Can plantar soft tissue mechanics enhance prognosis of diabetic foot ulcer?

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Abstract

Aim: To investigate if the parameters describing the mechanical properties of plantar soft tissue can increase the accuracy of predicting Diabetic Foot Ulceration (DFU).

Methods:

40 patients with diabetic neuropathy and no DFU were recruited. Commonly assessed clinical parameters along with plantar soft tissue stiffness and thickness were measured at baseline using ultrasound elastography technique. 7 patients developed foot ulceration during a 12 months follow-up. Logistic regression was used to identify parameters that contribute to predicting the DFU incidence. The effect of using parameters related to the mechanical behaviour of plantar soft tissue on the specificity, sensitivity, prediction strength and accuracy of predicting models for DFU were assessed.

Results

Patients with higher plantar soft tissue thickness and lower stiffness at the 1st Metatarsal head area showed an increased risk of DFU. Adding plantar soft tissue stiffness and thickness to the model improved its specificity (by 3%), sensitivity (by 14%), prediction accuracy (by 5%) and prognosis strength (by 1%). The model containing all predictors was able to effectively (χ^2 (8, N=40)=17.55,P<0.05) distinguish between the patients with and without DFU incidence.

Conclusion

The mechanical properties of plantar soft tissue can be used to improve the predictability of DFU in moderate/high risk patients.

Key words: Stratification; Risk Factors; Prospective studies, Ultrasonography, Diabetic Neuropathies, Diabetes Complications,

1. Introduction:

Diabetes mellitus (type 2) is the most frequent cause of non-traumatic lower-limb amputations [1] . With around one million amputations each year, this indicates a lost leg due to diabetes somewhere in the world every 20 seconds [2]. Up to 80% of these amputations could have been prevented with correct clinical management [3] and the issues relating to the diabetic foot disease presents a significant burden for health systems around the world.

Foot ulcers in people with Diabetes are multi-factorial and linked to a variety of risk factors like peripheral neuropathy, vascular insufficiency and physiological measures [4]. Whilst, some of the epidemiological studies demonstrate that the indicators of neuropathy like impaired sensation are predictors of ulceration [4–6], other studies show that peripheral vascular disease indicated by Ankle Brachial Index (ABI) [7,8], Blood biochemical measures like glycated haemoglobin level (HbA_{1c}) [5,8], total cholesterol [9], fasting blood sugar [10] to be among the risk factors for diabetic foot ulceration. Furthermore other readily identifiable parameters like age [7] and duration of diabetes [5] height [11], body weight [12] and Body Mass Index (BMI) [13] have been associated with ulceration risk in patients with diabetic neuropathy. Visual acuity (poor vision) score [10] and VPT score [12] (measure of neuropathy level) were reported to be associated with DFU.

A previous systematic review of risk stratification systems for diabetic foot ulceration identified: a) foot deformity, b) peripheral neuropathy (Vibration Perception Threshold -VPT or cutaneous insensitivity to monofilament), c) peripheral vascular disease (pulses and/or ABI), d) and previous amputation, e) the presence of callus, f) HbA1c, g) Tinea pedis, and h) onychomycosis as prognostic factors that are commonly used predicting the risk of ulceration [14]. Recently another systematic review and meta-analysis , reported that insensitivity to a 10-g monofilament or one absent pedal pulse will identify patients with moderate or intermediate risk of foot ulceration [15]. On the other hand in the same study Crawford and

co-workers [15] the history of foot ulcers or lower-extremity amputation were to be sufficient to identify those at high risk [15].

While the abovementioned parameters have been recognised as the common predictive risk factors for DFU, it has been established that the majority of the injuries to the foot happens as a result of mechanical trauma that the patient does not recognise due to neuropathy [16]. Hence peak plantar pressure [12] and a number of biomechanical parameters that elevate plantar pressure like limited joint mobility [10], forefoot deformities like hammer/claw toes, bony prominences, or Charcot feet [17], have been investigated as possible risk factors for DFU.

However, another parameter that could also increase the risk for mechanical trauma in the tissue, other than increased loading, is the detrimental changes in the mechanical properties of plantar soft tissue. These mechanical properties of the plantar soft tissue include the stiffness that quantifies the extent to which the tissue can resist deformation in response to an externally applied force. Previous studies have indicated that the mechanical properties of the plantar soft tissue change during the course of the disease but the causes of these changes as well as their possible implications are not yet understood. To be specific, studies comparing age matched populations of people with diabetes and people with no diabetes have shown that the plantar soft tissue in people with diabetes tends to be thicker [18], stiffer [19,20], harder [21] and also tends to have less energy return efficiency [22].

A possible mechanism for the abovementioned differences could be histological changes inside the tissues as a result of glycation [23]. This is also indirectly supported by the reported significantly higher heel pad stiffness in people with higher levels of Fasting Blood Sugar [24]. Another mechanism for altered tissue properties is the accumulation of internal tissue damage as a result of repetitive excessive loading of the plantar soft tissue due to impaired sensation.

While altered stiffness can potentially reduce the capacity of the plantar soft tissue to uniformly distribute loads, the changes in the internal structure and properties can also reduce its

mechanical strength. These changes in mechanical strength, that is the magnitude of mechanical stress the tissue can carry without sustaining damage, can make the plantar soft tissue more susceptible to trauma even without any change in plantar pressure.

From an engineering point of view, one would expect that a tissue, which is more vulnerable to trauma to exhibit a different mechanical behaviour when compared to tissues that are less vulnerable. However, the possible link between plantar soft tissue properties and the incidence of diabetic foot ulcers has not yet been investigated.

Investigating this requires reliable, safe and easy to use techniques that can be utilised in the clinic to measure the mechanical properties of the plantar soft tissue. To address this challenge, a testing protocol for using real time strain ultrasound elastography was recently developed and tested in the clinic [25]. This study by Naemi and co-workers [25] indicated that compared to non-ulcerated group, the ulcerated group had a significantly lower heel pad stiffness [25].

Although the results reported by Naemi and co-workers [25] can indicate a possible link between tissue mechanics and ulceration, the study design did not allow the researchers to recognise whether the observed differences are due to the physiological changes that contribute to ulceration or whether it reflects the pathophysiological changes that happen after ulceration. This could be due to the altered loading. or other pathophysiological phenomena [25].

Furthermore, the potential of using the parameters related to the mechanical properties of plantar soft tissue in identifying the risk of developing diabetic foot ulceration was never explored in patients with diabetic neuropathy.

Therefore, the purpose of the current study was to investigate if the parameters describing the mechanical properties of plantar soft tissue are associated with ulceration incidence and to examine if these parameters can be used to increase the prognosis accuracy for identifying patients at risk of diabetic foot ulceration.

2. Materials and Methods

2.1. Participants

A prospective study was conducted on 40 (M/F: 30/10) patients with diabetic neuropathy and with no current ulceration or severe foot shape abnormality i.e. (Charcot and Hammer toe) who attended the foot clinic at a diabetes hospital in South India (AR Diabetes Hospital, Chennai, India) in June 2015. Ethical approval was sought and granted by the institutional ethics committee and all volunteers provided full informed consent.

The inclusion criteria were: (1) Vibration Perception Threshold (VPT) of more than 25 V, (2) age 18 – 80 years, (3) at least one palpable pedal pulse on each foot. The exclusion criteria included current ulceration, previous amputation or active foot infection. The recruited group had average age of 64.1 ± 9.4 years, duration of diabetes 18.1 ± 9.7 years, height 1.66 ± 0.95 m, body mass 72.9 ± 14.9 Kg, and Body Mass Index (BMI) 26.2 ± 4.3 Kg/m^2 (Table 1).

Out of 40 recruited participants, six had a history of ulceration (Table 2). All patients at the point of recruitment to the study were advised by the clinician to use a standard sandal with a standard flat insole made of soft Microcellular Polymer. The recruited patients were followed up for a year after baseline data collection using routine clinical appointments.

2.2. *Data collection*

At baseline measurement, blood biochemical parameters were gathered from sample blood analysis on the day and included the Glycated Haemoglobin (HbA1c), Post Prandial Blood Sugar (PPBS), Fasting Blood Sugar (FBS), Cholesterol and Triglycerides, and the lipid profile of the participants were collected. The VPT, ABI measurements and soft tissue mechanics assessment were completed in a single session which took approximately 45 minutes per participant to complete.

All data were collected with participants laying on a couch in a supine position. VPT was measured at the hallux, 1st metatarsal head and the heel using a Biothesiometer (Kody Medical Electronics Private Ltd, Chennai, India). It needs to be emphasised that the range of VPT measurement reported with this device is up to 80 Volts. ABI was measured to quantify vascular sufficiency.

The plantar soft tissue was assessed at the sub calcaneal and underneath the 1st metatarsal head using ultrasound strain elastography (Esaote S.p.A., IT). According to the method proposed by Naemi et al (2016) [25], strain elastography (Esaote S.p.A., IT) was performed using a linear ultrasound probe (LA533, 13 MHz, Footprint: 53x11 mm) and a stand-off (Sonokit, Sonogel, Vertriebs, Gmbh) [25].

Manually applying low amplitude cyclic loading offers a qualitative assessment of the relative deformability of all imaged tissues/ materials [26]. The use of a stand-off material as reference enables a quantitative assessment of relevant stiffness for intra- and inter subject comparisons [25]. Considering the practicalities of the ultrasound elastography technique and informed by the methodology that was developed in our previous study, only the plantar soft tissues underneath the calcaneus and 1st Metatarsal head were assessed [25]. During strain elastography assessment, the soft tissue was compressed between the bony prominence and the standoff material that cover the probe head. With the available probe size, this could only be reliably implemented over bony prominence with adequate size i.e. Calcaneus and 1st Met head [25] .

2.3. Sample size calculation

In a previous study the average and standard deviation of the relative stiffness (Et) of plantar soft tissue at the 1st Metatarsal head area of five participants were calculated as 1.75 ± 0.34 [25]. Using this value and with an aim of detecting a 20% difference between the two groups

(those who would ulcerate and those who won't) with Alpha=0.05, Power=0.80, and considering the ratio of ulcerated over non-ulcerated patients' numbers in the clinic, a minimum sample size of 29 patients who would not ulcerate vs 6 patients who would ulcerate were required. With a possible attrition rate of 12 percent a total 40 participants were recruited to the study.

2.4. Follow up and ulceration incidence:

At the end of twelve months follow-up seven patients (6 male and 1 Female) developed a foot ulcer. Out of these, two patients had ulcers on the left foot and five patients on the right foot with the site of ulceration being: five on the forefoot and two on the rear foot. A foot ulcer was defined as a full-thickness wound as a result of localised injury to the skin and/or underlying tissue, below the ankle. The characteristics of the two groups of participants with and without ulceration incidence are shown in Table 1 and Table 2.

2.5. Statistical analyses:

All statistics were performed using IBM SPSS version 22.0 (Chicago, IL, USA). Normality test was performed on all continuous parameters. Descriptive statistics were reported for all parameters where, based on the results of normality test, a Mean and Standard deviation for normally distributed parameters (Kolmogorov-Smirnov test with $P >= 0.05$) and or Median for non-normally distributed parameters (Kolmogorov-Smirnov test with $P < 0.05$) were presented.

Furthermore parametric test of difference (Independent sample T-test) was performed for the normally distributed parameters where $P<0.05$ values (2-tailed) was considered as significant and Eta-squared was calculated as the effect size.

Non-parametric test of difference (Mann Whitney U-test) was performed for the non-normally distributed parameters where $P<0.05$ values (2-tailed) was considered as significant and *r* was calculated as the effect size. The association in categorical parameters were assessed using the Chi-square test of independence with Yates continuity correction and $P <0.05$ indicating significant association between the ulcerated and the non-ulcerated group. To assess the

strength of difference, effect size was calculated and reported as Phi coefficient. To select the covariates that contribute to predicting the risk of ulceration a univariate logistic regression model was fitted for each of the measured parameters including both the continuous and categorical parameters. From these parameters, those that had P values of less than 0.2 (Wald test) were included as covariates in multivariable logistic regression [27].

The covariate parameters that met the criterion of P<0.2 were included in logistics regression analysis, where covariates were added in consecutive blocks and the sensitivity (as the percentage of participants with ulceration incidence that are predicted correctly) and specificity (as the percentage on of participants with no ulceration incidence that are predicted correctly) along with the overall prediction accuracy (as the percentage of the entire cases that are predicted correctly) of the method were reported. Furthermore, the classification of accuracy of the final model, using the area under the receiver operating characteristic (ROC) curve with 95% confidence Ievel were calculated and referred to as the prognosis accuracy of the model.

3. Results:

3.1. Differences and potential for differentiation

The test of differences in continuous parameters between the group of patients who would ulcerate and those would not revealed significant ($p<0.05$) differences only in two parameters including height and Vibration Perception Threshold at the 1st metatarsal head area (Table 1).The continuous parameters from each group are highlighted in Table 1.

Among the continuous parameters, the results of univariate logistic regression indicated that five parameters showed to have P values (Wald test) of less than 0.2 (Table 1). These parameters were: 1) duration of diabetes, 2) VPT score at the 1st metatarsal head, 3) normalised tissue thickness 1st metatarsal head, 4) Tissue stiffness to thickness at the plantar

1st metatarsal area, and 5) Tissue stiffness to normalised tissue thickness at the plantar 1st metatarsal head area

Insert Table 1 here:

None of the categorical parameters showed to be significantly different between the group of patients who would ulcerate and those would not ($p>0.05$ for Chi square test of independence) (Table 2).

Insert Table 2 here.

Among the categorical parameters, history of ulcer, history of callus, insulin and OHA use, showed a P value (Wald test) less than 0.2 in Univariate regression analysis (Table 2). These three categorical parameters along the five continuous parameters mentioned above were used in multiple regression analyses according to the following.

3.2. Predictor model

Direct logistic regression indicated that the full model containing all eight predictors (3 categorical and 5 continuous) predictors was statistically significant χ^2 (8, N=40) = 17.55, P<0.05. Indicating that the model is able to distinguish the patients who would ulcerate within the next twelve months. The risk of ulceration was defined by a Score = 1.542 × (Presence of previous foot ulceration) + 2.632 × (Presence of previous Callus) + 0.444 × (the treatment regime code as OHA:1, Insulin: 2 and OHA and Insulin: 3) - 0.105 × (Duration of Diabetes) + + 0.124 × (VPT score at the 1st Met head) + 1.511 × (Normalised tissue Thickness at the 1st

Met) - 9.929 × (Tissue stiffness to tissue thickness at the 1st Met) + 5.956 × (Tissue stiffness to normalised tissue thickness at the 1st Met) – 9.266.

Figure 1 shows the specificity and sensitivity of the model in consecutive stages when different parameters were added to the model.

Insert Figure 1 here.

In block 1, while the duration of diabetes on its own did not predict any incidence of ulceration (0 out 7 incidence indicating sensitivity of 0%), the prognosis strength (the area under ROC) was 69.4% (Figure 1).

In block 2, by adding the history of ulcer the model showed to be able to predict three (out of 7) ulcerated cases (42.9 % sensitivity), while 32 (out of 33) non ulcerated cases were predicted correctly (97% specificity). The model at this stage has a prognosis strength (the area below ROC) of 77.5% (Figure 1). By adding the history of callus to the model in Block 3, the sensitivity dropped to 28.6% (only 2 out of 7 ulcerated cases were predicted), while the specificity was also decreased to 90.9% (30 out of 33 non-ulcerated cases were predicted correctly). In this case the prognosis strength (the area below ROC) was 80.1% (Figure 1).

In Block 4 in which the model includes: (A) Duration of Diabetes, (B) History of ulceration, (C) History of Callus, (D) Treatment code, the model has sensitivity and specificity of 57.1% and 97.0% respectively. The prognosis strength (the area below ROC) was 85.5% (Figure 1).

While this can indicate that only four ulcerations (out of total 7) in this cohort can be predicted based on the patients history and their treatment, in the consecutive blocks it is shown that the sensitivity of the model could be improved using foot specific data like the VPT score and plantar soft tissue parameters (Figure 1).

To achieve this in the next step (Block 5), when VPT score was added to the model the sensitivity did not change from 57.1 %, while the specificity was decreased to 93.9%. Furthermore this did not result in an enhanced prognosis strength of the model as the area under ROC stayed practically the same (i.e. nominal drop of 0.1% to 88.4%) (Figure 1).

It needs to be noted that the VPT in the 1st metatarsal head showed to be the only parameter which showed to be significantly ($P<0.05$) different between the two groups of DFU incidence and no DFU incidence with *r* value of 0.35 indicating a medium effect size. The odds ratios (Exp(B)) shows the changes in odds in being in one of the categories when the value of predictor increases by one unit. For this parameter it was found that when the VPT of the 1st metatarsal head increased by one Volt (and if every other parameter stays the same) the likelihood of that person developing a DFU within the next twelve months increases by 13% (i.e. EP(b)= 1.13).

In the next step (Block 6) by adding the normalised plantar tissue thickness at the 1st metatarsal head, to the model, neither of the sensitivity or specificity changed. While the prediction accuracy of the model did not change (87.5%), the area underneath the ROC was decreased to 0.625 (prognosis strength = 62.5%), indicating a decreased prognosis strength (Figure 1). For the soft tissue mechanical parameters it was found that when the normalised thickness of the sub-metatarsal fat pad increased by one unit (and if every other parameter stays the same) the likelihood of that person developing a DFU within the next twelve months increases by 350% (i.e. EP(b)= 4.5).

In the next step (Block 7), by adding the tissue stiffness to thickness at the 1st metatarsal head to the model, it was observed that the sensitivity of the model stayed the same, while the specificity increased to 97%. This also resulted in prognosis strength as the area below the ROC to increase to 78.1% (Figure 1).

Finally in the next stage (Block 8), the Tissue stiffness to normalised thickness was added to the model. Even though this did not change the specificity of the model, it led to an increase

in its sensitivity to 71.4%, prediction accuracy to 92.5% and the prognosis strength as the area below ROC to 89.7% (Figure 1).

Furthermore it was observed that with one unit increase in the stiffness to normalised tissue thickness at the 1st metatarsal head area (and if every other parameter stays the same) the likelihood of that person ulcerating within the next twelve months increases by 38500% (i.e. $EP_{(b)}$= 386).

The model as a whole could predict between 35.5 % (Cox and Snell R Square) and 58.7% (Nagelkerke R Square) of the variation in ulceration status. With specificity of 97%, and sensitivity of 71%, and the model correctly classified 93% of the cases (i.e. prediction accuracy = 93%).

Furthermore with the prognosis strength as the area under the receiver operating characteristic (ROC) curve was 90%.

4. Discussion:

4.1. Differences and potential for differentiation

Generally with regards to the differences between the group with and without ulceration incidence, only two parameters, namely: height and vibration perception threshold at the 1st metatarsal head area, showed to be significantly different ($P<0.05$). Overall, in terms of the potential for differentiation, three categorical parameters namely: OHA/Insulin use, Previous Ulceration and Previous Callus and five continuous parameters including: Height and Vibration Perception Thresholds along with the Normalised Tissue Thickness at the 1st Met, Stiffness to normalised tissue thickness at the 1st Met and Stiffness to tissue thickness at the 1st Met were found to show potential ($P<0.2$) for differentiation. The interaction of these eight parameters and their role in the multiple logistic regression model is discussed further down under the section 4.2.

The group with ulceration incidence was significantly taller than the group with no ulceration incidence (170 ± 3.4 cm vs 166 ± 10.1 cm) with a small to medium effect size (Eta-square = 0.14), however the results of univariate regression analyses indicated that height could not be used as a predictor of foot ulceration (P >0.2). This is contrary to the results reported by Iversen et al., (2008) [11] where increase in height was associated with a greater ulceration risk in patients with diabetic neuropathy.

The higher VPT in the group with ulceration incidence vs the group with no ulceration incidence (53.5 ± 7 Volt vs44.3 ± 33 Volt) with a medium effect size (r = 0.35), and the test for univariate regression analysis (P = 0.078), indicated that the VPT under the 1st metatarsal head can potentially be used to predict ulceration incidence in patients diabetic neuropathy.

Although the results of the current study contradicts what was reported by Ndip et al., (2010) who found no association between VPT at the Hallux and DFU, our findings are in line with the study by Kastenbauer & Sauseng, (2001) who found VPT at Malleoli to be significantly associated with the DFU development.

Whilst none of the categorical parameters showed to be significantly different between the two groups of participants with and without ulceration incidence, test of univariate regression analysis indicated that the presence of History of Ulcer (P = 0.039), and History of Callus (P = 0.180) can potentially be associated with ulceration incidence indicating that both these parameters can potentially be used to predict DFU. This is in line with the study by Crawford and co-workers [15] who reported a history of foot ulcers to be one of the factors sufficient to identify those at high risk of DFU.

The other categorical parameter that showed potential to differentiate between the groups with and without ulceration incidence, was the OHA/Insulin use indicated by univariate regression analysis (P= 0.137). This indicated that the participants who are on OHA can potentially have a higher risk of developing foot ulceration and this was significantly higher for those who used insulin. The results of this observation are in line with the observation by Boyko and colleagues

[5] who reported insulin but not oral medication to be associated with an increase in the risk of DFU incidence.

In addition to the continuous parameters, the duration of diabetes was observed to have a potential to be used in predicting DFU. Although this is in line with what was reported by Boyko and co-workers [5], the results of the current study predict a decreased risk as a result of increase in duration of diabetes that contradicts the findings reported by Boyko and co-workers. This may be affiliated to the moderate to high risk population who were studied in the current study (annual prevalence rate 17%) vs the 5% annual prevalence rate in population that were studied in the study by Boyko and co-workers [5].

The remaining continuous parameters that showed to have a potential to predict diabetic foot ulceration, were related to plantar soft tissue, namely: Normalised Tissue Thickness 1^{ST} metatarsal head (P= 0.169), Stiffness to normalised tissue thickness 1^{ST} metatarsal head (P= 0.143), and Stiffness to tissue thickness 1^{ST} metatarsal head (P= 0.142). Since these parameters were never investigated in assessing the risk of DFU, a comparison with existing literature is not possible.

4.2. Predictor model

Direct multiple logistic regression was performed to assess the impact of independent variables (covariates) that contribute to predicting the ulceration incidence in patients with diabetic neuropathy. The model contained eight variables including: (A) Duration of Diabetes, (B) History of ulceration, (C) History of Callus, (D) Treatment, (E) VPT score, (F) Normalised Plantar Tissue thickness at the 1^{st} metatarsal head, (G) Normalised plantar Tissue stiffness to thickness at the 1^{st} metatarsal head and (H) normalised plantar Tissue stiffness to normalised tissue thickness. These parameters were initially selected based on the results of the univariate logistic regression described under section 4.1.

It can be argued that the addition of the plantar soft tissue measures (as indicated in consecutive blocks 6, 7 and 8), overall resulted in an increase in sensitivity, specificity and

overall increased the prediction accuracy and prognosis strength of the model (Figure 1). Indeed the model could correctly predict 92.5% of the cases. However the model proposed here missed two cases (false negatives) who ulcerated in the twelve months follow up and there is one case of false positive which the model wrongly predicts an ulceration for a patient from the group with no ulceration incidence.

From the eight parameters in the logistic regression model, there are three categorical parameters including the ulceration history, callus history and the treatment code. From the remaining five covariates, three are related to the plantar soft tissue mechanical properties. Despite the significant predicting power of the model, none of the independent variables in the model made statistically significant contribution to the model (Table 1 and 2), none of the parameters on their own contribute significantly to the predictive ability of the model.

Overall, adding the plantar soft tissue stiffness and thickness to the model (i.e. improvement in the model from Block 5 to Block 8) improved the specificity (by 3%), the sensitivity (by 14%), the prediction accuracy (by 5%) and the prognosis strength (by 1%) of the predictor model.

4.3. Comparison with the existing models and the potential for future population-specific models

The prognosis strength of the multiple regression model that is proposed in this study (89.7%) was compared against the prognosis strength of the models proposed by Boyko et al (2006) [5] and that of what was proposed by Monteiro-Soares & Dinis-Ribeiro (2010) [8]. The G score was calculated using these two models indicated that the Area Under curve for ROC were 74.8% (Calculated based on Boyko et al, (2006)) and 63.2% for the models proposed by (Monteiro-Soares & Dinis-Ribeiro, (2010)).

The prognosis strength in predicting ulceration within twelve months following baseline assessment was higher in this study compared to existing models for the studied population, which can indicate that adding parameters related to the plantar soft tissue biomechanics can

potentially increase the accuracy of the models in DFU prediction in moderate to high risk patients.

It needs to be emphasised that the prevalence of diabetic foot ulceration in the sample size selected for this study was 17.5% that, although was a representative sample of an inpatient population, appears to be closer to the 18% prevalence rate that was reported for hospital-based population globally [15].

Furthermore, the lack of significant associations reported within this study on a number of parameters that are commonly associated with DFU may be a result of relatively small sample size, and it could also possibly be related to the ethnic population in this study. Previous studies report that peripheral nerve damage, visual acuity and fasting plasma glucose level were associated with enhanced level of ulceration risk in a Asian population [28].

However Ndip and co-workers [29] whilst looking at patients on dialysis therapy reported that the foot ulcers were more common in patients with white ethnicity when compared to patients of African descent.

4.4. Limitations and future direction

At this stage, it is not possible to identify the exact cause of the observed changes in the mechanical properties of the plantar soft tissue that contribute to ulceration. Indeed it is very likely that altered tissue biomechanics would be the combined effect of physiological changes in the tissue (i.e. glycation), and of structural changes as a result of repetitive excessive loading due to neuropathy. However, structured prospective studies with a large populations are required to investigate and isolate the effect of these two source on the mechanical properties of the plantar soft tissue.

Due to time limitations this study was focused on only two sites that are associated with high ulceration prevalence, namely the sub calcaneal region and the area below the 1st metatarsal head. However it needs to be emphasised that none of the ulceration incidents that are

reported in this study actually occurred in the abovementioned sites where the measurements were performed. This indicates that the observed association between tissue properties and ulceration is likely to be caused by phenomena that affect the entire plantar soft tissue in a uniform fashion. This interesting observation warrants further testing involving the entire plantar surface of the foot to verify and shed light on the exact nature of these phenomena.

While the findings of this study shed light on the association between the mechanical properties of plantar soft tissue and DFU, to use such parameters in global models further validation using large samples within multi-centre prospective cohort studies are warranted.

One could argue that the limitation of the current study is the small number of patients who were included in the study and the exclusion of patient with severe foot deformity [17] and amputation[15]. Previous studies and other clinical evidence indicate that that severe foot deformity and amputation are risk factors for ulceration incidence. While this limitation could decrease the validity of the proposed model for larger cohort studies, it clearly informs the design of future structured studies.

The approach employed within this study can further be developed into risk stratification systems, which can predict not only the likelihood but also the time of DFU incidence. The criticisms for including biomechanical parameters in ulceration risk models and as a diagnostic tool include the relatively long turnaround time for results, the availability of specialised equipment, patient safety etc. However with the availability of elastography in most of the current ultrasound machines and the relatively short turnaround time for assessing the mechanical properties of the plantar soft tissue, using tissue biomechanics in everyday clinical practice appears to be a realistic proposition.

5. Conclusion

The association between common risk factors for DFU was generally in line with previous studies. In addition, previous ulceration, previous callus, use of insulin, and VPT were found to be associated with increased risk of DFU. The major finding of this study and the proposed

model for prediction clearly shows that the mechanical properties of the plantar soft tissue have the potential to be used to complement existing risk factors to improve the predictability of DFU incidence in moderate to high risk populations.

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Conflicts of interest: none

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Parameters	No Ulceration Incidence		With Ulceration Incidence		P value Wald - Univariate Analyses	P Value Multi-variate Analyses	P Value for differences	Effect size for difference
	Mean or Median	S.D or N	Mean or Median	S.D or N				
Age (year) [a]	64	10.0	64	5.8	0.975		0.976 [c]	0.00 [e]
Duration of Diabetes (year) [b]	18	33	14	7	0.128	0.333	0.091 [d]	0.27 [f]
Height (cm) [a]	166	10.1	170	3.4	0.235		**0.043** [c]	**0.10** [e]
Weight (Kg) [a]	73.1	15.7	72.2	10.7	0.881		0.885 [c]	0.00 [e]
Body Mass Index (Kg/m^2) [a]	26.5	4.4	24.9	4.0	0.381		0.393 [c]	0.02 [e]
HbA1C (%) [b]	7.7	33	8.5	7	0.479		0.327 [d]	0.15 [f]
Fasting Blood Sugar level (mg/dl) [a]	164.5	61.9	172.4	48.1	0.744		0.751 [c]	0.00 [e]
Post Prandial Blood Sugar level (mg/dl) [a]	246.9	85.3	251.3	46.3	0.893		0.897 [c]	0.00 [e]
Triglyceride (mg/dl) [a]	116.2	36.9	129.1	34.6	0.392		0.400 [c]	0.02 [e]
Cholesterol (mg/dl) [a]	145.1	28.8	154.0	27.3	0.451		0.460 [c]	0.01 [e]
High Density Lipoprotein HDL (mg/dl) [a]	40.8	8.8	41.3	16.2	0.917		0.947 [c]	0.00 [e]
Low Density Lipoprotein LDL (mg/dl) [a]	80.9	26.7	91.9	31.3	0.337		0.343 [c]	0.02 [e]
Very Low Density lipoprotein VLDL (mg/dl) [b]	22.0	33	25.0	7	0.571		0.392 [d]	0.14 [f]
Ankle Brachial Index [b]	1.04	33	1.09	7	0.600		0.408 [d]	0.13 [f]
Vibration Perception Threshold Heel (V) [b]	44.9	33	52.5	7	0.086		0.078 [d]	0.28 [f]
Vibration Perception Threshold Hallux (V) [b]	43.9	33	52.8	7	0.076		0.130 [d]	0.24 [f]
Vibration Perception Threshold 1STMET (V) [b]	44.3	33	53.5	7	0.078	0.170	**0.025** [d]	**0.35** [f]
Tissue Thickness Heel (cm) [a]	1.37	0.22	1.42	0.24	0.547		0.557 [c]	0.01 [e]
Tissue Thickness 1st Met. (cm) [a]	0.76	0.21	0.91	0.16	0.107		0.095 [c]	0.07 [e]
Stiffness Heel [a]	1.30	0.25	1.34	0.33	0.683		0.691 [c]	0.00 [e]
Stiffness 1st Met [a]	2.21	0.82	1.84	0.54	0.265		0.262 [c]	0.03 [e]
Normalised Tissue Thickness Heel [a]	1.35	0.23	1.41	0.25	0.522		0.532 [c]	0.01 [e]
Normalised Tissue Thickness 1st Met. [a]	0.78	0.22	0.91	0.17	0.169	0.759	0.160 [c]	0.05 [e]
Stiffness to normalised tissue Thickness Heel [b]	0.94	33	0.99	7	0.715		0.929 [d]	0.01 [f]
Stiffness to normalised tissue thickness 1st Met [b]	2.34	33	2.35	7	0.143	0.292	0.346 [d]	0.15 [f]
Stiffness times Interface thickness Heel (cm) [a]	2.47	0.39	2.56	0.41	0.688		0.697 [c]	0.00 [e]
Stiffness times Interface Thickness 1st Met (cm) [a]	1.31	0.39	1.61	0.27	0.300		0.302 [c]	0.03 [e]
Stiffness to tissue thickness Heel (cm^{-1}) [a]	1.59	0.39	1.54	0.24	0.734		0.741 [c]	0.00 [e]
Stiffness to tissue thickness 1stMet(cm^{-1})[b]	1.40	33	1.31	7	0.142	0.241	0.294 [d]	0.17 [f]

Table 1: [a]- [Mean (Stdv)] for normally distributed variable – highlighted parameters; [b] [Median(N)] for non-normally distributed variable; [c] Sig. (2-tailed) - Independent Sample T-test; [d] Asymp.Sig (2 tailed) - Mann-Whitney; [e] Eta-squared = $t^2 / (t^2 + N_1 + N_2 - 2)$ where 0.01 small effect, 0.06 medium effect, 0.14 large effect, P values in bold are P< 0.05 ; [f] $r = z / (N_1 + N_2)^{0.5}$ where 0.1 small effect, 0.3 medium effect , 0.5 large effect ; Note that the selection of parameters in the logistic regression model was based on the univariate analyses in which parameters with P<0.2 were selected. The P values for these selected parameters are underlined in the table. Note that from the three VPT scores that showed to have a significant P value based on univariate analyses only VPT at the 1st Met was used due to colinearity between these parameters.

Categorical Variable	All (40)		No Ulceration Incidence (33)		With Ulceration Incidence (7)		P value Wald - Univariate Analyses	P Value Multi-variate Analyses	P Value for differences	Effect size for difference
	No	%	No	%	No	%				
Male	30	75.0	24	72.7	6	85.7	0.480		0.810 [a]	0.114 [b]
Smoking	4	10.0	4	12.1	0	0.0	0.999		0.781 [a]	-0.154 [b]
OHA only use	17	42.5	16	48.5	1	14.3	0.311		0.250 [a]	0.263 [b]
Insulin only use	8	20.0	6	18.2	2	28.6	0.931		0.250 [a]	0.263 [b]
OHA & Insulin use	15	37.5	11	33.3	4	57.1	0.137	0.667	0.250 [a]	0.263 [b]
Eye surgery	13	33.3	10	31.3	3	42.9	0.557		0.883 [a]	0.094 [b]
Edema	7	17.5	7	21.2	0	0.0	0.999		0.427 [a]	-0.212 [b]
Tineapedis	5	12.5	4	12.1	1	14.3	0.875		1.000 [a]	0.025 [b]
Onychomycosis	21	52.5	16	48.5	5	71.4	0.281		0.492 [a]	0.175 [b]
Callus	11	27.5	9	27.3	2	28.6	0.944		1.000 [a]	0.011 [b]
History of Ulcer	6	15.0	3	9.1	3	42.9	0.039	0.301	0.091 [a]	0.359 [b]
History of Callus	5	12.5	3	9.1	2	28.6	0.180	0.252	0.432 [a]	0.224 [b]
Foot shape abnormality	17	42.5	14	42.4	3	42.9	0.983		1.000 [a]	0.003 [b]

Table 2: Table 2 shows the categorical parameters including sex, Smoking, Oral Hypoglycaemic Agent (OHA) use, Insulin only use, OHA & Insulin use, history of eye surgery, Edema/swollen feet, Tineapedic, Onychomycosis, Callus, History of Ulcer, History of Callus and Foot shape abnormality for the participants and also for each group.

[a]- P values based on Chi-square test of independence (with Yates continuity correction) P <0.5 indicates significant association between ulcerated and non-ulcerated group on the parameter. [b]- Effect size as the Phi coefficient, with small =0.01, Medium = 0.30, Large = 0.50

Note that the selection of parameters in the logistic regression model was based on the univariate analyses in which parameters with P<0.2 were selected. The P values for these selected parameters are underlined in the table.

Figure 1: The Sensitivity (percentage of the group with ulceration occurrence that is correctly identified by the model) , Specificity (percentage of the group with No ulceration occurrence that is correctly identified by the model), Prediction Accuracy (percentage of the overall group that is correctly identified by the model), along with the Prognosis Strength (the areas below the Receiver Operation Curve) of the model when the covariates are added in sequential order from left to right. Block 1: The model includes covariate A only; Block 2: The model includes covariates A and B; 3: The model includes covariates A, B and C; Block 4: The model includes covariates A, B, C and D; Block 5: The model includes covariates A, B, C, D and E; Block 6: The model includes covariates A, B, C, D, E and F; Block 7: The model includes covariates A, B, C, D, E, F and G., Block 8: The model includes covariates A, B, C, D, E, F, G and H.

Where A: Duration of Diabetes, B: History of ulceration, C: History of Callus, D: Treatment code, E: VPT score, F: Planar Tissue thickness at the 1st met, G: Normalised plantar Tissue stiffness to thickness at the first met. H: Normalised plantar Tissue stiffness to normalised thickness.