Original Article

Trabecular Bone Score in Asian-Indian Post-menopausal Women Across the Spectrum of Hyperglycaemia: Insights from a Cross-Sectional Study

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Abstract

Introduction: Type 2 diabetes mellitus (T2DM) increases the risk of fragility fractures, despite the fact that areal bone mineral density (aBMD) is either increased or normal compared to healthy non-diabetic subjects. Hence, the trabecular bone score (TBS) is under investigation in this patient cohort as an alternative metric for the assessment of bone health. The present study aimed to determine TBS in post-menopausal women diagnosed with T2DM and in non-diabetic individuals. **Methods:** This cross-sectional study enrolled 101 individuals with T2DM and 101 individuals without overt T2DM (43 individuals with pre-diabetes and 58 normoglycaemic individuals). Participants underwent a comprehensive history and physical examination, biochemical investigations, and a dual-energy X-ray absorptiometry (DXA) scan with TBS measurement. **Results:** Post-menopausal women with T2DM did not exhibit any significant difference in aBMD levels in comparison to those with pre-diabetes or normoglycaemic individuals. Although there was no statistically significant difference in aBMD among the three groups, the mean TBS value was significantly lower in the T2DM group when compared to both comparison groups ($P < 0.001$). Additionally, glycated haemoglobin (HbA1c) and the duration of diabetes demonstrated a significant negative correlation with TBS. **Conclusion:** TBS may serve as a valuable tool for assessing bone health in individuals with T2DM, particularly when aBMD does not accurately predict the risk of fragility fractures. Both glycaemic control and the duration of diabetes significantly impact TBS values. In individuals with T2DM, incorporating TBS measurements alongside aBMD assessments could offer a more comprehensive evaluation of their bone health.

Keywords: Diabetes, India, post-menopausal, pre-diabetes, TBS

Introduction

Numerous studies over the past two decades have demonstrated that individuals with type 2 diabetes mellitus (T2DM) have an increased risk of fragility fractures, with ongoing research to determine optimal treatment options.[1-5] Diabetic neuropathy and retinopathy may heighten fall risk, contributing to fractures. However, even after adjusting for fall risk, T2DM remains associated with elevated fracture risk.[6,7] Areal bone mineral density (aBMD), measured by dual-energy X-ray absorptiometry (DXA), is the gold standard for fracture risk assessment. Despite most individuals with T2DM exhibiting normal or higher aBMD,[8] the increased fracture risk in these individuals suggests potential adverse effects of T2DM on bone quality rather than density. aBMD measurements focus solely on bone density, overlooking other critical determinants of bone strength, such as mineralisation, turnover, micro-damage, trabecular micro-architecture, and cortical macro-geometry.[9,10]

While the FRAX tool acknowledges type 1 diabetes mellitus (T1DM) as a secondary cause of osteoporosis, T2DM is not included.[11] Thus, an alternative tool for accurately assessing bone health in T2DM is needed.

Trabecular bone score (TBS) is a novel, non-invasive method for evaluating bone micro-architecture using DXA images

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of the lumbar spine which assesses pixel grey-level texture variations, reflecting trabecular structure.[12,13] Our study aims to use TBS as a marker of bone strength and compare it with traditional aBMD scores, hypothesising that TBS provides a superior prediction of skeletal deterioration and fracture risk in T2DM.

Materials and Methods

This cross-sectional, observational study was conducted in the Department of Endocrinology at a tertiary care centre, over an 18-month period from 1st July 2021 to 31st December 2022. The study cohort comprised post-menopausal women, categorised into two primary groups: those diagnosed with T2DM and those without overt T2DM. The latter group was further stratified into pre-diabetic and normoglycaemic individuals. Both groups were matched for gender and menopausal status to control for these potential confounders.

Sample size determination was based on a power analysis aimed at comparing the means of two independent groups (T2DM vs non-T2DM individuals). To achieve a power of 90% with a significance level (alpha) of 0.05, the minimum required sample size was calculated to be 98 participants (49 per group). This calculation utilised the means and standard deviations from a comparable study conducted by Paul *et al*. at Christian Medical College, Vellore, India.[14] During the study period, a total of 202 participants were enrolled, consisting of 101 individuals with T2DM and 101 without overt T2DM (43 pre-diabetic and 58 normoglycaemic individuals).

Patients diagnosed with T2DM according to the American Diabetes Association (ADA) diagnostic criteria, with a minimum disease duration of 5 years, were considered for enrolment. Eligible participants included those with or without co-morbidities, such as hypertension or hypo/hyperthyroidism, provided these conditions were well-controlled with appropriate medical therapy. Additionally, all participants were required to have experienced menopause for more than 5 years and to have provided informed consent to participate in the study.

Exclusion criteria were females with structural abnormalities of the lumbar spine (e.g., scoliosis), prior fractures, prior lumbar spine surgery, or arthritis of the lumbar spine. Other exclusion criteria included chronic kidney disease (defined as an estimated glomerular filtration rate [eGFR] <45 mL/min/1.73 m²), primary hyperparathyroidism, prolonged immobilisation, chronic liver disease, celiac disease, or other malabsorptive disorders. Participants with uncontrolled co-morbidities, such as uncontrolled diabetes (HbA1C >10%) or hypertension (blood pressure >160 / 100 mmHg despite treatment with three anti-hypertensive drugs), were also excluded.

Furthermore, individuals receiving or having previously received bone-altering drug therapies were not eligible. These therapies included sodium-glucose co-transporter 2 inhibitors (e.g., canagliflozin), thiazolidinediones, proton pump inhibitors, teriparatide, bisphosphonates, denosumab, hormone replacement therapies, loop diuretics, corticosteroids, synthetic retinoids, anti-epileptic drugs, anti-coagulants (e.g., heparin, low-molecular-weight heparin [LMWH], warfarin), cyclosporine, chemotherapeutic agents (e.g., methotrexate, ifosfamide, imatinib), and anti-retroviral therapy. Post-menopausal females diagnosed with T2DM and without T2DM were enrolled from the Endocrinology Outpatient Department (OPD) and healthcare workers from the hospital. After verifying eligibility based on the inclusion criteria, study participants underwent DXA scans. Comprehensive medical histories, including co-morbidities, family history, treatment history, and adherence to therapy, were documented. The mean value of the last three glycated haemoglobin (HbA1c) readings was recorded for all participants.

TBS and aBMD T-scores were assessed using a fan-beam DXA machine (Hologic Discovery A), which was equipped with Hologic APEX software version 4.0.2 and TBS software version 3.0. Standardised procedures were followed during the measurements. The machine underwent regular calibration, and its performance was monitored in accordance with the manufacturer's quality assurance protocol. The DXA scan was conducted with participants in the supine position, and TBS and T-scores were calculated from the lumbar vertebrae (L1–L4) region.

Statistical analysis

Statistical analysis was conducted using IBM Statistical Package for Social Sciences (SPSS) software version 29.0 (SPSS, Inc., Chicago, IL). Quantitative variables were reported as mean ± SD. Categorical variables were presented as proportions or percentages. The normality assumption for continuous variables was tested using the Kolmogorov-Smirnov (KS) test. For normally distributed variables, a *t*-test was used to compare two groups, while the Wilcoxon-Mann-Whitney U test was used for non-normally distributed variables. To assess differences in aBMD and TBS among diabetic, pre-diabetic, and non-diabetic post-menopausal women, Quade's test (non-parametric ANCOVA) was employed. The dependent variables were aBMD and TBS, while the independent variable was the glycaemic status of the participants (diabetic, pre-diabetic, and non-diabetic groups). Covariates included in the analysis to control for potential confounding factors were age, time since menopause, body mass index (BMI), and 25-hydroxyvitamin D (25OHD) levels.

Post-hoc comparisons were conducted as applicable. Categorical variables were analysed using the Chi-square test or Fisher's exact test, as appropriate. Pearson's correlation was used to explore the linear correlation between two normally distributed continuous variables, while Spearman's correlation was used for non-normally distributed variables. Univariate and binary logistic regression were performed to assess risk factors.

A *P* value of less than 0.050 was considered statistically significant.

Ethical aspects

This study was approved by the Institutional Ethics Committee of PGIMER, Chandigarh, and all procedures were conducted in accordance with the ethical standards set forth in the 1964 Declaration of Helsinki. Written informed consent was obtained from all participants. Ethical Clearance was obtained from the institute (NK/7633/MD/253).

Results

A total of 202 participants were enrolled in the study, comprising 101 individuals with T2DM and 101 individuals without T2DM, which included 43 pre-diabetic individuals and 58 normoglycaemic individuals. The baseline parameters of all three groups are shown in Table 1.

All females in the three groups had been post-menopausal for at least 5 years at study recruitment, with no significant difference in the duration since menopause (P = 0.607). The diabetic group had significantly higher age and BMI. They also had significantly higher mean fasting plasma glucose (FPG), post-prandial plasma glucose (PPG), and HbA1C levels (P < 0.001), along with a significantly lower mean haemoglobin level by 0.64 g/dL (P = 0.004). The mean estimated glomerular filtration rate (eGFR) was also significantly lower in the diabetic group. Serum calcium and phosphorus levels were similar across all groups. Total cholesterol and LDL levels were significantly lower in the diabetic group (P < 0.001). There were no significant differences in ALP, vitamin D, intact parathyroid hormone (iPTH), or thyroid profile values among the groups.

Approximately 77.2% of post-menopausal females with T2DM had co-existing hypertension, nearly double the prevalence in the pre-diabetes group (41.4%) and the normoglycaemic group (39.5%) (P < 0.001). The incidence of coronary artery disease (CAD) and cerebrovascular accidents (CVAs) was higher in the diabetic cohort, with CAD prevalence at 11.9% for T2DM, 9.3% for pre-diabetes, and 3.4% for normal individuals. However, these differences were not statistically significant. Quades's test analysis revealed that aBMD did not significantly differ across the three groups after adjustment for age, time since menopause, BMI, and 25OHD levels. In contrast, TBS showed a significant difference among the groups (F = 13.52, P < 0.001). Subgroup analysis indicated that the diabetic group had significantly lower TBS compared to both the pre-diabetic and non-diabetic groups (P < 0.001), with no significant difference between the pre-diabetic and non-diabetic groups (P = 0.999).

As shown in Table 2, the prevalence of osteopenia and osteoporosis was similar across all three groups, with no significant differences (P = 0.301). Both HbA1c levels and the duration since diabetes diagnosis exhibited a statistically

Table 1: Baseline characteristics of post-menopausal females: Data are represented as Mean±SD

Parameters	Diabetes (Group 1) (n=101)	Pre-diabetes (Group 2) (n=43)	Normal (Group 3) (n=58)	P
Age (yrs)	60.3±7.7	57.5±7.5	57.4±6.7	0.025*
Time Since Menopause (yrs)	12.09±7.03	11.05±5.85	11.31±5.85	0.607
BMI (kg/m^2)	27.63±3.67	27.50±4.18	25.88±3.62	0.015*
FPG (mg/dL)	146.18±48.39	95.95±10.13	93.70±8.71	<0.001*
PPG (mg/dL)	206.60±64.50	120.19±17.31	117.30±19.83	<0.001*
Creatinine (mg/dL)	0.77±0.20	0.72±0.14	0.72±0.16	0.056
eGFR (mL/min)	83.71±25.91	95.37±24.09	90.16±25.02	0.032#
Corrected Calcium (mg/dL)	9.42±0.41	9.54±0.42	9.56±0.53	0.108
Phosphorous (mg/dL)	3.63±0.59	3.47±0.61	3.42±0.52	0.055
ALP (U/L)	116.67±43.85	112.53±32.94	116.10±39.75	0.850
LDL (mg/dL)	88.95±36.86	115.30±33.54	120.05±44.07	<0.001*
HDL (mg/dL)	49.06±13.53	49.28±15.14	50.13±11.06	0.883
HbA1C (%)	7.76±1.27	5.91±0.20	5.38±0.22	<0.001$
TSH (uIU/mL)	2.52±1.18	2.43±1.19	2.33±1.26	0.639
T4 (ug/dL)	8.40±1.64	7.97±1.23	8.02±1.51	0.180
T3 (ng/dL)	1.27±0.37	1.26±0.22	1.20±0.26	0.455
25(OH)D_3 (ng/mL)	36.05±21.30	33.25±14.87	37.84±18.31	0.496
PTH (pg/mL)	54.42±25.58	56.79±22.99	56.64±24.83	0.808
aBMD (g/cm^2) (L1–L4)	0.835±0.130	0.865±0.126	0.817±0.183	0.398
aBMD (g/cm^2) (Neck of femur)	0.656±0.113	0.708±0.110	0.667±0.126	0.087
aBMD (g/cm^2) (Lower 1/3 Radius)	0.543±0.113	0.573±0.734	0.560±0.956	0.769
TBS	1.269±0.099	1.340±0.081	1.346±0.097	<0.001*

* - Significant difference between group 1 vs group 2 and 3; no significant difference between group 2 vs 3. # - Significant difference for group 1 vs group 2, no significant difference for other groups. $ - Significant difference for all intergroup comparisons (group 1 vs 2, 1 vs 3, 2 vs 3). BMI: Body Mass Index, FPG: Fasting Plasma Glucose, PPG: Post-prandial Plasma Glucose, eGFR: Estimated Glomerular Filtration Rate, ALP: Alkaline Phosphatase, LDL: Low-Density Lipoprotein, HDL: High-Density Lipoprotein, HbA1C: Glycated Haemoglobin, TSH: Thyroid-Stimulating Hormone, T4: Thyroxine, T3: Triiodothyronine, 25(OH)D_3: 25-Hydroxyvitamin D_3, PTH: Parathyroid Hormone, aBMD: Bone Mineral Density, TBS: Trabecular Bone Score

significant negative correlation with TBS, as illustrated in Figures 1 and 2. Pearson's correlation coefficients were −0.315 ($P < 0.001$) for HbA1c and −0.197 ($P = 0.048$) for time since diagnosis.

The average 10-year probability of major osteoporotic fractures (MOFs) in the diabetes group was 5.22% using conventional FRAX. When adjusted for TBS, this probability increased to approximately 6.23% ($P < 0.001$), as shown in Figure 3. Similarly, the 10-year probability of hip fractures was 1.65% with conventional FRAX, increasing to 1.90% with TBS adjustment ($P < 0.001$). Twelve patients out of 101 (11.88%) met the FRAX cut-off for treatment of osteoporosis according to ISBMR guidelines (major osteoporotic fracture more than or equal to 10.5% and hip fracture more than or equal to 3.5%) without adjustment for TBS which increased to 16 (15.84%) after adjustment for TBS, an increase of 33%.

Discussion

This cross-sectional study, utilising three-site DXA along with TBS, revealed no significant difference in aBMD among the diabetes, pre-diabetes, and normal groups. However, TBS values showed a significant difference, with the diabetes group exhibiting significantly lower TBS compared to both the pre-diabetic and non-diabetic groups. Notably, no difference was found in TBS between the pre-diabetic and non-diabetic groups. Moreover, a significant linear correlation was observed between TBS and both HbA1c levels and the duration of diabetes. There was association of higher HbA1c levels and longer duration of diabetes with lower TBS values. This suggests that TBS may serve to be a more sensitive marker of bone health in individuals with T2DM compared to aBMD.

T2DM is associated with impaired bone health and an elevated risk of pathological fractures.[1-8] While aBMD measured by DXA is a commonly used method for assessment of fracture risk, it does not account for bone micro-architecture's role in predicting fracture risk. Notably, individuals with T2DM may exhibit normal or even higher aBMD values,[8] underscoring the need for a marker that evaluates bone quality alongside routine aBMD measurements. Recent studies, including a meta-analysis, have demonstrated the utility of TBS as an independent marker of bone quality and strength.[15-17] In our study, we assessed TBS in post-menopausal females with T2DM to evaluate its effectiveness in assessing bone health in this population. Patients with T2DM had higher BMI, more prevalence of hypertension, and lower total and LDL cholesterol at baseline as compared to the other two groups. This is expected due to the known association of diabetes with the above co-morbidities and higher statin use in the T2DM cohort.[18-20]

Numerous studies have demonstrated an increased risk of pathological fractures in T2DM, even at normal aBMD levels.[21,22] While aBMD was similar across all groups in our study, TBS was significantly lower in the diabetic group. Previous research has demonstrated that TBS is a superior marker of bone health in diabetics compared to aBMD. For instance, in the

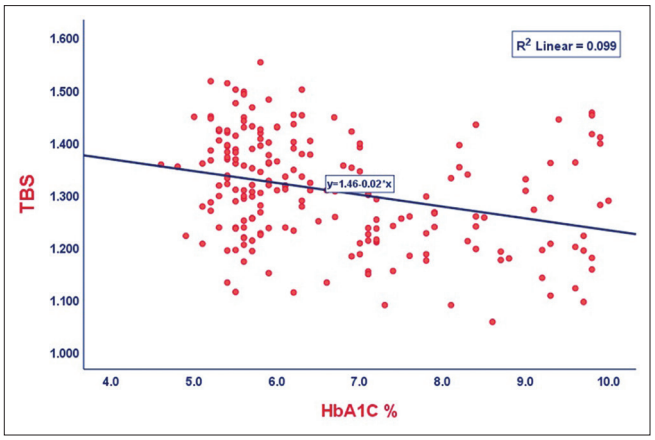

Figure 1: Inverse Linear Correlation of TBS with HbA1C

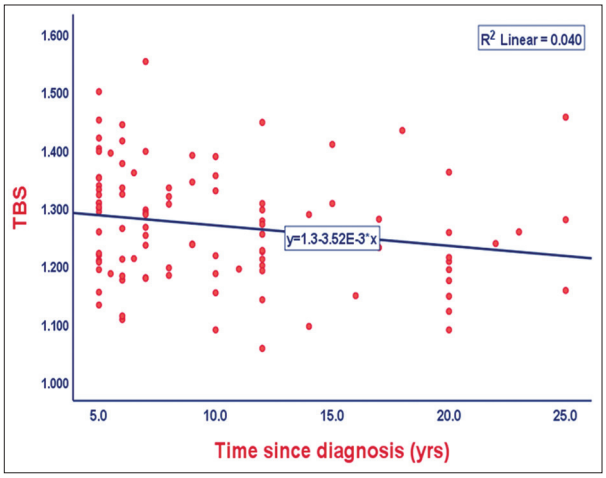

Figure 2: Inverse Linear Correlation of TBS with Time Since Diagnosis of Diabetes

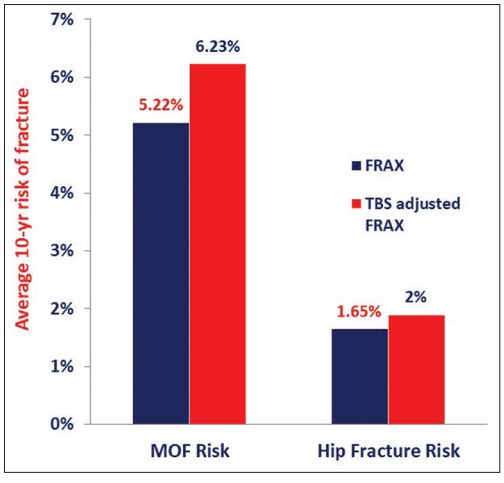

Figure 3: FRAX versus TBS-adjusted FRAX in Post-menopausal Females with T2DM

study by Yamamoto *et al.*,[23] there was a significant correlation between low TBS and vertebral fractures in T2DM, which was irrespective of aBMD. Similarly, Lin *et al.*[24] reported that TBS had a higher AUC for the detection of vertebral fractures when

Table 2: Prevalence of Osteoporosis and Osteopenia in Diabetes, Pre-diabetes, and Normal Individuals

Osteopenia			Osteoporosis			*P*
Diabetes (*n*=103)	Pre-diabetes (*n*=43)	Normal (*n*=58)	Diabetes (*n*=103)	Pre-diabetes (*n*=43)	Normal (*n*=58)	
42.0%	46.5%	39%	41.1%	27.9%	46%	0.301

compared to aBMD in T2DM patients. These findings suggest that TBS may provide a more accurate clinical assessment of bone micro-architectural deterioration in T2DM.

In our study, both the duration of diabetes as well as HbA1c levels were inversely associated with TBS, consistent with previous research.[25] A previous study demonstrated improvement in TBS but not in aBMD with good glycaemic control.[26] Moreover, studies have demonstrated that TBS-adjusted FRAX is a better predictor of fracture risk in post-menopausal females with T2DM than conventional FRAX.[27,28] In one of the studies, among the diabetic subgroup, the adjustment of FRAX for TBS led to a net reclassification improvement of 3.9% for incident MOF and 2.5% for hip fracture.[29] Our findings also indicated that TBS-adjusted FRAX provided a significantly different fracture risk assessment compared to conventional FRAX in the diabetes group, suggesting its potential utility in clinical practice. In our cohort of diabetics, it led to a 33% increase in the number of people qualifying for treatment as per Indian osteoporosis guidelines, which leads to a significant increase in the number of individuals who can potentially benefit from treatment.[30]

This study has several strengths, including the inclusion of a well-defined study population and the adjustment for relevant covariates such as age, BMI, time since menopause, and 25-hydroxyvitamin D levels, enhancing the validity of our findings. However, the study has some limitations. The cross-sectional design of the present study limits its ability to establish a causal relationship between T2DM and changes in bone health parameters. The inclusion of only post-menopausal women with T2DM and the exclusion of participants with certain co-morbidities and those on specific medications may limit the generalisability of the findings to the broader population of individuals with T2DM. Lastly, the reliance on self-reported data for certain medical history elements may introduce recall bias, potentially affecting the accuracy of some associations observed. Future longitudinal studies with larger, more diverse populations are warranted to confirm and extend these findings.

Conclusion

In summary, our study underscores the importance of comprehensive bone health assessments in individuals with T2DM. TBS serves as a valuable marker of bone quality and strength, complementing aBMD measurements to provide a more complete evaluation of bone health in this population. Further research is warranted with larger sample sizes to validate these results and to establish the broader clinical utility of TBS-adjusted FRAX in fracture risk prediction for individuals with T2DM.

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Supervision: Rimesh Pal, Sanjay K. Bhadada

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author, Prof Dr. Sanjay K. Bhadada, upon reasonable request.

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