (IQR)**	**Diabetic Median (IQR)**	**Two sample t-test p-value**	***T*-test *P*-value**
AGE	37.5 (33 - 54) yrs.	54 (47 - 61) yrs.	0.01	0.01
eGFR	108 (95.5 - 127.5) ml/min/1.73m^2	112 (96.0 - 126.0) ml/min/1.73m^2	0.8	0.8
HbA_{1c}	5.6 (5.1 - 6.0)%	6.3 (6.0 - 8.0)%	0.0	0.0
Normal demographic data				
Parameter	**Mean (sd) non-diabetics**	**Mean (sd) Diabetics**	**Two sample t-test p-value**	**Cohen's d effect sizes**
BMI	29 (7.0)	29.2 (7.0)	0.7	0.1
ABI	1.1 (0.1)	1.1(0.1)	0.8	0.1

Table 2. Relationship between high blood pressure and Diabetic status (confounding variable).

High Blood Pressure	Status		*P*-value
	Non-diabetic	Diabetic	
No	29 (81.0%)	7 (20.0%)	
Yes	7 (19.4%)	28 (80.0%)	<0.001
Total	36	35	

4. Results

4.1. Demographic Findings

In a cohort of 71 Black-African participants, 36 (51%) were non-diabetic controls and 35 (49%) were diabetic participants with early-stage PAD. The median for age was significantly higher in diabetic patients compared to non-diabetic patients (Table 1).

The *means* (*SD*) for Body Mass Index were neither significantly nor meaningfully different between diabetic patients and the non-diabetic controls (Table 1), however, the *median* HbA_{1c} levels were significantly higher in diabetic patients when compared to do the non-diabetic controls (Table 1). There were neither significant nor meaningful differences in Ankle Brachial Index between diabetic patients and non-diabetic controls (Table 1).

4.2. Relationship between High Blood Pressure and Diabetic Status (Confounding Variable)

Table 2 shows that there was a noted significant association between high blood pressure and diabetic participants with early-stage PAD in their lower limb arteries, as reflected by 28 diabetic participants out of 35 had high blood pressure when compared to only 7 non-diabetic participants out of 36 had high blood pressure, ($P < 0.001$).

4.3. The Popliteal Artery Findings

In the popliteal arteries, the means (SD) for peak systolic velocity, resistive index and pulsatility index was significantly and meaningfully higher in diabetic patients compared to non-diabetic controls (Table 3). Again, peak systolic velocity, pulsatility index and resistive index showed very well to excellent reliability within sessions for diabetic patients and non-diabetic controls and the measurements of all the parameters showed acceptably low variability within sessions for both groups (Table 3).

4.4. Anterior Tibial Artery (ATA) Findings

In the anterior tibial arteries, the means (sd) for peak systolic velocity, pulsatility index and the resistive index was significantly and meaningfully higher in diabetic patients compared to non-diabetic patients (Table 4). Again, peak systolic velocity, pulsatility index and resistive index showed good to excellent reliability

Table 3. Descriptive statistics and within sessions reliability of ultrasound parameters in the diabetic and non-diabetic popliteal artery.

Variable	Mean (SD) diabetic patients	Mean (SD) non-diabetic patients	% mean difference	*T*-test *P*-value	Cohen's *d* effect	ICC within sessions of diabetic patients 95% CI	ICC within sessions non-diabetic patients 95% CI	% CV within sessions of diabetic patients	% CV within sessions non-diabetic patients
Peak systolic velocity	73.0 (10.3) cm/s	56.3 (5.3) cm/s	16.2%	<0.001	2.0	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.0)	1.1%	0.3%
Pulsatility index	8.2 (2.3)	9.0(3.0)	0.4%	0.5	0.2	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.0)	9.0%	5.4%
Resistive index	1.1 (0.1)	1.0 (0.1)	6.1%	<0.001	1.0	0.9 (0.7 - 1.0)	0.7 (0.5 - 0.8)	7.0%	2.3%

SD = standard deviation; ICC = intraclass correlation coefficient; % CV = percentage coefficient of variation; %mean difference = percentage mean difference.

Table 4. Descriptive statistics and within sessions reliability of ultrasound parameters in the diabetic and non-diabetic anterior tibial arteries.

Variable	Mean(SD) diabetic patients	Mean (SD) non-diabetic patients	% mean difference	*T*-test *P*-value	Cohen's *d* effect sizes	ICC within sessions diabetic patients 95% CI	ICC within sessions non-diabetic patients 95% CI	% CV within sessions diabetic patients	% CV within sessions non-diabetic patients
Peak systolic velocity	47.0 (9.0) cm/s	40.0 (7.2) cm/s	17.0 %	<0.001	0.8	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.0)	1.7%	0.7%
Pulsatility index	8.0 (2.2)	7.0 (2.0)	12.0%	<0.001	0.4	0.9 (0.8 - 1.0)	1.0 (1.0 - 1.0)	**11.1%**	4.5%
Resistive index	1.1 (0.1)	1.0 (0.1)	6.0%	<0.001	0.6	0.7 (0.5 - 0.8)	0.7 (0.4 - 0.8	5.2%	3.0%

SD = standard deviation; ICC = intraclass correlation coefficient; % CV = percentage coefficient of variation; SDD = smallest detectable difference; SEM = standard error of measurement.

within sessions for all the groups and acceptably low variability was noted in the measurements of all the parameters in both groups except in pulsatility index for the diabetic patients (Table 4).

4.5. Posterior Tibial Artery (PTA) Findings

In the posterior tibial arteries, the means (SD) for peak systolic velocity and the resistive index was significantly and meaningfully higher in diabetic patients compared to non-diabetic patients, other than pulsatility index (Table 5). Again, peak systolic velocity, pulsatility index and resistive index showed good to excellent within sessions reliability in both groups and the variability amongst measurements of all parameters in both groups was acceptable (Table 5).

Table 5. Descriptive statistics and within sessions reliability of ultrasound parameters in the diabetic and non-diabetic posterior tibial artery.

Variable	Mean (SD) diabetic patients	Mean (SD) non-diabetic patients	% Mean Difference	*T*-test *P*-value	Cohen's *d* effect sizes	ICC within sessions of diabetic patients 95% CI	ICC within sessions non-diabetic patients 95% CI	% CV within sessions of diabetic patients	% CV within sessions non-diabetic patients
Peak systolic velocity	44.0 (12.0) cm/s	41.0 (8.0) cm/s	9.0%	0.01	0.3	1.0 (1.0 - 1.0)	1.0 (1.0 - 1.0)	2.0%	0.6%
Pulsatility index	7.0 (5.1)	7.0 (2.0)	3.0%	**0.7**	**0.1**	1.0 (1.0 - 1.0)	1.0 (0.9 - 1.0)	10.0%	4.3%
Resistive index	1.1 (0.2)	1.0 (0.1)	5.1%	<0.001	0.4	0.7 (0.5 - 0.85)	0.8 (0.7 - 0.9)	8.0%	3.0%

SD = standard deviation; ICC = intraclass correlation coefficient; % CV = percentage coefficient of variation; %mean difference = percentage mean difference.

5. Discussion

The aim of this investigation was to compare blood flow between the diabetic and non-diabetic participants' groups as reflected by the Doppler ultrasound parameters consisting of peak systolic velocity, pulsatility index and resistive index in the right popliteal, anterior tibial and posterior tibial arteries. In this study, peak systolic velocity, pulsatility index and resistive index were significantly and meaningfully higher ($P < 0.001$; $d \geq 0.3$), in diabetic patients compared to non-diabetic controls except for pulsatility index of the posterior tibial arteries ($P = 0.7$; $d = 0.1$). All the ultrasound parameters showed good ($ICC \geq 0.7$; *0.5 - 0.9, 95% CI*) to excellent ($ICC \leq 1.0$; *1.0 - 1.0, 95% CI*) within sessions reliability as well as acceptable variability (<*10% CV*) within groups except pulsatility index of the anterior tibial arteries for diabetic patients (*11.1% CV*) (Tables 3-5).

A Significant and meaningful difference in blood flow between the two groups was interpreted as the ability to demonstrate the effects of early-stage PAD on the lower limb blood flow of diabetic patients while the non-significant and non-meaningful difference in blood flow between the two groups was interpreted as an inability to demonstrate the effects of early-stage PAD on the lower limb blood flow of diabetic patients. In this investigation PSV and RI came out as the ultrasound parameters able to detect a difference in blood flow between diabetic patients with early-stage (asymptomatic) PAD and non-diabetic controls. Thus, PSV and RI were concluded as the ones able to demonstrate the effects of early-stage PAD on the lower limb blood flow of diabetic patients with the exclusion of the pulsatility index.

In this investigation, within sessions reliability ranged from good (ICC ≥0.7; 0.5- 0.9, 95% CI) with a % CV of less than 10%. These findings reflected that the ultrasound parameters were repeatable in measuring blood flow in the lower limbs of diabetic patients with early-stage PAD as well as in the non-diabetic

controls.

Peak systolic velocity and resistive index showed a significantly high mean percentage difference (≥5.1%; $P \leq 0.01$) between groups in the popliteal, anterior tibial and posterior tibial arteries. This reflected that these parameters were able to detect a difference in blood flow due to early stage PAD and also that this change in blood flow was of clinical significance and would call for a change in the management of the diabetic patients (**Tables 3-5**).

However, pulsatility index showed a non-significantly low mean percentage difference (≤0.4%; $P \geq 0.5$) between groups in the popliteal and posterior tibial arteries reflecting incapability to show blood flow changes due to PAD between groups except in the anterior tibial arteries (12.0%; $P < 0.001$).

A study by [34] reported that resistive index of the acral finger was significantly higher ($P < 0.001$) in diabetic patients with late-stage (symptomatic) PAD compared to non-diabetic controls. Similarly, their results also reflected that RI in diabetic patients became even higher as the duration of diabetes mellitus increased ($P < 0.01$). [35] also showed that pulsatility index was reduced in late-stage PAD, their findings showed that a pulsatility index of <1.2 indicated critical limb ischaemia with a sensitivity of 0.9 and a specificity of 0.6 while Ankle Brachial Index of less than 0.9 showed a sensitivity of 0.7 and a specificity of 0.4. However, the findings of the study by [35] were diabetic patients with late-stage (symptomatic) PAD and in this scenario peak systolic velocity reduced while end-diastolic velocity was mostly absent due to increasing resistance to blood flow thus resulting in a smaller value of pulsatility index. Therefore, the findings of these prior studies all provided evidence on the utilisation of ultrasound parameters to detect and grade late-stage (symptomatic) PAD.

The findings of this investigation showed different mean values for ultrasound parameters in the lower limb blood flow of Zimbabwean Black/African diabetic patients with early-stage PAD and non-diabetic controls when compared to prior published range values of healthy individuals and diabetic patients with late-stage PAD. In this investigation, the mean value for peak systolic velocity in the popliteal arteries of diabetic patients with early-stage PAD was 72.9 ± 10.3 cm/s while prior evidence [28] [36], has shown a peak systolic velocity of greater than 180 cm/s elicited in arterial stenosis of greater or equal to 50%. This confirmed that the sample participants for this investigation could have been having arterial stenosis of way less than 50% stenosis but the ultrasound parameters still managed to demonstrate the effects of early-stage PAD in the blood flow of these diabetic patients. Therefore, this evidence justifies the need to utilise duplex ultrasound parameters to enhance screening and quantification of early-stage PAD in asymptomatic diabetic patients alongside the prior recommended Ankle Brachial Index.

In this investigation, the mean value for peak systolic velocity in the popliteal arteries of non-diabetic controls was lower (55.3 ± 3 cm/s) compared to prior published mean normal value for other healthy populations (68 ± 1 cm/s). These different values for blood flow shown by peak systolic velocity maybe be due to

different ethnicities, lifestyles or diets of the sample populations of participants in the different studies [37]. In their study, [37] it was noted that the epidemiology of PAD was not homogenous amongst different ethnic populations with diverse lifestyles and diets.

Prior evidence [36] [38] has shown the normal ranges for the peak systolic velocity of tibial arteries in healthy individuals as 55 ± 1 cm/s while this investigation finding in non-diabetic controls showed a lower mean peak systolic velocity of the anterior tibial arteries (40.0 ± 7.2 cm/s) and the mean peak systolic velocity for the posterior tibial arteries was lower as well (40.6 ± 8.0 cm/s).

The pulsatility index for the popliteal arteries in healthy individuals in prior studies was shown as greater than 8 [36] [38] while in this investigation the mean pulsatility index value for the non-diabetic controls was lower and shown as (6.9 ± 2.1).

The principal investigator utilised Ankle-Brachial index as a complementary parallel test alongside the ultrasound protocol parameters to detect early-stage PAD in asymptomatic diabetic patients [4] [6] [9] [39]. The test has been recommended in practice guidelines to screen and quantify early stage (symptomatic) PAD in prior studies undertaken in other populations, and Ankle Brachial Index values less or equal to 0.9 reflect PAD in the lower limb arteries [4] [6] [9] [39].

In this investigation, there was no significant difference, ($P > 0.05$) in the values for Ankle Brachial Index between the groups thus Ankle Brachial Index was a weaker test in demonstrating early stage (asymptomatic) PAD in diabetic patients while ultrasound parameters including peak systolic velocity and resistive index demonstrated the effects of early-stage PAD in the blood flow of participants with Ankle Brachial Index values greater than 0.9 (**Table 1** and **Tables 3-5**).

Late-stage PAD results in pressure reduction in the ankle arteries while the upper arm arteries will still be yet unaffected, thus resulting in a lower value for Ankle Brachial Index in such patients. However, the case is not true in early-stage PAD because pressure reduction will not yet be taking place in the ankle arteries, thus when they get divided by the unaffected upper arms pressure the result would reflect normal values of ankle brachial index of around 1.

In this investigation, the noted confounding variable was high blood pressure and findings showed a positive correlative relationship between diabetes and medical history of high blood pressure.

The strength of this investigation was that it was carried out under controlled settings which limited performance, recall, selection and misclassification of exposure and outcomes bias as well as measurement error. The ultrasound parameters measurements of blood flow between the two groups were in comparable conditions when the measurements were taken, such that the lapse period of one month over which the data was gathered did not matter even if it could have allowed the accumulation of a thicker plaque than initial values at the beginning of the month since the investigation was not aimed at investigating any changes in

blood flow with time.

Since PSV and RI were capable of demonstrating the effects of early stage PAD on the blood flow of asymptomatic diabetic patients, the future work after this investigation utilised PSV and RI in the third investigation which aimed to establish any detectable blood flow effects in the popliteal artery following beetroot juice ingestion. Therefore, according to the findings of this investigation, PSV and RI could be utilised in augmenting ankle brachial index in screening and quantification of the effects of early-stage PAD on the blood flow of asymptomatic diabetic patients with an Ankle Brachial Index value of greater than 0.90.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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