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Quantification of bone marrow fat fraction in type 2 Diabetes Mellitus using Dixon MRI

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Abstract

Background: *Due its chronic nature, diabetes mellitus (DM) can damage several body systems. In the skeletal system it can cause bone loss, increased bone marrow fat content and even osteoporosis. Recently, skeletal fragility is a known complication of type2 DM (T2DM) with increased fracture risk.*

Aim of study: *to evaluate the utility of Dixon magnetic resonance imaging (MRI) to quantify bone marrow fat (BMF) and assess the fat fraction (FF). As evidence that non mineralized component may be important in the evaluation of bone strength.*

Patients and methods*: This case control study included 50 participants classified into 2 groups: group A (control group) included 30 normal healthy age, sex and body mass index (BMI) matched population, group B included 20 T2DM patients without microvascular complications, Dixon MRI of lumbosacral spine and both hips was performed for all participants with quantification of bone marrow FF.*

Results: *The study showed a statistically significant difference in FF measured by Dixon MRI in lumbar vertebrae but not femoral neck between T2DM cases and control. This may be explained by higher FF normally in femur more than the vertebrae. Pairwise comparisons with Bonferroni correction for multiple tests revealed that FF in lumbar vertebrae was statistically significantly higher in the non-complicated diabetic group vs. the control group (p-value =.001)*

Conclusion*: T2DM is associated with changes in the bone quality with increased bone marrow FF that can be reliably assessed using Dixon MRI. This can be used as potential therapeutic target for drugs to restore proper BMF content, early prevention and treatment of fractures.*

Keywords*: Dixon MRI, fat fraction, diabetic bone disease, vertebrae, femoral neck.*

1. Introduction

DM has become the third non-communicable life threatening disease, after cardiac diseases and cancers. During its prolonged course, DM can damage multiple systems throughout the body causing several acute and chronic complications [1].

In addition to nephropathy, retinopathy, and cardiac diseases, DM can also cause damage to the skeletal system, bone loss, microarchitecture changes and even osteoporosis with subsequent

increased bone fragility which in addition to diabetes induced falls increase susceptibility to fractures [2].

In T2DM patients, fractures can occur with normal or even increased bone mineral density (BMD). Thus, T2DM induced bone fragility is influenced by bone quality alterations rather than decreased BMD, and is defined as "diabetic osteopathy". Therefore, BMD assessment alone is not a reliable method to evaluate fracture risk [3, 4].

Bone consists of mineralized (cortex and trabeculae) and non-mineralized (bone marrow) components. Previously, BMF was thought to be an inert fat depot. Recently it has been identified to have several mechanisms for diabetes-induced skeletal fragility. Firstly, BMF is recently known to have an endocrine functions that secretes adipokines with systemic and local effect and potential to negatively impact bone metabolism [5, 6].

Secondly, BMF has a negative effect on bone by producing imbalance between adipogenesis and osteoblastogenesis. Both adipocytes and osteoblasts have a common mesenchymal stem cell precursor, and differentiation toward adipogenesis occurs at the expense of osteoblastogenesis with subsequent decrease in bone formation. There is abundance of BMF in old people and is now interpreted as a part of whole-body ageing process [7, 8].

Only a few number of studies have investigated BMF content in patients with DM. The literature suggests that DM may be a state of increased BMF and accelerated ageing. Changes in BMF composition with T2DM are likely to result from multiple interacting factors. Poor glycemic control, circulating lipids, peroxisome proliferator-activator receptor-γ (PPARγ), visceral adiposity, Pioglitazone and hypoleptinemia are suggested mechanisms for the putative effects of DM on BMF. These lead to increase adipogenesis, decrease osteoblastogenesis, decrease bone formation, increase osteoclastogenesis, and osteocyte apoptosis [9, 10].

Despite the focus on the mineralized bone component, recent researches have highlighted the important role of non-mineralized component in maintaining bone health. Quantitative measurements of BMD have been performed by using dual-energy-X-ray-absorptiometry (DEXA) or quantitative computed tomography (QCT). But these methods have a low predictive value of fracture risk caused by assessing exclusively the mineral component neglecting bone microstructure and bone marrow [11].

Historically, clinical measures of BMF required a bone biopsy. A recent study to estimate BMF in the distal tibia using high resolution peripheral computed tomography (HR-pQCT). False high marrow density in sites adjacent to bone cortex and trabeculae due to partial volume, low resolution, radiation risk and currently only available on distal sites limits the usefulness of this technique. As a result, HR-pQCT is still only used as a research tool [12].

In this context, imaging techniques are emerging to non-invasively evaluate the criteria of the non-mineralized bony component. MRI allows the quantitative evaluation of bone marrow without radiation risk with multiplanar, volumetric and three-dimensional (3D) acquisitions. [13].

Magnetic resonance spectroscopy (MRS) is considered the gold standard imaging technique for localized fat quantification. However, MRS is time-consuming, technically demanding, subject to sampling errors, has low spatial resolution and characterized by overlapping water and fat peaks in bone marrow due to susceptibility effects from the trabecular bone [14, 15].

The Dixon technique was first developed in 1984 and named after its inventor. Dixon is a chemical shift-based water/fat separation sequences that joined the abilities of MRI and spectroscopy to separate fat and water signals to generate water-only (WO) and fat-only (FO) images in one acquisition. [16].

In several studies, Fat quantification comparison of Dixon technique with MRS showed excellent agreement and good correlation between both. Also, was significantly correlated with histology [17-19].

Recently, the Dixon method has gained interest in musculoskeletal imaging. Single-point, two-point or multi-point Dixon techniques are acquired at different echo times (TEs) with certain spacing time, so that fat- and water signals measured when they are in-phase (IP) and out-of-phase (OP). This gives Thus, Dixon is able to quantify fat amount within a region of interest (ROI). Through post-processing, WO and FO images are reconstructed by adding and subtracting the IP signal and OP signal respectively. Thus, four sets of images are obtained per acquisition [20].

Generally, Dixon MRI fat quantification technique has multiple advantages over MRS. It does not need a high experience in voxel prescription. In addition, an automatic reconstruction of FF map is provided as an easy and fast quantification method. In spine, Dixon is used to produce more homogeneous fat suppression, a higher signal-to-noise ratio image with shorter scan time and a larger field of view (FOV). Unlike MRS, Dixon technique is not suitable to discriminate between different lipid molecules [18, 20, 21].

Dixon is characterized by being fat-sensitive and fat-specific (fat is the only source of signal in FO images). While T1 sequence is sensitive to fat signal in addition to all other short T1 substances. Finally, the specificity of FO images to the fat signal allow image acquisition in association with any type of pulse sequence. Dixon provides not only qualitative, but also quantitative information: on the relative amount of fat and water and map the fat fraction [22, 23].

To the best of our knowledge, there is lack of previous researches studying the role of Dixon technique in assessment of BMF in T2DM.

The aim of our study: to evaluate the utility of Dixon magnetic resonance imaging (MRI) to quantify bone marrow fat (BMF) and assess the fat fraction (FF). As evidence that non-mineralized component may be important in the evaluation of bone strength.

METHODS

The institutional review board of ethics approval was obtained and informed consents from all participants was waived. This case control study was carried out during the period from January 2021 to July 2023 and initially included 50 participants referred from Diabetes and Endocrinology unit to Radiology divided into two groups:

Group A: 30 age, sex and BMI-matched healthy control subjects.

Group B: 20 T2DM adult patients without microvascular complications. (premenopausal females and ≤50 year males)

Exclusion criteria: thyroid and parathyroid disorder, liver or renal failure, bronchial asthma, connective tissue disorders, patients with any hematological disease, bone disease, malignancy, pregnancy, smoking, patients taking drugs such steroids, anticoagulant and Pioglitazone, female receiving hormonal contraception, macro vascular complication (ischemic heart disease, peripheral vascular disease, stroke) as well as contraindications for MRI acquisition, such as cardiac pacemaker, implants or other metallic prosthesis and claustrophobia.

All participants were subjected to history taking, clinical examination, and laboratory investigation (TSH, HBA1C, Calcium, Phosphorus, serum creatinine, lipid profile, CBC). For T2DM patients, history includes duration of diabetes and treatment of diabetes (oral metformin and sulphonyl urea or insulin)

MR imaging

MRI was performed using a 1.5 T magnet (SIEMENS healthineers, Aera, Germany, serial numer142230) using a lumbar and hip coils.

T1 volume interpolated breath-hold examination (VIBE) Dixon MRI is acquired for both lumbosacral region in sagittal plane (TR=7.05ms, TE=2.39ms, FOV=300 × 300 mm, slice thickness=2.5 mm, scan time about 47 seconds) and for both hips in coronal plane (TR=7.05ms, TE=2.39ms, FOV=410 × 410 mm, slice thickness=3.5 mm). Additional sagittal T2 weighted images are acquired (TR 2850 ms, TE 100 ms, 4 mm slice thickness, FOV 320 × 320 mm)

Four images are generated: IP, OP, WO (WO = IP + OP) and FO (FO = IP–OP). Images are transferred to work station provided by the vendor and are interpreted by an expert radiologist (15 years' experience in MR imaging). FF was calculated as a percentage (FF = FO/FO+WO) using Syngo.via® software.

Equal ROIs avoiding the cortex and endplates are placed in L1-L4 vertebrae in mid-sagittal plane. These regions were selected to be representative of the BMF of the whole axial skeleton. The final FF percentage for each subject used in statistical analysis was the average of these 4 values divided by FF of an equal size ROI placed in the subcutaneous fat.

Similarly, equal ROIs are placed in neck of both femurs avoiding the cortex in coronal plane. Fat fraction percentage measured equal the mean FF of femoral neck on both sides divided by FF of equal rectangular ROI placed in the subcutaneous tissue.

Statistical analysis:

Data were analyzed using: IBM-SPSS software (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). MedCalc® Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021).

Data expression:

-Qualitative data were expressed as N (%).

-Quantitative data were tested for normality using Shapiro-Wilk's test with data being normally distributed if p>0.050. The presence of significant outliers (extreme values) was tested for by inspecting boxplots.

-Quantitative data were expressed as mean ± SD if normally distributed or median and interquartile range (Q1 or 25th percentile – Q3 or 75th percentile) if not

Data comparison:

-Qualitative data between groups:

For more than 2X2 cross tabulation, Fisher-Freeman-Halton test was used as the expected count in cells was < 5 in some of the cells.

-Quantitative data between more than two groups:

The one-way ANOVA test was used to compare normally distributed quantitative data between more than two groups.

The Kruskal-Wallis H-test was used to compare non-normally distributed quantitative data between more than two groups.

Receiver Operating Characteristic (ROC) curve analysis: was used to find a cutoff value of a continuous variable that can discriminate between two conditions.

Associations:

Point biserial correlation: test was used to determine whether there is an association between a dichotomous variable (i.e., nominal variable with two categories) and a quantitative variable. The strength of association was considered low, medium, or high if the correlation coefficient (rpb) was > 0.1 to <0.3, 0.3 to <0.5, or 0.5 or more, respectively.

The Spearman's correlation test was used to determine whether there is a linear relationship / association between two non-normally distributed quantitative data. The strength of association was considered low, medium, or high if the correlation coefficient (r) was > 0.1 to <0.3, 0.3 to <0.5, or 0.5 or more, respectively.

Significance level: for any of the used tests, results were considered as statistically significant if p value ≤ 0.050.

RESULTS:

This study involved 50 participants divided into two groups:

Group A: 30 age, sex and BMI-matched control subjects.

Group B: 20 T2DM patients without microvascular complications.

Age, sex and BMI of the 2 groups are shown in (table 1). This table shows no statistically significant difference in age, sex and BMI between the two groups.

Table (1): Age, sex, and BMI in the study groups.

Parameter	Group A N=30	Group B N=20	p-value
Sex Female Male	 25 (83.3%) 5 (16.7%)	 18 (90%) 2 (10%)	.860
Age (years)	42.8 ± 4.8	42.7 ± 5.4	.104
BMI (kg/m2)	33.9 ± 4.3	33.9 ± 3.7	.999

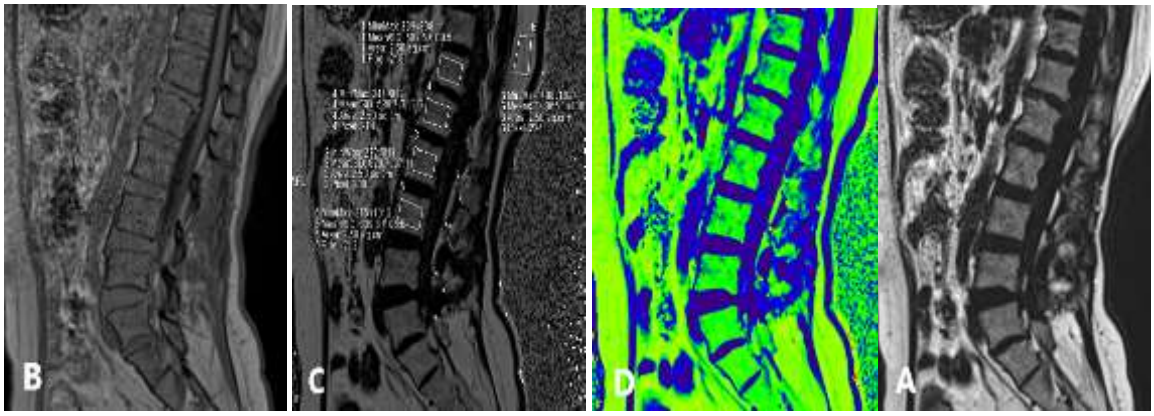
Notes: Sex data is N (%), and the test of significance is Fisher-Freeman-Halton Exact Test. Age and BMI data is mean ± SD, and the test of significance is one-way ANOVA.

As regard FF measured on Dixon MRI, a statistically significant difference is noted in lumbar vertebrae but not femoral neck between the two groups. Pairwise comparisons with Bonferroni correction for multiple tests revealed that FF by Dixon MRI in lumbar vertebre was statistically significantly higher in group B vs. A (p-value=.001) (Table 2) (Figure 1, 2).

Table (2): Fat fraction measured by Dixon MRI in the study groups

Parameter	Group A N=30	Group B N=20	P1-value
% FF (lumbar vertebrae)	40.8 (35.4-47.2)	58.2 (46.3-64.3)	0.001
% FF (femoral neck)	77 (72.5-81.6)	81.2 (77.8-83.9)	>0.05

Notes: Data is median (Q1-Q3), and the test of significance is Kruskal-Wallis H-Test.
P1 value group A Vs. B



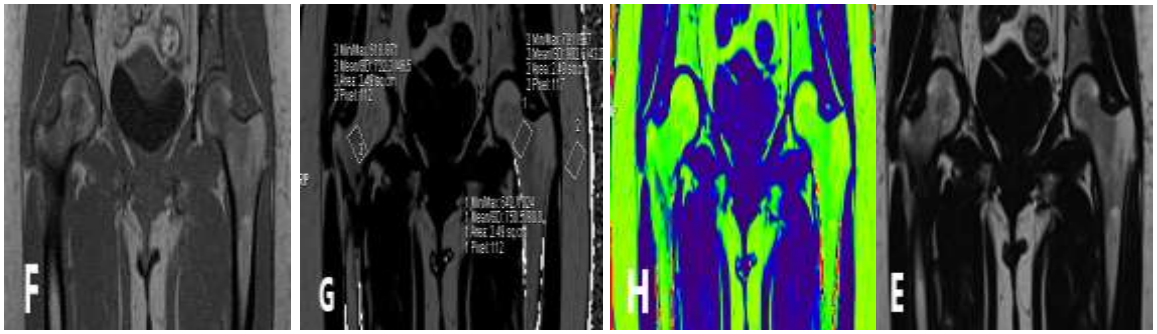


Figure 1: T2DM patient (A, B, C, D) Sagittal lumbosacral spine Dixon MRI fat only image, add (fat + water), divide image (FF = FO/FO+WO) and colored fat fraction map respectively showing increased FF measuring about 70%. (E, F, G, H) Coronal both hips Dixon MRI fat only image, add image (fat + water), divide image (FF = FO/FO+WO) and colored fat fraction map respectively showing increased FF measuring about 82%.

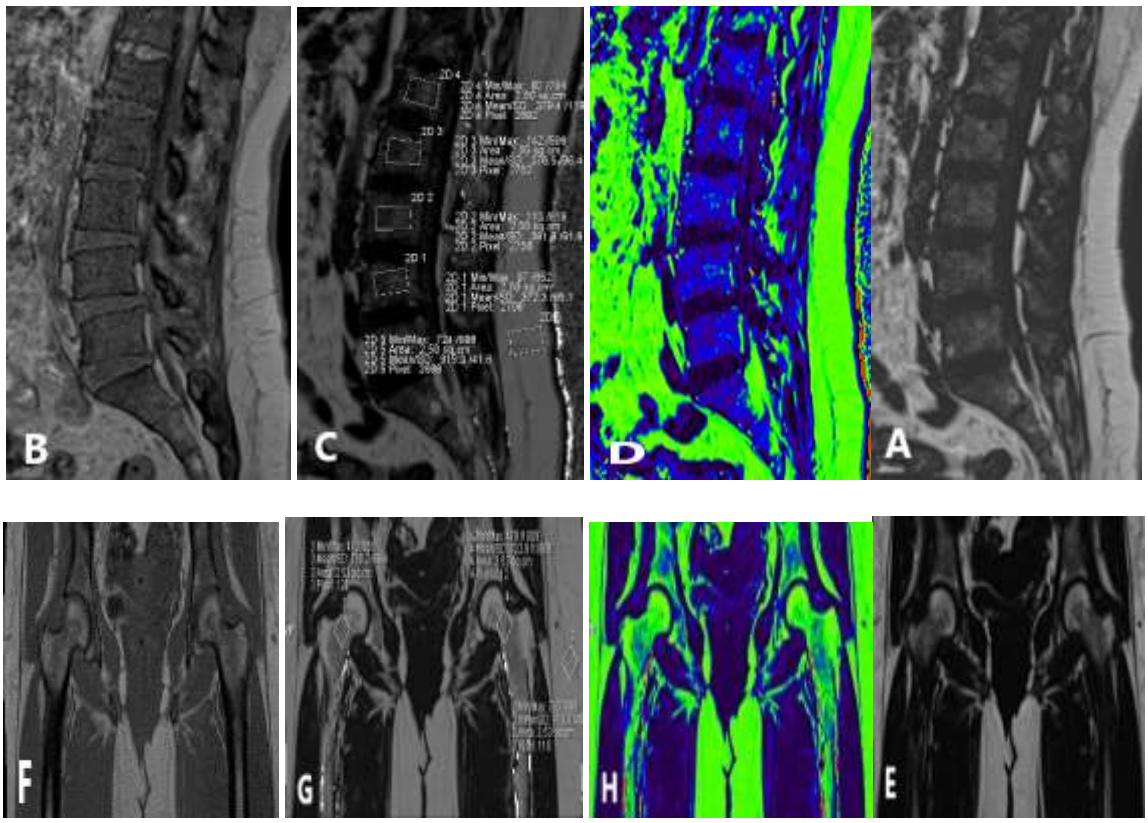


Figure 2: Control case (A, B, C, D) Sagittal lumbosacral spine Dixon MRI fat only image, add (fat + water), divide image (FF = FO/FO+WO) and colored fat fraction map respectively with FF measuring about 40%. (E, F, G, H) Coronal both hips Dixon MRI fat only image, add image (fat + water), divide image (FF = FO/FO+WO) and colored fat fraction map respectively with FF measuring about 78%.

Correlation of FF by Dixon MRI with study parameters revealed a statistically significant positive correlation between the FF at lumbar vertebrae vs. FF at femoral neck and also age in years. Also, there was a statistically significant positive correlation between the FF at femoral neck vs fat fraction at lumbar vertebrae, age and sex being higher in males (table 4).

Table (3): Correlation of Fat fraction by Dixon MRI with study parameters

Parameter	Lumbar vertebrae		Femoral neck	
	Coefficient	**p-value**	**Coefficient**	**p-value**
FF (lumbar)	**-**	**-**	**.561**	**<.001**
FF (hip)	**.561**	**<.001**	**-**	**-**
Age (years)	**0.235**	**0.028**	**0.314**	**0.003**
Sex	**0.151**	**0.160**	**0.475**	**<.001**
BMI (kg\m2)	**-0.022**	**0.837**	**-0.194**	**0.07**
Hemoglobin A1C	**0.050**	**0.707**	**-0.149**	**0.256**
DM duration (years)	**-0.006**	**0.967**	**0.105**	**0.432**

Notes: The test of significance is Spearman's correlation for quantitative data and point bi-serial for nominal data.

ROC curve analysis for group B vs. A: A ROC curve of FF used for differentiating increase BMF in uncomplicated diabetic patients from control group with cutoff in lumbar vertebrae >0.5297 yield area under the curve (AUC) 0.792, p-value <0.001, sensitivity of 66% and specificity of 93.3%. Cutoff in femoral nrck >0.8037 yield AUC 0.672, p-value 0.035, sensitivity of 75% and specificity of 73.3% (figure 3).

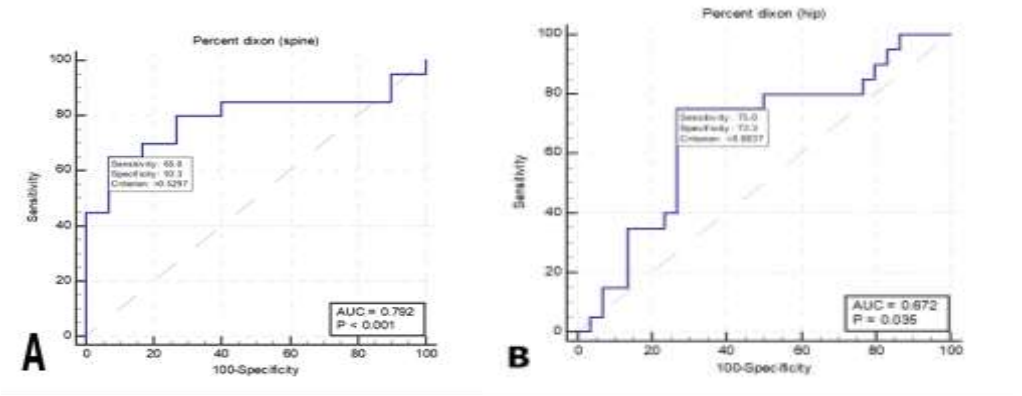


Figure 3: ROC curve analysis for uncomplicated DM group vs. control (A:lumbar vertebrae, B:femoral neck)

DISCUSSION

Nowadays, skeletal fragility is considered a complication of T2DM with increased risk of hip, vertebral, forearm wrist and foot fractures [24, 25]. Increased BMF has been involved in several conditions that negatively affect bone quality like osteoporosis and T2DM. BMF quantification could potentially enhance the diagnosis and improve prediction, early prevention and treatment of fractures [26].

Patients can benefit from multiple therapeutic approaches to inhibit BM adiposity. Improving the glycaemic control, exercise regulation parathyroid hormone treatment and several bioactive molecules (vitamin D, Puerarin, Strontium ranelate, and Senolytic agent) are reported to stimulate osteogenic differentiation while inhibiting BM adipogenesis and lipotoxicity [27-30].

Recently, Dixon method, a chemical shift-encoding water-fat imaging, has been used for rapid evaluation of fat content with high signal-to-noise ratio and high spatial resolution. Therefore, Dixon method is suitable for large FOV evaluation such as imaging of spine or the extremities. Dixon MRI can be used to quantify BMF and for assessing its FF [31]. FF quantification by Dixon MRI was used in several studies (to detect vertebral bone marrow metastasis, assess subchondral bone marrow in knee osteoarthritis and skeletal involvement in Gaucher's disease) [32-34].

Today, Dixon-type pulse sequences are widely offered by nearly every manufacturer with their specific patented names. For example, Siemens uses the generic name DIXON, Philips provides multi-point or mDixon and General Electric (GE) has iterative decomposition of water and fat with echo asymmetry and least squares estimation (IDEAL). Dixon images can be acquired with any pulse sequence (spin-echo, fast spin echo, gradient-echo, T1, T2, proton density, or even postcontrast T1 sequences) [22, 35].

Our study included 50 participant classified into 2 groups: group A (30 age, sex and BMI matched control, group B (20 T2DM patients without microvascular complications).

Dixon MRI can be used to quantify BMF and assess its FF. As evidence that non-mineralized component may be important in the evaluation of bone strength. Our study showed a statistically significant difference in FF by Dixon MRI in lumbar vertebrae but not femoral neck between the two groups. This may be explained by higher FF normally present in femur more than the vertebrae [36].

Pairwise comparisons with Bonferroni correction for multiple tests revealed that FF by Dixon MRI in lumbar vertebrae was statistically significantly higher in group B vs. group A (p-value,.001)

To the best of our knowledge, there is lack of previous studies used Dixon MRI for assessment of BMF in diabetic patients. Our study comes in agreement with a larger study on BMF in T2DM that showed a statistically significant difference as regard BMF between diabetic patients and control. This study included 156 men, 24 % of them with T2DM. Lumbar vertebrae BMF was assessed by MRS. In age-adjusted analysis, T2DM was associated with higher BMF (59 %) compared with controls (55 %, p = 0.03) [37].

Our study is also supported by several studies on mouse models that showed increased marrow adiposity associated with DM. Also, morphometric analysis of the human bone marrow showed significant increase in both adipocyte number and size (1.7-fold larger) in diabetic patients compared with healthy controls [38-40].

In a multiple regression model, abundance of BMF was associated with diabetes, independent of age, sex and BMI. This suggests that DM has an impact on BMF accumulation that is independent from other major determinants of marrow adiposity [39]. Increased BMF in diabetic patients is considered as a facet of the whole-body accelerated senescence process. On the other hand, BMF aging can amplify senescence through direct endocrine mechanisms and also indirectly impinging upon regenerative mechanisms provided by mesenchymal stromal and hematopoietic cells [41, 42].

In a study on 128 cases of patients with T2DM (37 cases with normal bone mass, 43 cases with osteopenia and 48 cases with osteoporosis), IDEAL-IQ sequence was used to evaluate vertebral microstructure changes in T2DM patients and found that FF % in normal bone mass was 50.325 ± 7.243, in osteopenia 54.288 ± 6.736, and in osteoporosis 60.825 ± 6.783 (P-Valueb <0.001) [43].

In his study using m-Dixon-Quant method, Chang et al, 2020 found that FF% values were significantly higher in the osteoporosis (OP) group than that of the normal control group (FF% = 51.25 ± 7.38 in the control group; 54.70 ± 8.30 in the osteopenia group; and 62.53 ± 5.02 in the OP group; OP versus control, P < 0.05) [44].

In the present study, there was a statistically significant positive correlation between the FF at lumbar vertebrae vs. FF at femoral neck and also age in years. There was a statistically significant positive correlation between the FF at femoral neck vs age and sex being higher in males.

This is supported by Slade et al., 2012 study that also found that BMF sites had good correlation with one another (r = 0.411, p < 0.05 for vertebra and tibia) [45].

Chang et al., 2020 found that FF% exhibited a positive correlation with age ($F1,74 = 16.35$, $P < 0.0001$, $Y = 0.3807*X + 34.97$, Pearson's $R2 = 0.1810$) [44]. Also, Al Saedi et al., 2020 stated that there is increasing levels of BMF evident in aging and osteoporosis and is associated with decreased bone mass due to reduced osteoblastogenesis [46]. Another study on BMF stated that aging has been associated with an increase of adipocyte numbers, so higher BMF in T2DM is considered early aging of bone [47].

Other similar studies also reported an age-related increase of vertebral BMF measured by MRS found in different sample sizes (20 men with age range: 30–65 years and 24 women with age range: 30–69 years) [48], 154 healthy subjects (age range: 11–95 years) [49], and 259 healthy subjects (age range: 62–90 years) [50]. These studies also demonstrated a gender variance of the vertebral BMF, with higher values in males compared to females. Kugel et al., 2001 reported that males having approximately 6–10% more fat than females of comparable age between the ages of 20 and 60 years. Thereafter, vertebral BMF sharply increases in females between 55 and 65 years of age, i.e after menopause [49, 50].

Limitations

Lack of previous study assessing role of Dixon MRI in BMF quantification in T2DM for comparison, and less number of male patients compared to female. Multicenter study with larger number of cases and control are needed to validate the studies.

CONCLUSIONS:

DM leads to increased bone marrow fat and early bone aging. Dixon MRI is a fast reliable noninvasive method for quantification of BMF and measuring its FF and thus can be used as a potential alternative for gold standard MRS in routine clinical settings. BMF has been identified as a potential marker for DM induced skeletal fragility that can be used as potential therapeutic target for drugs. Restoring proper BMF content in diabetic patients may benefit, bone strength, metabolic homeostasis and hematopoiesis.

Abbreviations:

DM: diabetes mellitus
T2DM: type 2 diabetes mellitus.
MRI: magnetic resonance imaging
BMF: bone marrow fat
FF: fat fraction
BMI: body mass index
BMD: bone mineral density
BMAT: bone marrow adipose tissue
PPARγ: peroxisome proliferator-activator receptor-γ
DEXA: dual energy X-ray absorptiometry
QCT: quantitative computed tomography
HR-pQCT: high resolution peripheral computed tomography
3D: three dimentional
MRS: magnetic resonance spectroscopy
WO: water only
FO: fat only
TE: echo time
IP: in phase
OP: out of phase
ROI: region of interest
FOV: field of view
TSH: thyroid stimulating hormone
HB A1C: hemoglobin A1C
PTH: parathyroid hormone
CBC: complete blood count
VIBE: volume interpolated breath-hold examination
TR: repetition time
IDEAL: iterative decomposition of water and fat with echo asymmetry and least squares estimation
ROC: receiver operating characteristic
SI: signal intensity
AUC: area under the curve
OP: osteoporosis

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Medical Research Ethics Committee: IRB approval code number: MD.20.12.393

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