Assessment of Bone Mineral Density (BMD) by DEXA (Dual Energy Xray-Absorbiometry) in Type 1 Diabetes Mellitus patients and Co-relation with Glycemic and Non-glycemic parameters; An Observational study

THESIS

Submitted to

All India Institute of Medical Sciences, Jodhpur

In partial fulfilment of the requirement for the degree of

DOCTORATE OF MEDICINE (DM)

(ENDOCRINOLOGY)

BATCH: JULY 2020 **DR. SHINJAN PATRA**

AIIMS, JODHPUR

All India Institute of Medical Sciences, Jodhpur

DECLARATION

I hereby declare that the thesis titled **"Assessment of Bone Mineral Density (BMD) by DEXA (Dual Energy Xray-Absorbiometry) in Type 1 Diabetes Mellitus patients and Co-relation with Glycemic and Non-glycemic parameters; An Observational study"** embodies the original work carried out by the undersigned in All India Institute of Medical Sciences, Jodhpur**.**

DR SHINJAN PATRA
Department of Endocrinology
All India Institute of Medical Sciences
Jodhpur

All India Institute of Medical Sciences, Jodhpur

CERTIFICATE

This is to certify that the thesis titled **"Assessment of Bone Mineral Density (BMD) by DEXA (Dual Energy Xray-Absorbiometry) in Type 1 Diabetes Mellitus patients and Co-relation with Glycemic and Non-glycemic parameters; An Observational study"** is the bonafide work of **Dr. Shinjan Patra** carried out under our guidance and supervision, in the Department of Endocrinology, All India Institute of Medical Sciences, Jodhpur.

Guide

Dr. Ravindra Shukla
Additional Professor
Department of Endocrinology
AIIMS, Jodhpur

Co-Guides:

Dr. Mahendra Kumar Garg
Professor & Head
Department of Endocrinology & Metabolism
All India Institute of Medical Sciences
Jodhpur

Dr. Mithu Banerjee
Additional Professor & Head
Department of Biochemistry
All India Institute of Medical Sciences
Jodhpur

Dr. Madhukar Mittal
Additional Professor
Department of Endocrinology and metabolism
All India Institute of Medical Sciences

Jodhpur

All India Institute of Medical Sciences, Jodhpur

CERTIFICATE

This is to certify that the thesis titled **"Assessment of Bone Mineral Density (BMD) by DEXA (Dual Energy Xray-Absorbiometry) in Type 1 Diabetes Mellitus patients and Co-relation with Glycemic and Non-glycemic parameters; An Observational study"** is the bonafide work of **DR SHINJAN PATRA** carried out in the Department of Endocrinology, All India Institute of Medical Sciences, Jodhpur. It is further certified that the candidate has fulfilled the pre-requisites necessary for the submission of this thesis work.

DR. M.K.GARG
MBBS, MD, DM
Professor & Head
Department of Endocrinology & Medicine
AIIMS Jodhpur

All India Institute of Medical Sciences, Jodhpur

DECLARATION

I hereby declare that the thesis titled **"Assessment of Bone Mineral Density (BMD) by DEXA (Dual Energy Xray-Absorbiometry) in Type 1 Diabetes Mellitus patients and Co-relation with Glycemic and Non-glycemic parameters; An Observational study"** embodies the original work carried out by the undersigned in All India Institute of Medical Sciences, Jodhpur.

DR SHINJAN PATRA
Department of Endocrinology
All India Institute of Medical Sciences
Jodhpur

2

All India Institute of Medical Sciences, Jodhpur

CERTIFICATE

This is to certify that the thesis titled **"Assessment of Bone Mineral Density (BMD) by DEXA (Dual Energy Xray-Absorbiometry) in Type 1 Diabetes Mellitus patients and Co-relation with Glycemic and Non-glycemic parameters; An Observational study"** is the bonafide work of **Dr. Shinjan Patra** carried out under our guidance and supervision, in the Department of Endocrinology, All India Institute of Medical Sciences, Jodhpur.

Guide

Dr. Ravindra Shukla
Additional Professor
Department of Endocrinology
AIIMS, Jodhpur

Co-Guides:

Dr. Mahendra Kumar Garg
Professor & Head
Department of Endocrinology & Metabolism
All India Institute of Medical Sciences
Jodhpur

Dr. Mithu Banerjee
Additional Professor & Head
Department of Biochemistry
All India Institute of Medical Sciences
Jodhpur

Dr. Madhukar Mittal
Additional Professor
Department of Endocrinology and metabolism
All India Institute of Medical Sciences
Jodhpur

All India Institute of Medical Sciences, Jodhpur

CERTIFICATE

This is to certify that the thesis titled **"Assessment of Bone Mineral Density (BMD) by DEXA (Dual Energy Xray-Absorbiometry) in Type 1 Diabetes Mellitus patients and Co-relation with Glycemic and Non-glycemic parameters; An Observational study"** is the bonafide work of **DR SHINJAN PATRA** carried out in the Department of Endocrinology, All India Institute of Medical Sciences, Jodhpur. It is further certified that the candidate has fulfilled the pre-requisites necessary for the submission of this thesis work.

DR. M.K.GARG
MBBS, MD, DM
Professor & Head
Department of Endocrinology & Medicine
AIIMS Jodhpur

ACKNOWLEDGEMENT

"Satisfaction lies in the effort, not in the attainment"

–Mahatma Gandhi

The completion of this Thesis has been a journey of mettle and patience and it is with immense humility that I extend my gratitude to the people who aided me in this journey.

I would not be at this landmark moment without the trust and patient patronage of Dr. Ravindra Shukla, Additional Professor, Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, Jodhpur, my DM thesis Guide. His step-by-step support and guidance have been instrumental in completing this dissertation. I will always remain grateful and obliged to him for his guidance.

I would like to especially thank Dr. Mahendra Kumar Garg sir, Professor & Head, Department of Endocrinology & Medicine, AIIMS Jodhpur for his constant support and encouragement. His clarity of thought, vision and extensive knowledge in research has been a great source of inspiration for me. I am equally indebted to Dr. Madhukar Mittal, Additional Professor, Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, Jodhpur for his wisdom and constant words of encouragement.

I would like to take this opportunity to thank Dr. Mithu Banerjee, Additional Professor and Head, Department of Biochemistry, AIIMS Jodhpur for her guidance and for being always ready to help. I am truly honoured and grateful for the opportunity to learn under these wonderful teachers. I want to express my sincere thanks to Dr Ayan Roy, Assistant Professor in the department Endocrinology & metabolism in AIIMS Kalyani for giving a wider perspective to this dissertation.

I am also thankful to Dr. Prasoon Rastogi, my batchmate who made it easy for me to include patients for my research by stepping up in times of need to help balance the OPD and ward work and the commitments to my thesis. I would also like to thank my respected seniors Dr. Parul Gupta and Dr. Vanishri Ganakumar and beloved Juniors Dr Parth, Dr Eram, Dr Anupriya and Dr Pradakshna, Dr. Azher, Dr. Charmi and Dr. Saloni for their support during my

recruitment and follow up. All their constant cooperation and support had made my work a lot easier and comfortable.

I want to express my deepest regard to all our endocrinology department staffs, Mr. Sailesh Bhagat Pundari, Mr. Shivendra, Mr. Subhash Bishnoi, Mr. Ramswaroop Garwa, Mr. Sunil Dara, Mrs. Roshni, Mr. Raju Bhati for their kind help and support in any need for my thesis work.

Family is the backbone which has helped me to sail through this journey of residency. I am immensely thankful to my wife Mrs. Titas Saha Patra to stay with me in this tough 3 years period of residency 2000 kms away from our respective homes. It was very difficult to settle and execute all my work in the department and simultaneously study and finish the thesis. My wife had been there with mine and to overcome all such difficulties. I cannot be more thankful to her. My parents Professor (Dr.) Bidhan Chandra Patra and Dr. Shampa Patra have been instrumental in my academic journey so far. Their wisdom for knowledge, constant encouragement and caring for their only son has been the biggest blessing for me. I need to thank my in law's, Mr. Pradip Kumar Saha and Mrs. Joyita Saha for their huge contribution in making my residency a lot easier and feasible by staying back with ourselves and helping me out in all needs. I convey my deepest love and gratitude to my 6 months old boy, Shriyan for his lovely presence in my life and making my life as fruitful as it can be.

Last but by far not the least, I express thanks to all my patients without whom this milestone was not possible. Listening to them and understanding the problems they face daily, gave me the true insight about the management of Type 1 diabetes. To educate them regarding their bone health was one of the most satisfying moments for me.

I would like to end with the saying that nothing worth achieving has ever been achieved without the guidance of our teachers and the support of family and friends and patients.

"*Coming together is a beginning, staying together is progress, and working together is success*"

Dr Shinjan Patra

BMD DXA machine and our work

The Hologic QDR DXA machine has been the cornerstone of my thesis journey. My whole thesis work has revolved around this machine. We can proudly say that DXA machine of this calibre is not available in most of the leading institutes in India. It has been a fascinating journey with this DXA machine and we have learnt so much during this 2 years work. I convey my heartfelt gratitude and thanks to DXA technician Mr. Girish and Mr. Sunny for their profound co-operation. They have been instrumental in our study's completion success and no words of praise in enough for them to describe their tenacity and commitment to DXA work in our department. Mr. Girish has been very helpful in analysing of all 120 patients scans and taking out numerous mathematical data for my thesis. I convey my sincere gratitude for this.

Long live our BMD DXA machine and our technicians.

Dedicated to my soulmate Titas

and our adorable kid Shriyan

INDEX

LIST OF ABBREVIATIONS

T1DM	Type 1 Diabetes Mellitus
BMD	Bone Mineral density
DXA	Dual Energy Xray Absorptiometry
PPAR-λ	Peroxisome Proliferator Activated Receptor-γ
IGF-1	Insulin Like growth factor-1
GLP-1	Glucagon like peptide 1
GAD-65	Glutamate Acid Decarboxylase-65
IA-2	Islet Antigen-2
ZnT8	Zinc transporter 8
HRpQCT	High-resolution peripheral quantitative computed tomography
TBS	Trabecular Bone score
RR	Relative Risk
SPA	Single Photon Absorptiometry
DPA	Dual Photon Absorptiometry
BMC	Bone mineral content
BTM	Bone turnover marker
CTX	C-terminal cross links of collagen
vBMD	Volumetric bone mineral density
TBS	Trabecular Bone Score
AGE	Advanced Glycation end products
Runx2	Runt-related transcription factor 2
SMAD	Small mothers against decapentaplegic.
MMP	Matrix metallopeptidase
LRP	Lipoprotein receptor related protein
STZ	Streptozocin
IGFBP	Insulin like growth factor binding protein
IAPP	Islet Amyloid Polypeptide
MEPE	Matrix extracellular phosphoglycoprotein
RANKL	Receptor activator of nuclear factor-kappa B ligand
OPG	Osteoprotegerin
NOD	Non obese Diabetic

FRAX	Fracture risk assessment tool
HOMA-IR	Homeostatic model of assessment Insulin resistance
ANOVA	Analysis of variance
LS BMC	Lumbar spine bone mineral concentration
FN BMC	Femoral neck bone mineral concentration
WB BMC	Whole body bone mineral concentration
VAT	Visceral adipose tissue
TBF	Total body fat

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXURES

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder resulting in loss of pancreatic insulin-producing cells that presents in childhood or early adulthood. This tigger factor for autoimmunity has been proposed to be various viral infections to various external factors. Along with increased risk of complications including retinopathy, nephropathy, neuropathy, and cardiovascular events, adults with type 1 diabetes have decreased bone mineral density (BMD) compared with control subjects (1)(2)(3).

The increased magnitude of diabetes worldwide has compelled us to investigate more on bone health of such patients. In fact, osteoporosis is the most significant metabolic bone disease in individuals with diabetes. Patients with diabetes are at risk for osteoporosis and its complications, including hip fracture (4). Various studies have demonstrated that type 1 diabetes is associated with alterations in bone health in children and adolescents. Pre-pubertal, pubertal as well as post-pubertal patients with type 1 diabetes have decreased bone mass measured both by Dual-Energy X-ray Absorptiometry (DXA) scan and quantitative ultrasound (5). These observations suggest that adverse effects on bone health may occur early after the diabetes diagnosis itself. Understanding the natural history of BMD changes in young adults with T1DM may elucidate how the disease progresses and provide opportunities for prevention of significant bone loss and, presumably, fracture.

Potential pathogenic mechanisms of T1DM related bone damage may include hyperglycemia, autoimmune inflammation, increased marrow adiposity through increased Peroxisome Proliferator Activated Receptor-γ activity (PPAR-λ), Hypoinsulinemia and Hypoamylinemia, deficit of insulin like growth factor-1 (IGF-I), hypovitaminosis D and the non-enzymatic glycosylation of type 1 collagen with subsequent formation of advanced glycation end products (6). One factor that may contribute to a reduction in cross-linking in bone is the change in incretin hormones. It has been seen that impaired incretin function after the oral ingestion of food or glucose is associated with a decrease in cross-linking of collagens (7). The ingestion of food leads to an increase in glucagon-like peptide type 2 (GLP2) and GLP1.In both T1DM and T2DM, GLP1-related insulin release may be impaired. This may be associated with a decreased formation of crosslinks of collagen and thus possibly causes accumulation of fragile bone matrix. This improper bone formation and remodelling can also lead to less resistance to load.

In some studies, the early-onset and long duration of the disease has played a role in the T1DM associated bone loss, whereas in other studies, chronic diabetes complications predicted the high risk of fractures in T1DM patients. These differences are probably due to the non-homogeneous samples studied (i.e., evaluating eugonadal and hypogonadal together). Moreover, osteoporosis is a multifactorial disease in which different factors and environments interact.

Current knowledge gap and rationale of our study:

There is lot of knowledge gaps in the discussion of T1DM and BMD changes. First and foremost, the relationship between the glycemic status & the BMD is very conflicting in various studies. Expectedly poor glycemic status should lead to poor bone health, but it is not that simple as it is thought to be. Another very important aspect is the co-relation of BMD of a T1DM patient with duration of diabetes as well as age at diagnosis. Various studies have revealed conflicting results like mentioned above. The bio-chemical parameters like serum calcium, phosphorus, Bone-specific Alkaline-Phosphatase (bs-ALP) and 25 –Hydroxy-Vitamin-D doesn't co-relate well the bone findings as well in T1DM patients. Very inadequate evidences persist regarding relationship between pubertal status and BMD changes in T1DM patients. There have been no sufficient evidences establish relationship between the various auto-antibodies inT1DM patients like GAD-65, Anti-Insulin Ab, IA-2 and ZnT8 with BMD. Most of the studies have reported BMD at spine and hip as a standard protocol, whereas BMD at distal radius has been under-reported. BMD at various sites is reduced in presence of other auto-immune diseases like celiac disease, rheumatoid arthritis and auto-immune hypothyroidism. As a very common association with Type 1 DM, these diseases need to be evaluated as well during BMD work-up and effect over bone changes must be documented.

Another very important knowledge gap that persists is the relationship between bone quality scores and T1DM. Many of the studies including Indian studies have heavily focused on bone mineral density only in T1DM. The other bone parameters like trabecular bone score (TBS) and relationship of BMD with body mass indices, total lean body mass and total fat mass have not been discussed much in literatures. As per as the Indian studies are concerned, Joshi et al. had conducted a landmark study on T1DM and bone mineral density about a decade back (8). This was a remarkable study which had shown low bone density in all parts of the body.

However, the technology of TBS and body mass percentages were lacking at that time. The upcoming modality in studying bone histomorphometry in T1DM and other bone diseases have been HRpQCT. Various studies have evaluated this modality as a tool to understand the T1DM bony defects.

As a tertiary care centre in a North-western part of India, we have felt a huge unmet need for a study of BMD changes in T1DM patients. There have not been much comprehensive studies reported from this part of India with respect to BMD changes and T1DM patients. Some anecdotal case reports have mentioned about reduced BMD in T1DM patients from India, but a detailed analysis about the above said clinical, biochemical, and immunological parameters are largely lacking. Hence this study is planned with aim to evaluate BMD comprehensively in T1DM patients including physical, biochemical, and immunologic parameters which have been under reported in the literature. Our study is one of the first kind of Indian study which not only observes the BMD in T1DM, but also TBS and body fat and lean body mass analysis in T1DM.

Review of literatures

Global Prevalence data of T1DM:

The global prevalence and incidence of type 1 diabetes mellitus (T1DM) in children and adults is increasing. More than 1 million children were affected by T1DM by 2019, and the greatest increase in incidence was observed in children younger than 5 years (9,10). The global prevalence of type 1 and type 2 diabetes in adults is currently estimated to be close to 425 million, with an expected increase to 629 million by 2045 (3). The subsequent cost of treatment has increased in many folds for T1DM all over the world. India is certainly not an exception at any level. A meta-analysis including nearly 140,000 subjects with fractures reported a pooled relative risk (RR) of any fracture of 3.16 (95% CI 1.51–6.63; $p = 0.002$) (8) hip fractures of 3.78 (95% CI 2.05–6.98; $p < 0.001$), and spine fractures of 2.88 (95% CI 1.71–4.82; $p < 0.001$) in type 1 diabetes (4).

Indian Prevalence data of T1DM:

The available published studies from different parts of India reveal the prevalence of T1DM in the range of 3.7 to 10.2 per 100,000 populations, with higher prevalence in urban area and in males. The improved health care facilities across the nation, the increased awareness among commoner, the establishment of various tertiary care centers in various parts of country has ensure more detection of T1DM and their proper treatment (9,10) (11,12). Along with improvement of healthcare and easy availability of insulin, much cheaper in rate compared to western counterparts, India has progressed firmly on its way to treat T1DM effectively. There has been significant decline in the prevalence of microvascular complications along with ketoacidosis episodes in Indian patients (13,14). There is definite lack of data in bone involvement in T1DM from India.

Osteoporosis/Low bone mass data from Indian T1DM patients:

Before the advent of DXA machine, BMD was measured by single photon absorptiometry (SPA) or dual photon absorptiometry (DPA). In all those measurements, T1DM with adolescents had shown poor bone mineral mass (15,16). Even with normal bone mass in T1DM, the fractures risks have found to be higher. Various studies have confirmed this disproportion in a great way (8). However, this data's have not been consistent across all the studies. The only published study from western India by Joshi et al., compared 86 patients of T1DM between 12- 45 years of age and mean disease duration of 14.6 years with age, sex and

body mass index (BMI) matched controls. BMD of total body and lumbar spine was significantly lower in patients with T1DM compared to controls. Furthermore, patients with T1DM had 10% less bone mineral content (BMC) in comparison with controls (17). Furthermore, patients with T1DM had 10% less bone mineral content (BMC) in comparison with controls (17).

Bone phenotype in T1DM:

The abnormal bone phenotype of T1DM can be discussed into 4 categories:

- Low BMD
- Disruption of the bone microarchitecture
- Increased fracture risk even with normal BMD resulting in 6-fold increased prevalence of hip fractures in individuals with T1DM compared to healthy adults
- A low bone turnover status (18,19) (20).

Diabetes has been related to osteoporosis and low bone mass for a long time. Type 1 diabetes has been specifically associated with low bone mass for various reasons. The exact etiopathogenesis has not been elucidated properly till now but has been extensively discussed in various review articles and meta-analysis. The basic concept is bone mass is generally reduced in T1DM but preserved in T2DM. Even high bone masses can be found in T2DM, but all of these situations lead to increased fracture risks (3). Recurrent hypoglycemic attacks leading to recurrent fall and trauma, microvascular complications like neuropathy, vision loss due to retinopathy, impaired renal function leading to active vitamin d deficiency, secondary hyperparathyroidism, uremic osteodystrophy can all lead to increased chances of fall and fractures in T2DM (21). All these mechanisms apply for T1DM also but the bone architecture has also been proven to be affected in T1DM (22). This low bone mass persists for whole life of such patients and the risk of fracture aggravates around 3rd or 4th decade (23). Though the bone loss has been seen to co relate with the glycemic control, but it does not seem to co relate well with the duration of the diabetes. Various studies have failed to establish this relationship (19).

Physiology of bone accrual in adolescents and children:

Peak bone mass accrual is the major determinant of bone density and future fracture risks. It increases continuously following a steep increase during the pubertal stage. In girls, peak bone mineral accrual occurs 2 years earlier than in boys, approximately 0.7 years after reaching peak height velocity, and synchronously with menarche. The bone mineral content reaches the plateau around 6 years after achieving the peak height velocity and it remained till 2nd or 3rd decade of life (7). Males have generally higher bone density than females due to various reasons. Males have larger bones. They have periosteal bone deposition compared to endosteal bone deposition in females. The gain in bone concentrations continues beyond puberty in males in contrast to females where the bone density falls after 2nd decade mostly. Surprisingly men with T1DM are more prone to low bone mass compared to their female counterparts. Hypogonadism is more prevalent in T1DM men which could possibly explain the relative low bone mass in men T1DM (24).

Height and height velocity in T1DM:

The reductions in serum IGF-1 levels found in T1DM were therefore thought to be the cause for growth abnormalities in these subjects. Detailed discussion regarding abnormality in GH and IGF-1 axis in T1DM is done in osteoblast sections. However, this was not obvious in all studies as some reports indicated improved and others impaired growth velocity.

Large retrospective studies have shown that children with T1DM gained more weight during infancy and showed a higher linear growth when compared to controls at the time of diagnosis (25,26). Songer et al. had observed that T1DM patients had longer linear height compared to controls at the time of diagnosis. This study was carried out among 200 odd patients (27). However, during pubertal growth T1DM children showed attenuation of height gain, which was subsequently shown to be sex-dependent, and attributed to reductions in serum IGF-1 levels.

There are conflicting reports showing normal or slightly reduced final height in T1DM subjects (28). In the Oxford study, final heights were not significantly different from the mid-parental height SDS despite a reduced growth spurt during puberty. The poor growth velocity is seen in only very poor glycemic control patients.

Altered Bone biomechanical competence in T1DM:

The factors that underlie altered bone biomechanical competence may include: i) altered matrix competence (e.g., through the glycation of collagen or an altered crystal structure); ii) altered bone turnover; or iii) altered bone density or microarchitecture or structure, which may be a result of the previous two factors. These mechanisms have been proposed to be a chief pathogenic cause for low bone mass in Type 1 diabetes. Some factors that affect bone biomechanical competence and/or turnover may be common among T1D and T2D, as is the case with hyperglycemia. Others, such as the lack of endogenous insulin secretion in T1D and insulin resistance and, in some cases, frank hyperinsulinemia in T2D, may be less common (29).

Altered bone turnover:

Diabetes has been described with low bone turnover in various rodent and human studies (30) (31) (32) (33) (34). However this difference have not been noted in all T1DM studies till date (5). Various biomolecules released into the circulation during bone resorption and formation are called Bone Turnover markers (BTM). Assessment of the BTM's are an indirect or surrogate marker of bone turnover status. Decreases in osteocalcin and C-terminal crosslinks of collagen (CTX) were seen, whereas other markers in general were not altered; there was large heterogeneity in all of the biochemical markers of bone turnover because of the differences in the assays that were used (35). The link to CTX may be interesting, because it represents the crosslinking of collagen and is thus a contributor to bone biomechanical competence, which may not be fully reflected in bone mass, density, and structure. However, a recent meta-analysis found an increase rather than a reduction in NTX. In both T1D and T2D, GLP1-related insulin release may be impaired. This may be associated with a decreased formation of crosslinks (including, among others, CTX) and thus possibly with the accumulation of fragile bone matrix. This decreased resorption could in theory also lead to an accumulation of old bone, which may perhaps be less resistant to load (36) (37). The duration of the suppression of CTX (and, to some degree, P1NP) is short-lived and seems to last for only 3–6 h. Frequent meals may be prescribed for some patients with T1D in the form of, say, snacks in order to prevent hypoglycemia. This may perhaps also explain why patients with metabolic syndrome have lower bone mineral density (BMD) than expected after adjustment for confounders and a slightly increased risk of fractures. Unadjusted, the higher body mass index (BMI) that occurs in metabolic syndrome is associated with higher BMD (38). With presence of persistent hyperglycemia, bone cells are less likely to get adhered (39). T1DM bone

remodelling in stark contrast to the post-menopausal osteoporosis where there is high remodelling of the bone (3).

Bone density and microarchitecture:

As previously described bone density is generally reduced in T1DM and preserved in T2DM but the fracture risks increase in both the cases (29). These findings suggested to be a reduced mechanical impedance of bone in diabetes. But to describe this increased fracture risk, bone mineral density is often found to be insufficient.

BMD DXA as a tool for bone density:

The major limitation of DXA in children and adults is that it measures bone mineral content (BMC) and not true volumetric BMD (vBMD). As a surrogate, BMC is divided by the area investigated to reveal areal BMD, which becomes a surrogate of true BMD. Consequently, areal BMD underestimates the vBMD of small bones, whereas it overestimates vBMD in children with tall stature. In contrast, the method of peripheral quantitative computed tomography (pQCT) directly calculates vBMD, measures the cortical and trabecular compartment, and assesses bone geometry as well as the bone muscle unit (40). Thus, DXA machine is having the limitation of measuring cortical and trabecular bone, not separately.

Additional tools for bone mineral density:

Trabecular bone score has been developed as an additional tool to describe the bone microarchitecture which can more effectively determine the fracture risk (41). Some of the studies have not found differences in BMD in many sites among T1DM and controls, but TBS have been found to be significantly low in T1DM compared to controls (42). Computerized tomography (CT)-based techniques include high-resolution peripheral quantitative CT (HRpQCT), which may scan the forearm or tibia, and QCT, which may scan the lumbar spine, hip, or forearm are another set of non-invasive investigations that can detect the microarchitectural alteration of the bone (35). These techniques have been found to be useful to detect bone microarchitectural differences which are not picked up by BMD DXA. These HRpQCT techniques have detected increased cortical porosity in diabetic patients in some studies however this findings have been refuted in other studies as well (43). These HRpQCT techniques have detected increased cortical porosity in diabetic patients in some studies

however this findings have been refuted in other studies as well (44). The prime limitation of HRpQCT measurements is its cost which makes this installation difficult even in the best of tertiary care centres in western world. The improved and newer DXA machine use is quite prevalent in India now, available in some central government autonomous institutions in tertiary levels. The availability of HRpQCT machine is quite scarce now. There is very few data's of HRpQCT from India even in the post-menopausal women, and practically no data's on T1DM. This is an unmet need which needs to be addressed.

MRI in one of young T1DM female studies have confirmed increased bone porosity and structural alterations (6). Increased trabecular spacing has been described in such studies. Increased presence of microvascular complications like retinopathy has been found to be associated with such findings (6). The possible explanations behind this increased porosity has not been well elucidated till now as more research is warranted.

Guidelines in diagnosing osteoporosis in T1DM:

There are no guidelines available to clinicians on how and when to measure the BMD in children with T1DM. The International Society of Clinical Densitometry (ISCD) recommends that bone mass measured by DXA should be reported as BMC or areal BMD which can be compared with reference values from children of similar age, gender and if possible, race/ethnicity to calculate Z-score. The basic problem lies in this point as normative data of such age group population is scarce. This is especially worrisome in Indian context as no normative data exist in terms of pediatric population. The DXA machine which we use, the discovery hologic, uses Asian ethnicities data to calculate the "z" and "t" score. Though this Asian ethnicities population includes Indian, the reliability of the data is doubtful (45). The FRAX toll data we use is also derived from Singaporean Indians, which is again not that reliable.

Basic factors influencing the bone mineral density alteration in T1DM:

Altered bone matrix competence:

Bone matrix is a composite material consisting of hydroxyapatite, cells, and organic matrix, which among other components contains collagen. These various components all contribute to biomechanical competence, such as resistance to pressure, traction, or torsion. The resistance to these strains may vary, and bone may be less resistant to, say, torsion than it is to pressure. Calcium hydroxy appetite crystals add to the matrix competence of the bone. Collagen adds to the support and strength (46). Mouse model studies have shown that peak bone mass achievement in T1DM have not hampered but the presence of advanced glycation end products can hamper this process (47). It causes reduction in stiffness, energy absorption, elastic modulus, and maximum load. Accumulation of pentacidine and poor cross linking of collagen adds to the poor bone matrix competence. Imperfect formation of calcium hydroxyapatite crystals is also a potential pathogenic mechanism (48). Increased pathological cracks are associated with this phenomenon. High osmotic pressure (mannitol), osteoblasts increase the secretion of type 1 collagen by a 12-fold factor, which indicates an excess of organic matrix production (49).

Impact of insulin status and glucose levels:

Insulin exerts its anabolic action on osteoblasts and hypoinsulinemia leads to low bone mass specially in T1DM (50). Persistent hyperglycemia leads to hypercalciuria status which can adds to the poor health of bone (51).

Impact of anti-diabetic medications:

Pioglitazone, an important drug used in T2DM to counteract the insulin resistance, converts stem cell development to adipose cells in place of osteoblasts. This can explain the potential bone density losing property of such class of drug (52). Constant hyperglycemia leads to upregulated peroxisome proliferator-activated receptor type gamma activity which can itself cause the same mechanism as like pioglitazone for bone loss (53). As per as only type 1 diabetes is concerned, inulin usage per se does not cause any bone density alteration. But inappropriate doses leading to recurrent hypoglycemic attacks and frequent falls can lead to increased risk of fractures (3).

Impact of alterations in the Wnt and LDL receptor-related protein 5 pathways:

The anabolic Wnt pathway is affected in diabetes. LDL is known to play a role in bone cells, and the Wnt pathway may be a mechanism for increased fracture risk with low LDL levels as a result of statin treatment. The LRP 5,6,8 has been designated to be a positive regulator of Wnt pathway and osteoblast differentiation (54,55). LDL binds to the LRP through apolipoprotein B and E moieties and may therefore stimulate the Wnt pathway. Randomized controlled trials have shown that statin treatment, leading to decreased LDL, can lead to significant bone loss. Though this evidences are insufficient (56).

Basic differences between T1DM and T2DM:

A host of factors differentiate the bone pathobiology among these two entities. The constant hyperglycemic state, advanced glycation end products (AGE), presence of microvascular complications like retinopathy, renal involvement and neurological involvement can lead to poor bone mass and quality. The cross linking of collagen is also severely impaired. These are common mechanisms in both type of diabetes. What differentiates T1DM from T2DM is the failure to attain the peak bone mass during pubertal development in T1DM (22). These patients mostly got detected diabetes in their 2nd decade and failed attain the bone accrual. So, this comparatively 'thin' bone can have falsely low BMD in BMD DXA which uses 2D technology to calculate the bone mass, however matching the T1DM population with same body mass and body proportions can avoid these problems. All cases need to be matched controls with same BMI. HRpQCT can be a good tool to differentiate between such cases (57).

Low BMI in T1DM and relationship with BMD:

BMI is a major influencing factor in determining BMD in T1DM. The adipose tissue, apart from providing mechanical loading, also increases BMD through the activity of adipocytokines (58). A constant catabolic state of T1DM leads to very few adipocytokines in patient body; expectedly these patients are having low BMI and chances of low bone mass. Studies measuring the adipose tissue mass and lean body mass and relationship with BMD is very scarce. No Indian studies have evaluated this point so far.

Association of other auto-immune diseases and association with BMD in T1DM:

Auto-immune thyroid disease and celiac disease ae the commonest autoimmune association in T1DM. Both these diseases are associated with low bone mass in T1DM. However, the effect of diabetes on bone exclusively cannot be determined by the presence of such diseases. So an ideal study should be formulated with exclusion of such diseases to see the effect of T1DM on bone (59).

Muscle bone cross talk:

Bone and muscles are integrated organs that exert a mutual control and are in turn controlled by several factors, such as the GH-IGF-1 axis, sex steroids, adipokines (e.g., leptin, adiponectin, visfatin, resistin) and vitamin D. In addition to mediating the muscle-bone crosstalk, muscles release myokines that affect other organs and tissues, including the liver, intestine, and adipose tissue, which in turn release cytokines and hormones responsible for regulating bone homeostasis. Among the myokines, irisin is a small peptide derived from the proteolytic cleavage of fibronectin III domain-containing protein 5, produced during physical exercise (3,24).

Alteration in bone molecular level in T1DM:

Effects of T1DM on osteoblast:

Osteoblasts (OBs) are essential to bone formation; they synthesize collagen, mineralize osteoid and participate in bone remodelling. T1D effects on OBs and their progenitor cells have been studied extensively and appear to involve various mechanisms that synergistically act to cause osteoblast dysfunction (60). Genes encoding various osteoblast progenitor cells affected in T1DM. Runt-related transcription factor 2 (Runx2), the master regulator of bone development, directs differentiation of mesenchymal cells into pre-osteoblasts, promotes the formation of the immature osteoblast and inhibits differentiation of mesenchymal cells into adipocytes and chondrocytes (61). Runx2 is positively regulated by β-catenin and negatively regulated by Peroxisome Proliferator Activated Receptor Gamma (PPARγ), SMAD Family Member 3 (Smad3) (62). In animal models of insulin deficiency, there is definite reduction of Runx2 transcripts, where correction of uncontrolled hyperglycemia has reversed its levels. Along with down regulation of Runx2, matrix metallopeptidase 9 (MMP-9), matrix metallopeptidase 13

(MMP-13), Ibsp, Col1, phosphate regulating endopeptidase homolog, X-Linked (Phex), dentin matrix acidic phosphoprotein 1 (DMP-1), osteopontin and OC all are downregulated (63). However, these results have not been very consistent throughout the studies. In cell culture, osteoblasts respond to insulin by increasing collagen production and increasing osteocalcin which in turn has a "feed-forward" role in stimulating pancreatic beta cell proliferation and insulin secretion. The Wnt/beta catenin pathway, which is essential for osteoblast differentiation and regulation of bone formation in differentiated osteoblasts, has also been shown to be down regulated in the bone of STZ-induced diabetic animals (64).

IGF-1 as trophic factor for osteoblast:

Diminished gene expression of IGF-1, IGF-1 receptor (IGF-1R) and insulin receptor (IR) in bone marrow stromal cells (BMSCs) and low protein levels of IGF-1 and IGF-1R have been seen in STZ-induced diabetic rats. Linear and radial bone growth as well as gains of bone mineral density (BMD), are greatly affected by GH, the endocrine (serum) IGF-1, and bone-tissue derived IGF-1 (autocrine/paracrine IGF-1) (65). Together with insulin, GH and IGF-1 also regulate carbohydrate and lipid metabolism to facilitate body growth, puberty, and maturation.

GH-IGF-1 axis abnormalities in T1DM:

Typically, T1DM patients display high GH with low IGF-1. This is the prime example of GH resistance which is seen in various chronic diseases like chronic liver disease, malnutrition etc. GH response exaggerated during puberty in T1DM where insulin resistance is observed maximally. This insulin resistance is mostly a physiological response (66). Low IGF-1 is possibly related to hepatic GH resistance and portal insulinopenia (67). Increase in inflammatory cytokines like IL-6 and IL-8 can affect the low IGF-1 state. The IGF-1 levels were also negatively correlated with glycemic control, particularly during puberty. Various case control studies have established this association (68). Most of the studies have established low IGFBP1 which can explain the growth impairments in T1DM. Increased body adiposity causes rise in IGFBP2. IGFBP3 seems to remain unaltered in T1DM though there are some studies which have shown low IGFBP3. Free IGF-1 is shown to be normal to high in patients with T1DM which can possibly explain unaltered adult height in moderately controlled T1DM

(69,70). In 1930, Mauriac described a girl with T1DM who had poorly controlled diabetes and showed hepatomegaly and short stature. Nowadays, Mauriac syndrome is rare (71).

IGF-1 stimulates the production of osteoblasts from the mesenchymal stromal cells; essential for bone mass development (72). It exerts its action through IGF-1R. As insulin shares a definite homology with IGF-1, it can activate the IGF-1R with lower affinity. The IR and IGF-1R are present in the osteoblasts which are responsible for the osteoblastic cell production for mesenchymal stem cells. IR knock out mice have shown to be associated with poor trabecular bone status, poor post-natal growth and low bone density (73,74). Decreased insulin and poor IGF-1 thus accounts for an important causative factor behind low bone mass in T1DM. In patients with uncontrolled T1DM, the levels of free IGF-1 are low due to increase in IGF-binding proteins particularly IGFBP3. Hence, low IGF-1, as a result of insulin deficiency may result in low accrual of peak bone mass (58). Glucose is a necessary nutrient for most cells including osteoblasts and osteoclasts. In vitro, osteoclasts and osteoclasts are inhibited by excess glucose in the media. In vivo, the effects of glucose are more complicated with either a direct effect of glucose or an indirect effect through insulin release or inflammation (75).

Amylin deficiency in T1DM:

In addition to insulin, patients with T1DM are deficient in amylin (IAPP); a hormone co-secreted with insulin by pancreatic beta cells. In rodent models of T1DM, amylin has been shown to increase osteoblastic and chondrocyte proliferation activity, while suppressing osteoclastic proliferation and activity (76).

T1DM on number and survival of osteoblasts:

Low numbers of osteoblasts accompanied by low osteoid formation and mineralization have been documented in bones of rats and mice with autoimmune- or STZ-mediated diabetes (77). Apoptosis of the osteoprogenitor cells due to continuous oxidative stress can explain such findings whereas administration of insulin and IGF-1 have been found to reverse these situations. An interesting finding about PTH has also been found in such studies. In poorly controlled T1DM iPTH has been found to be low which can partially explain the poor osteoblastogenesis in T1DM (78).

Effect of T1DM osteoblast activity and osteocalcin:

T1DM significantly retards the action of osteoblasts. Osteocalcin, a marker of late-stage osteoblast development and action, is severely decreased in T1DM indirectly establishes the negative association between osteocalcin and poorly controlled diabetes. Human studies have also proven this association (76,79). Poor collagen synthesis is associated with poor osteoblastogenesis.

Hyperglycemia and inflammatory changes on osteoblast activity:

AGEs impair mineralization when present in a high glucose environment in vitro and they increase bone marrow stromal cells (BMSC) apoptosis through inflammatory and oxidative stress-inducing mechanisms (80). Pancreatic beta cells got destroyed by autoinflammatory cytokines and leading to absolute insulin deficiency. These inflammatory cytokines impair the functions of osteoblasts. Poor glycemic control leading to increased levels of TNF-α, IL-1, IL-6 which have been shown to negatively impair osteoblast differentiation and functions (81,82).

Leptin action on osteoblasts:

In-vitro studies demonstrate that leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. Some studies have shown high serum leptin levels as well as some studies have shown low leptin levels in T1DM (83). However, with treatment of insulin leptin levels increase in T1DM. Possible high leptin levels can also be associated with low bone mass in T1DM. This needs to be more investigated before final conclusion (84).

Effects on T1DM on osteocytes:

As new bone mineralizes, terminally differentiated osteoblasts become embedded within lacunae of mineralized tissue transforming into osteocytes, the most abundant of bone cells. These embedded cells maintain their intercellular communication by multiple filopodial cellular processes which extend throughout the lacunar-canalicular pore system, linked by gap junctions (85). Osteocytes mainly acts as a chief mechanosensory cells of the bone which is responsible for the sensing the biomechanical forces (85). Their role in mechano-sensation is paramount to inducing skeletal shape, density and size adjustments. However, data's on osteocytes are quite limited due to relative inaccessibility.

Osteocyte activity, sclerostin and T1DM:

Osteocytes secrete sclerostin, an inhibitor of the Wnt pathway. It impedes the osteoblast synthesis and thus acts as a negative regulator of bone synthesis (86). Mechanical loading, high estrogen, high PTH decreases sclerostin levels, whereas mechanical unloading, advancing age, detrimental bone quality all increases sclerostin levels. Most of the studies, be it be streptozocin induced diabetic rats or human models, have depicted high sclerostin in T1DM patients (87,88). Sclerostin neutralizing antibodies have shown to reverse the bone findings in T1DM. The reasons behind increased sclerostin is not clear in T1DM however advancing age in puberty, high glycated hemoglobin, associated insulin resistance can give rise to exaggerated sclerostin response (89,90). The co-relation of high sclerostin and increased fracture risks in T1DM is however is debated in some studies (91).

DMP1, FGF-23, MEPE in T1DM:

Not much is known about the effect of T1D on other osteocyte-expressed proteins, such as dentin matrix protein-1 (DMP1), fibroblast growth factor 23 (FGF23) or matrix extracellular phosphoglycoprotein (MEPE). Insulin clamp technique has shown that FGF-23 doesn't differ significantly in T1DM (92).

Osteocyte number and survival in T1DM:

A significant reduction of lacunar structure is seen in animal models of T1DM. Perhaps the failure to synthesize osteoblasts in adequate number is the key underlying reason (93). A rat model of insulin deficiency has also proved deficient sclerostin positive osteocyte numbers (94). Insulin sensitizer had reversed the findings in this study.

Mechanical loading and T1DM:

Male Akita mice, a model of severe insulin-deficiency diabetes, demonstrate impaired bone formation in response to repetitive mechanical loading of the ulna, when compared to wild-type mice or to female Akita mice (a model of milder blood glucose elevation). The constant hyperglycemic state impairs the mechanosensory stimulus for osteocytes development (95).

Effect of T1DM on osteoclasts:

Bone resorption, a key role played by osteoclasts, is altered and increased in t1DM. Poor glycemic status leads to increased bone turnover and bone loss in T1DM (96).

Modulation of RANK/RANKL/OPG system in T1DM :

Receptor activator of nuclear factor-kappa B ligand (RANKL) binds receptor activator of nuclear factor-kappa B (RANK) in pre-osteoclasts and promotes osteoclast differentiation, survival, and activation. The interaction between RANKL and OPG, its decoy receptor plays the vital part in regulating osteoclastogenesis. Various studies have unequivocally demonstrated increased RANKL activity in T1DM. RANKLS has been shown to be less sensitive to OPG in T1DM than controls (97). Most of the other studies have found out reduced OPG synthesis in poorly controlled T1DM. Osteoclastogenesis is exaggerated by chronic hyperglycemic state, insulin deficiency states as in T1DM. Advanced glycation end products also causes similar pathobiology (98).

Other markers of increased osteoclastogenesis in T1DM:

Increased cathepsin K, TRAP (tartrate-resistant acid phosphatase) expression is seen in T1DM. In addition, osteoclast cultures from NOD mice contain smaller osteoclasts, yet they resorb more bone than control osteoclasts and express more cathepsin K, MMP-9 and pro-osteoclastogenic mediators (99). However, some other studies have refuted these findings. With so many contrasting findings, it is postulated that the timing of onset of diabetes can predict the osteoclastogenesis response (100).

Type 1 diabetes and osteoporosis: the molecular pathway:

Though the post-menopausal status has been the front runner cause for osteoporosis, the bone fragility in diabetes has come up mostly in the last decade. The researches which have been carried out in last 2 decades have equivocally proposes low bone density in type 1 diabetes but mechanisms, types, pathobiology have not been very well explained.

T1DM and bone matrix:

Besides having direct effects on the cells involved in bone remodelling, DM1 also affects the bone matrix, thereby modulating bone quality. These effects are mediated by the formation of

advanced glycation end (AGEs) products, which are produced due to nonenzymatic glycosylation of proteins or lipids and are implicated in multiple diabetes complications, including bone fragility (101).

Biochemical markers of bone fragility:

The bone content of pentosidine, the most abundant AGE in non-diabetics with hip fracture was greater than in those without hip fracture. Bone pentosidine levels are related to the strength of the human vertebra, independent of BMD (102). Increased bone pentosidine have been associated with increased vertebral as well as non-vertebral fractures in diabetes. This AGE has been mostly linked and studied in T2DM but its role in T1DM is also proposed.

Histomorphometry in T1DM:

Histomorphometry of fluorochrome double-labeled (typically with tetracycline or demeclocycline) trans-ilial bone biopsy is still considered the gold standard for measuring bone turnover. The bony specimen is about 7 mm in width and spans both cortices as the sample is taken from below the iliac crest. Bone sections embedded in plastic; it is seen under microscope. The most common reported derived dynamic variables are bone formation rates (BFR), estimates of bone volume and surface that are replaced every year, and activation frequency (AcF) which is the best index of bone remodeling.

Histomorphometry studies in T1DM:

Arma's et al have investigated 84 patients of T1DM, where they have not found any histomorphometric difference between cases and controls. The authors later published the data's of the complete cohort which did not show any difference. This cohort was consisted of relatively young diabetics with adequate glycemic control without much complicating features. Structural histomorphometry measures from this study also correlated with Trabecular Bone Score of the spine measured by DXA (103).

PTH and vitamin D axis:

PTH-levels may be low or hypo-responsive in adults with diabetes, in line with the low bone turnover status. Hypomagnesaemia-related modulation of the calcium- sensing receptor

sensitivity may contribute to relative hypoparathyroidism contributing to low bone turnover. Though the exact epidemiological data's are not present, a state of relative PTH resistance is found in T1DM (51) (104). A tri-sodium-citrate calcium clamp study performed in 15 adult T1D participants and 19 matched controls suggested that T1D participants had a lower set-point for PTH secretion, but normal PTH responsiveness to hypocalcemia (105). Renal dysfunction associated with T1DM can alter the PTH status and act as a confounding factor.

Relationship of vitamin D status with T1DM:

The status of vitamin D is also vary across various geographical regions. Australia and African nations along with notably India comes under the endemicity of vitamin d deficiency. In an Australian study (27.5°S), 10% of the participants were vitamin D deficient and the mean 25-OH-D3 level was significantly lower in children with T1DM than in controls ($p < 0.002$). In a Swiss cohort (46.6°N) 60% of the participants were vitamin D deficient by the end of summer (106,107). Generally, vitamin D supplements do not improve bone mass in vitamin D sufficient children. However, according to 2 Cochrane Reviews in children with 25-OHD3 levels below 35 nmol/L, an increase in total body BMC and lumbar spine BMD of ∼2% over a follow-up time of 1–2 years was recorded upon supplementation. Therefore, adequate supply of vitamin D seems an important factor to limit diabetic bone disease (108,109). A registry study conducted in US children found that 36 % of T1D participants were deficient in vitamin D; however, the prevalence was similar to what was reported in similarly aged children from the nationally representative National Health and Nutrition Examination Survey (110). However another large cross sectional study from Denmark catering over 1000 patients of T1DM did not reveal any deficiency of vitamin D status (111). Associated with poor sunlight exposure and nutritional deficiency, another factor that stands out as a causative factor behind hypovitaminosis D is increased excretion of vitamin d binding proteins. With persistent hyperglycemia due to poor glycemic status, and associated microvascular complications, this protein is getting lost in urines. This factor is often overlooked in T1DM. However one notable study have refuted this claim by proving no relationship of serum 25 (OH) D levels with hba1c (112).

Microvascular complications in diabetes:

Campos-Pastor et al. showed that adults with T1DM with microvascular disease had progressive bone loss despite good glycaemic control (113).showed that adults with T1DM with microvascular disease had progressive bone loss despite good glycaemic control (113). Though T1DM can present initially with bone loss but this bone loss can exacerbate during later stages of complications. Authors have suggested two distinct stages for bone loss for such patients. In 1st phase there is bone loss due to various bone related mechanisms followed by plateau phase. The 2nd phase initiated after the onset of various microvascular complications (114). Differences between trabecular and cortical remodelling may explain why the trabecular compartment is more involved in the first phase and the cortical compartment more in the second phase of the disease. Cortical bone formation depends on vascular pericytes; microvascular complications causes cortical bone loss due to defect in the osteoblastogenesis.

The Framingham Heart Study showed that loss in cortical bone is associated with progression of atherosclerotic disease over a 25 year of follow-up period. The role of VEGF in microvascular complications like retinopathy, nephropathy and neuropathy have been well documented and discussed across various literatures (115,116). In addition to angiogenesis, VEGF is a key determinant of bone vascularization regulating osteoblast differentiation, bone repair and post-natal bone homeostasis.

Diabetic nephropathy:

Almost 40% patients of T1DM will develop diabetic nephropathy in 30 years of disease. Progression to nephropathy is associated with impaired calcium-phosphorus metabolism, and therefore bone metabolism, primarily renal osteodystrophy. Adynamic bone disease is a quite prevalent feature in a T1DM patient (117). This is characterized by low bone turnover, low bone volume and markedly decreased cellularity, but almost normal mineralization. This manifests in decreased trabecular bone density determined by pQCT and increased cortical bone density compared to patients with high-turnover lesions (118). Such instances are the classic examples of increased fracture risks even without decreased BMD. Diabetic nephropathy comorbidities leading to PTH deficiency, together with the Vitamin D deficiency occurring synergistically propel the individual towards reduced bone turnover and bone fragility (22,104).

Diabetic retinopathy:

Retinopathy is seen in about all patients after 40 years of T1DM. It is directly related to excess VEGF (119). The presence of proliferative diabetic retinopathy unequivocally causes poor bone quality in T1DM which is established in various studies (120) (114). An intimate relationship between bone microvasculature and bone cells mediated by endothelial cells does exist. The proximity of the bone-forming "basic multicellular unit" (BMU) to the microvasculature is critical in maintaining bone anabolism. Alterations of bony architecture does happen in presence of high VEGF and abnormal bone morphogenetic proteins such in case of diabetic retinopathy (121).

Diabetic neuropathy:

Diffuse symmetric polyneuropathy and autonomic neuropathy are the commonest neurological association in T1DM. The incidence rate is pretty like retinopathy. Autonomic neuropathy is caused by the damage of small (Aδ, B and C) nerve fibers and characterized by the imbalance of sympathetic and parasympathetic activities. The dysfunction affects the α & β receptors which are heavily expressed on osteoblasts. So presence of significant autonomic neuropathy impairs the actions of osteoblasts and resultant altered bone turnover affects the bone quality in T1DM. This is supported by evidence from rodent models which shows activation of these adrenoceptors causes impairment in trabecular bone microarchitecture bone mass and strength (122,123).

Macrovascular complications in Diabetes:

Cardiovascular diseases constitute the major cause of mortality among T1DM patients. These patients have co existing high-grade inflammation and oxidative stress. Endothelial dysfunction is a common accompaniment in such cases along with presence of retinopathy and neuropathy (124). Several studies have found out higher cardiovascular mortality with low bone mass in T1DM. This link has been documented in the Prospective Epidemiological Risk Factors Study which found an increased risk for hip fracture with aortic calcification (adjusted RR: 2.3 95%: [1.1–4.8], p=0.03) and in a Swedish population-based case-control in which women without diabetes but with CVD were twice as likely as those to have fractured than those who did not (adjusted odds ratio for hip fracture 2.38 [95CI: 1.92–2.94]) (125). Significant VEGF responses are seen in hypoxic conditions leading to increased osteoclast

absorption. Chronic hypoxia leads to anaerobic conditions leading to impaired osteoblastogenesis. Factors including inflammatory cytokines, oxidative stress, vitamin K and vitamin D deficiency, bone morphogenetic proteins (BMPs), osteocalcin and sclerostin have been implicated separately in both the pathogenesis of CVD and bone fragility (126).

Figure 1. Schematical summary of the various mechanisms that can affect the bone health in T1D:

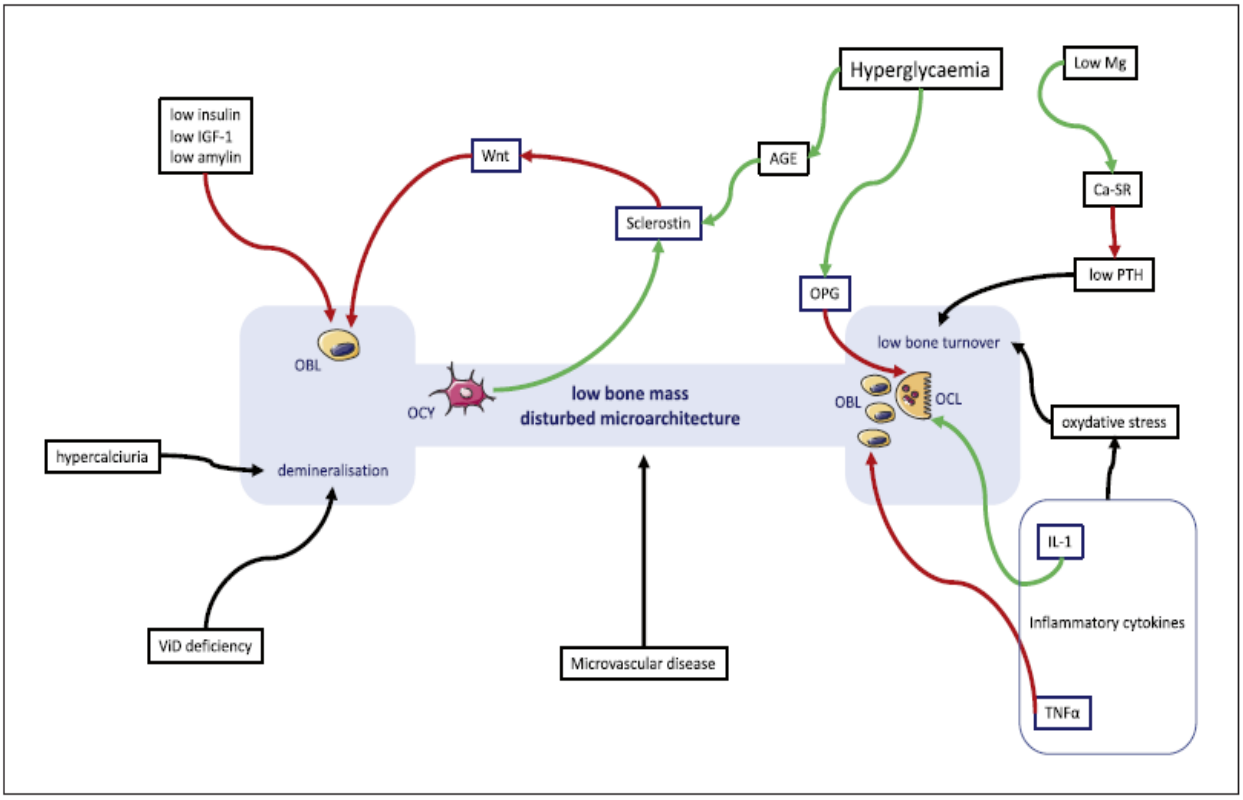
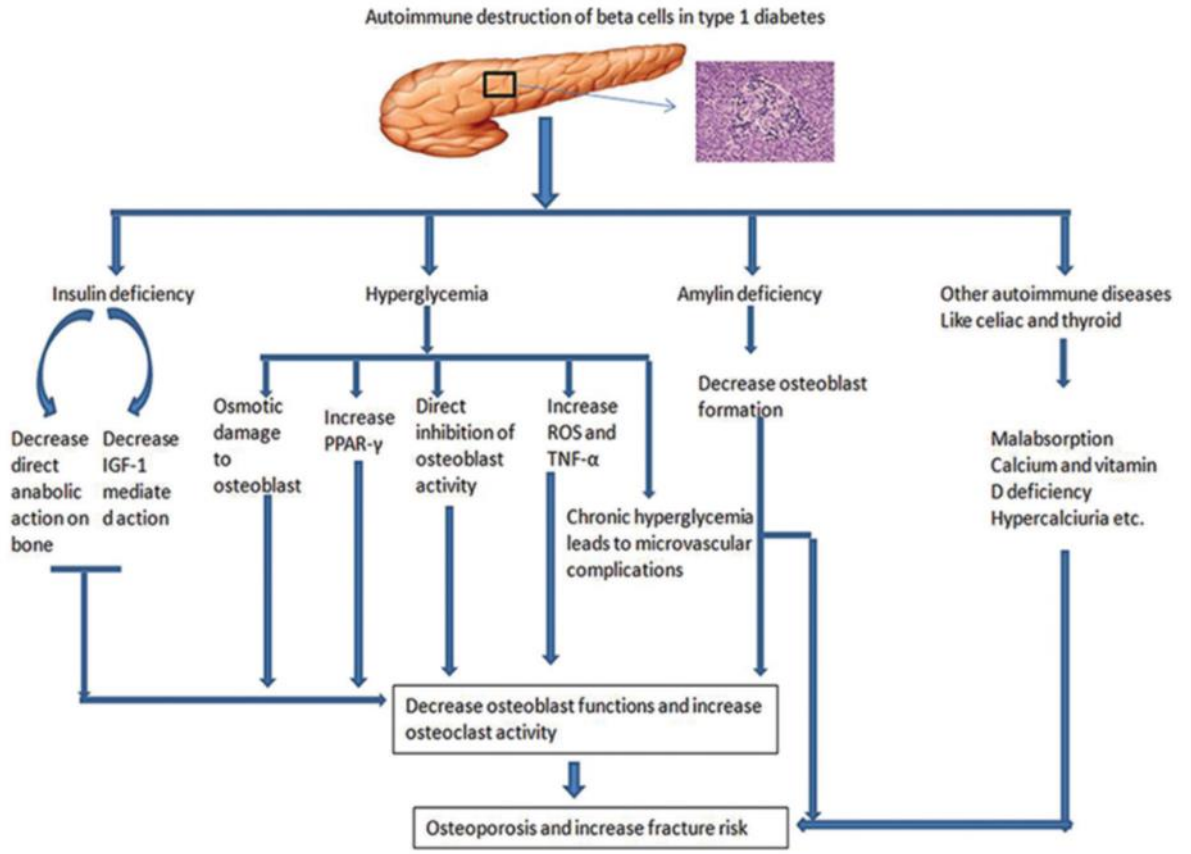
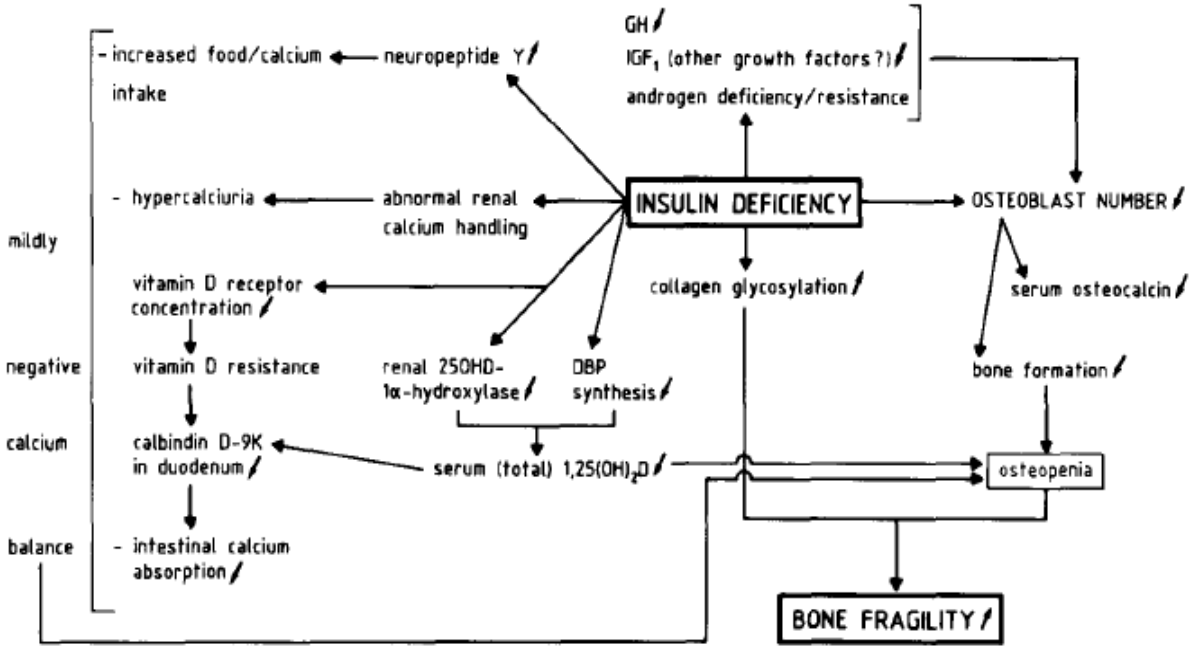


Figure 2. Schematical diagram showing auto-immune destruction of beta cells in T1DM leading to adverse effect on bone physiology:





Summarising the points leading to poor bone health in T1DM:

- Insulin deficiency leading to low anabolic state and low IGF-1; reduced osteoblast formations and actions
- Osteoblast damages due to chronic hyperglycemic state and advanced glycation end products
- Chronic inflammatory state; high TNF-α, IL-1 leading to increase osteoclastic activity along with reduced osteoblastic actions
- Amylin deficiency leading to direct suppression of osteoblasts
- Hypercalciuria, low vitamin D status, resistance at the receptor level contributes to poor bone health
- Low normal PTH along with resistance at the bone level
- Other associated co morbid conditions leading to low bone mass such as celiac disease, thyrotoxicosis etc.

Increased rate of falls in T1DM:

Any form of diabetes is associated with increased risk of falls. Associated sensory neuropathy and neuromuscular impairment is the prime reason for increased fall. Furthermore, diabetic neuropathy may lead to falls that are more severe and falls that are sideways, as opposed to forward or backward (127,128). A higher risk of falls has also been seen in diabetes of longer duration, and as discussed above, longer duration of diabetes is also associated with a greater fracture risk. Higher degree of microvascular complications like diabetic retinopathy leads to poor vision and resultant frequent falls in diabetes. T1DM patients on insulin are very prone for frequent and recurrent hypoglycemic episodes which is another cause of hypoglycemia (129). The attacks of frequent hypoglycemia is far more frequent in T1DM compared to T2DM; so this increased fall leads to increased fracture rates.

Prevention of bone mineral loss in T1DM:

Physical activity is the best way to promote the bone accrual and bone strength during childhood and adolescence. In one study, regular weight-bearing physical activity (180 min/wk, including ball games, jumping activities, and gymnastics) improved total and lumbar bone mineral accretion in children with T1DM, in a similar magnitude to healthy subjects (130). Therefore, children with T1DM should be encouraged to have regular physical activity.

As already discussed, vitamin D deficiency is a prevalent cause for low bone mass in T1DM, optimum supplementation of vitamin D is pivotal to reverse the bone loss. Therefore, all children with T1DM should be recommended to have adequate calcium intake (1200 mg/day) and replacement of vitamin D, if they are deficient.

Avoidance of microvascular complications is also key to prevent this bone loss.

Treatment perspective:

As of now no anti-osteoporotic management has been approved by any osteoporosis international body. There have been a very few randomized controlled studies which have evaluated the efficacy of such agents (19).

Table 1 Measures to Detect Increased Fracture Risk and Intervention to Prevent Fracture in Patients with T1D

Measures to detect fracture risk in patients with type 1 diabetes
Dual-energy X-ray absorptiometry scan
Detection of vertebral fractures by vertebral fracture assessment or X-ray of the thoracic and lumbar spine
Assessment of falls and hypoglycemic events
Intervention against bone fragility in patients with type 1 diabetes
Vitamin D and calcium supplementation in T1D following national guidelines.
Anti-osteoporotic treatment at T-score <-2.0. Alendronate is the first line choice.
Reduce falls and hypoglycemic events

Abbreviations: DXA, Dual-energy X-ray absorptiometry; T1D, Type 1 diabetes.

Figure 3. Various mechanisms leading to increased skeletal fragility in T1DM patients:

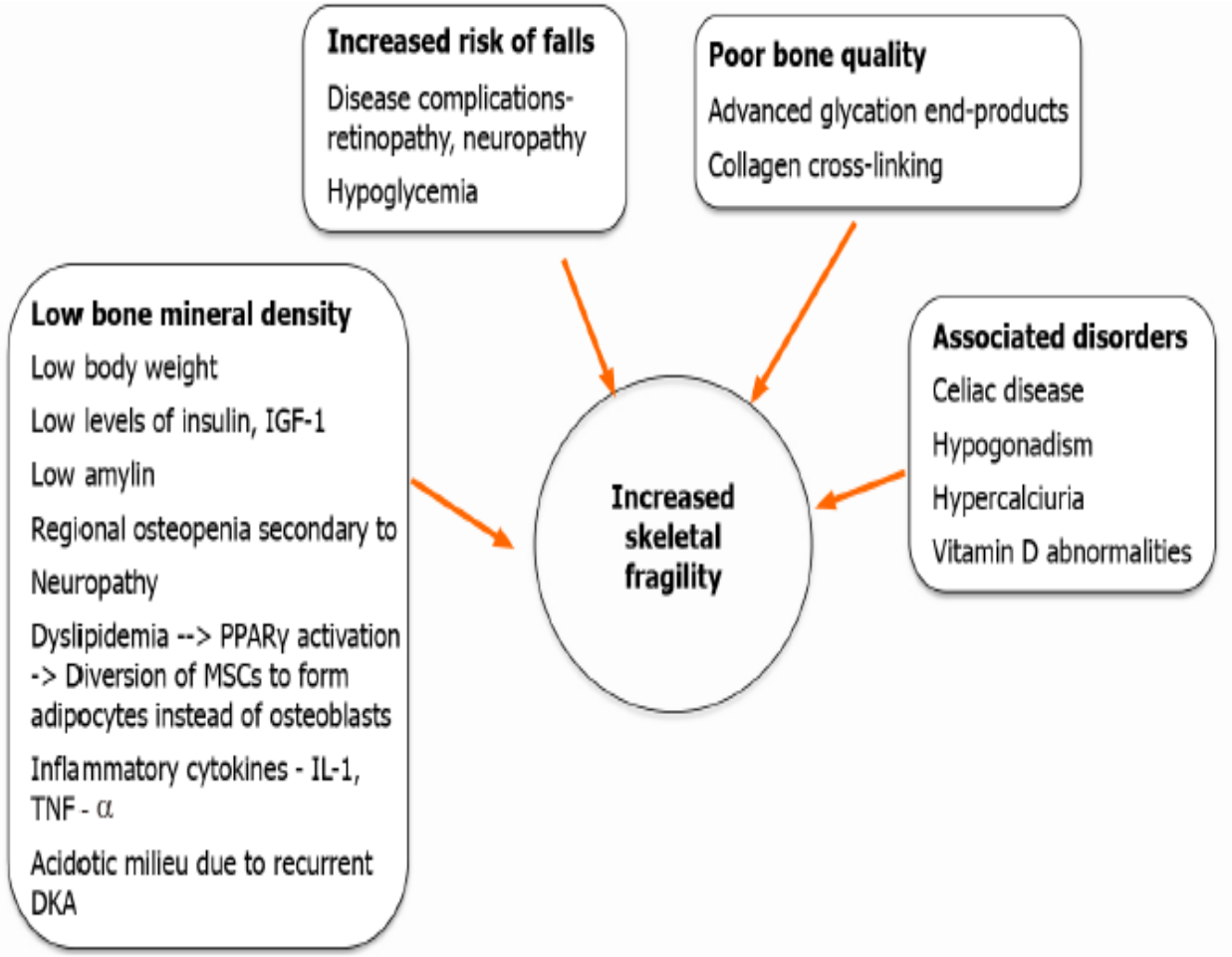
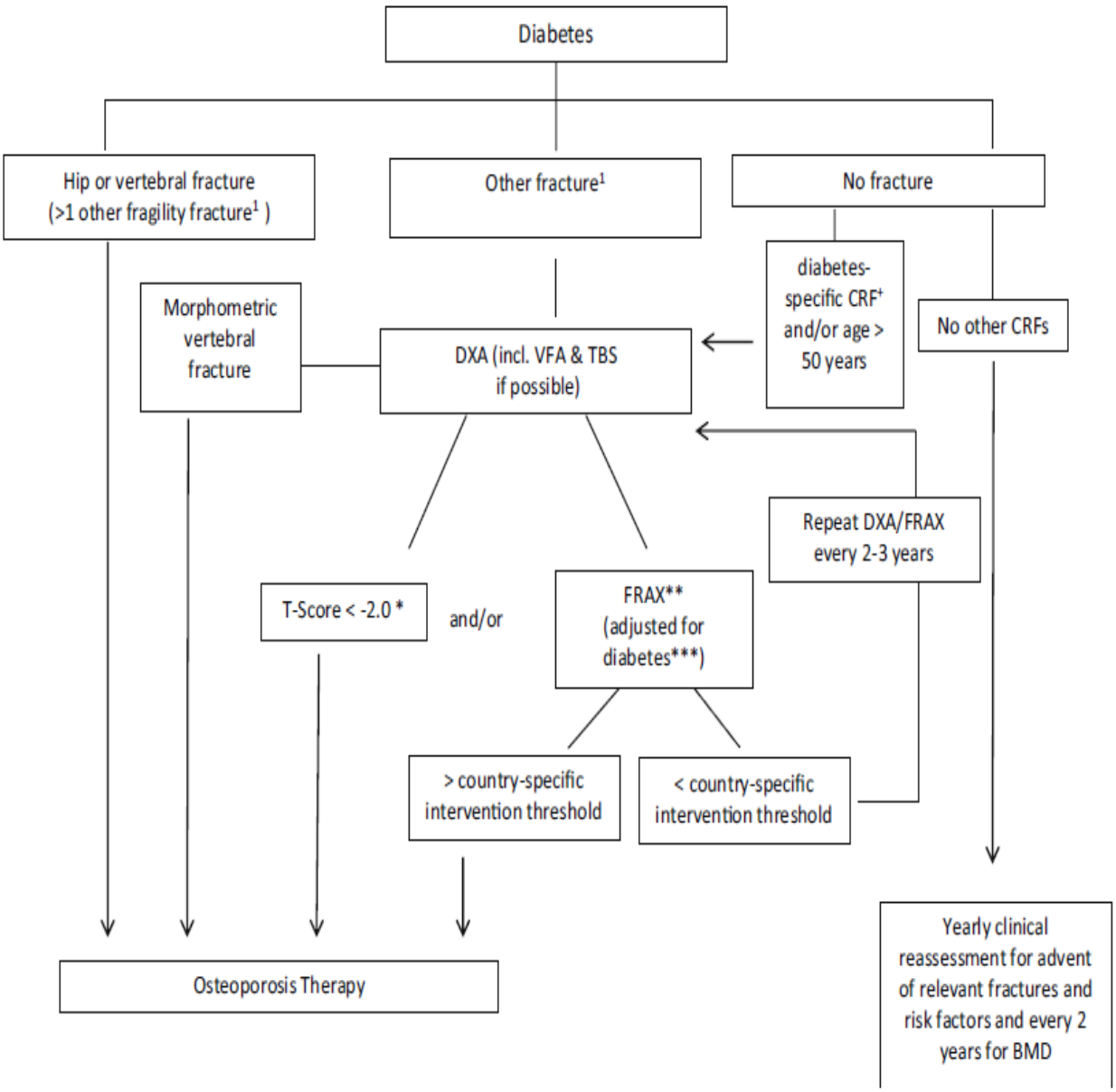


Figure 4. Proposed algorithm of evaluation of bone health in T1DM patients:



Analysis of the various case-control studies on skeletal health on Type 1 Diabetes:

Population-based studies have consistently found that individuals with TID are at greater risk for fracture compared to the general population. If we can summarize the findings of 10 major studies spanning 7 different nations, we can get the following results:

Taken together, these studies utilized data from seven different countries and included participants from both sexes and across the entire age range (24) ((65) (131) (132) (133) (134) (135) (136) (137) (138) (139) (140) (17) (141) (142).

Table 2. Various studies on BMD of T1DM which have calculated z score in respect to control populations:

Year	Author	Country	Sex	Age (years)	n	Reported z score	LBM prevalence
1996	Munoz-Torres et al.	Spain	M/F	30 ± 9	94	LS BMD Z: −0.89 ±1.2 FN BMD Z: −0.99 ±1.2	N/A
1997	Pascual et al.	Spain	M	9.7 ± 4.3	26	LS BMD Z: −0.34 ± 1.1	N/A
1999	Lunt et al.	New Zealand	F	43 (26-66)	99	LS BMD Z: −0.2 (−0.4-0) FN BMDZ: −0.12 (−0.4-0.1)	N/A
2000	Pastor et al.	Spain	F	28.6 ± 8.9	62	LS BMD Z: −0.84 ± 1.3 FN BMD Z: −0.93 ± 1.3	22%
2000	Rozadilla et al.	Spain	M/F	28.9 ± 8.8	88	LS BMD Z: −0.38 ± 1.1 FN: −0.37 ± 1.1	NA
2001	Gunczler et al.	Venezuela	M/F	9.5 ± 2.2	23	TB BMD Z: 0.27 ± 0.61 LS BMD Z:−0.89 ± 1.2	45%
2001	Lopez-Ibarra et al.	Spain	M/F	28.4 ± 5.4	32	LS BMD Z: −0.61 ± 1.2 FN BMD Z: −0.32 ± 1	NA

2002	Valerio et al	Italy	M/F	13.1 ± 1.7	27	LS BMD Z: −0.44 ± 1.02	37%
2004	Ingberg et al.	Sweden	M F	43.1 ± 5 41.2 ± 5	18 20	LS BMD Z: −0.7 ± 1.6 FN BMD Z: −0.7 ± 1.4 LS BMD Z: 0.6 ± 0.9 FN BMD Z: 0.1 ± 0.9	N/A
2006	Leger et al.	France	M F	13 (10–16) 14 (12–17)	73 54	TB BMC Z:−0.2 (−0.82-0.58) LS BMC Z:−0.02 (−0.44-0.57) TB BMC Z: −0.34 (−0.92-0.54) LS BMC Z: −0.37 (−1.29-0.53)	N/A
2007	Miazgowski et al.	Poland	M	43.6 ± 5.1	36	LS BMD Z: −0.71 ± 1.1 FN BMD Z: −0.67 ± 0.7	N/A
2009	Hamilton et al.	Australia	M F	43.4 ± 15.9 37.9 ± 13.8	50	LS BMD Z: −0.27 ± 0.2 FN BMD:−0.38 ± 1.1 LS BMD Z: 0.31 ± 1.2FN BMD Z: −0.04 ± 1.3	N/A
2011	Eller-Vainicher et al.	Belarus	M/F	32.8 ± 8.4	175	LS BMD Z: −0.11 ± 1.2 FN BMD Z: −0.32 ± 1.4	LS-26.3% FN-33.4%
2013	Joshi et al.	India	M/F	27.2 ± 11.2	75	TB BMD Z: −1.10 ± 1.5 LS BMD Z: −1.03 ± 1.2	N/A
2013	Zhukouskaya et al.	Belarus	M/F	31.1 ± 8.6	82	LS BMD Z: −0.56 ± 1.3 FN BMD Z: −0.64 ± 1.1	LS-37% FN-30%

2015	Parthasarathy et al.	India	M F	11.4 ± 3.6 10.8 ± 3.9	77 93	LS BMAD Z: 0 ± 1.1 TB BMC Z:3 −0.2 ± 1.1 LS BMAD Z: 0 ± 1 TB BMC Z:3 −0.5 ± 1.12	22%

Another meta-analysis done by Guilina Valerio et al. have recorded various studies incorporating various factors pertaining to low bone mass in type 1 diabetes. This study has tabulated various study findings regarding T1DM (15) (143) (144) (145) (146) (147) (148) (149) (133) (150) (131) :

Table 3. Tabulation of meta-analysis results in Guilina Valerio study

Authors	**N**	**Age (years)**	**Duration of diabetes**	**Methods of BMD**	**Findings**
Levin et al.	35	15.8 (range 9–20) 29.4 (range 22–57)	0.25–13 10–43	SPA	>10% forearm mass loss in 54% of the patients; greater deficiency of cortical and trabecular bone mass in younger patients than in older ones
Wiske et al.	78	15.2 (range 8–25)	1–18	SPA radiogrammetry	Midshaft mass at the right radius –1.24 SD ($p < 0.001$ vs. normal mean); percent cortical area –0.22 SD and cross-sectional cortical area –0.25 SD at the 2nd metacarpal bone of the

					left hand (p <0.05 vs. normal mean)
Leon et al.	87	11.2 ± 3.9	3.5 ± 4.1	radiogrammetry	Percent cortical area at 2nd metacarpal bone of the left hand –0.25 SD, values 1–2 SD in 9.5% of the patients
Weber et al	66	longitudinal cross-sectional	3	SPA	Reduction in bone mineral content in 6.6% of the patients reduction in BMC in 12% of the patients
Ersoy et al.	30	range 1–16 years	Various	DPA	Reduced lumbar BMD: 11% (anteroposterior) and 16% (lateral) lower than in controls (p < 0.05)
Ponder et al.	56	12.3 (range 5–18)	0.1–14.8	DPA	Normal lumbar BMD
Roe et al	48	12.8 ± 3.4	5.2± 3.6	qCT	Reduced cortical lumbar BMD: 3.5% lower than in controls (p < 0.02)
Lettgen et al.	21	12.6± 3.7	5.2± 4.3	qCT	Reduced total BMD: 18.9% lower than in controls (p < 0.01) at the ultradistal forearm (radius)
Gunczler et al.	26	12.1 ± 3.1	4.3 ± 2.9	DXA	Lumbar BMD z score –1.06B0.2 (22% lower than in controls; p < 0.01); 54% <–1 SD

De Schepper et al.	23	12.5 ± 3.7	2.8 ± 1.5	DXA	Lumbar BMD z score –0.31B0.95 (30% <–1 SD, 4% <–2 SD)
Pascual et al	84	boys 9.7 ± 4.3 girls 11.2 ± 3.8	1-13.8	DXA	Normal BMD distribution at spine and forearm

Discussions about some specific case control studies on BMD in T1DM:

In a study of ***Ingbarg et al*** in long standing diabetes and BMD:

This study was conducted on 38 patients of t1DM and BMD was measured by DXA. The results of the cases were compared with age matched controls. The mean age was 43 years (range 33–55 years) and the mean duration of diabetes was 33 years (range 28–37 years). Besides a tendency to a reduced abdominal fat mass in diabetic males, no difference was observed in fat mass, muscle mass, or BMD between the groups. Significant correlations were found between insulin dosage and whole-body fat mass in diabetic females and between serum cholesterol levels and abdominal fat mass in diabetic males. This is one of the earliest studies which had shown no difference in body mass index among T1DM and controls. This study had also tried to investigate the phenomenon of central obesity in young adolescent females (136). It is not clear whether there is any relationship between insulin resistance, body composition and hormonal abnormalities in adult patients with type 1 diabetes. This study had observed significantly low IGF-1 compared to controls which is in liazo with the other studies. In this study the body fat estimated in DXA co related with the skin fold thickness measurement. However, this finding has been refuted by other studies as well so skinfold thickness seems to be an inadequate measure of body fat measurement.

Daniel Novak et al. in their study of around 24 swedish diabetic patients showed long term diabetes reduces cortical bone strength, periosteal circumference, endosteal circumference and body composition. 23 patients were recruited from swedish diabetes registry for this study with a mean age of around 23 years. Age gender and geography matched controls were recruited. T1DM patients had lower lean body mass and shorter compared to their age matched BMI

matched controls. The cases had also lower muscle area also. Surprisingly this study failed to show any difference in bone mineral density among cases and controls. There were no difference in fracture data's between cases and controls. Smaller periosteal circumference was also evident after adjustments for physical activity, BMI, height, and Hochberg multiple comparison. Here in this study the altered cortical bone strength refers to the modified bone microarchitecture in T1DM (151).

Namrata Sanjeevi et al. in their study evaluated T1DM patients prospectively for 18 months. The relationships between adiposity and inflammation and bone mineral density in T1DM was investigated in this study. The main finding was CRP was inversely associated with BMD of the total body, pelvis and leg (n=136). Percent body fat was inversely associated with BMD of the total body and pelvis; whereas percent trunk fat was related only to total body BMD. So they concluded Greater inflammation and adiposity were related to lower BMD in youth with type 1 diabetes. Investigating the impact of inflammation and adiposity on bone turnover markers could provide insights on mechanisms that contribute to this relationship.

Ameya Joshi et al had studied 86 consecutive T1DM cases and 140 unrelated age and sex matched healthy nondiabetic controls. It is one of the only comprehensive case control study done in India. After history and examination, BMD and body composition were assessed by dual energy X-ray absorptiometry (DXA). Serum samples were analysed for calcium, phosphorus, albumin, creatinine, alkaline phosphatase, 25 (OH) vitamin D3, intact parathormone (PTH) levels (both cases and controls) and HbA1c, antimicrosomal and IgA tissue transglutaminase (IgA TTG) antibodies, cortisol, follicle stimulating hormone (FSH), testosterone, sex hormone binding globulin (SHBG), tetraiodothyronine (T4), thyroid stimulating hormone (TSH), growth hormone (GH), insulin-like growth factor-1 (IGF-1), and insulin-like growth factor binding protein 3 (IGFBP3) (cases only). T1DM cases had a lower BMD as compared to controls at both total body (TB) and lumbar spine (LS) ($P < 0.05$). Patients with celiac autoimmunity (CA) had significantly, lower BMD as compared to age, sex, and body mass index (BMI) matched T1DM controls. Linear regression analysis showed that low BMD in T1DM patients was associated with poor glycaemic control, lower IGF-1 levels, less physical activity (in total population as well as in male and female subgroups), and lower

body fat percentage (in females) and higher alkaline phosphatase level (in males) ($P < 0.05$) (17).

Cristina Eller-Vainicher et al. in their study evaluated a total of 175 eugonadal type 1 diabetic patients (age 32.8 +/-8.4 years) and 151 age & BMI-matched control subjects. In all subjects, BMI and BMD (as Z score) at the lumbar spine (LS-BMD) and femur (F-BMD) were measured. Daily insulin dose (DID), age at diagnosis, presence of complications, creatinine clearance (ClCr) and HbA1c were determined along with this. They got LS-BMD and F-BMD levels to be lower in patients of T1DM than in control subjects. LS-BMD was independently associated with BMI and DID, whereas F-BMD was associated with BMI and ClCr. The presence of all of these risk factors had a positive predictive value and their absence had a negative predictive value of 62.9 and 84.2%, respectively. Authors had also analyzed the data's using the TWIST system in combination with supervised artificial neural networks and a semantic connectivity map. The TWIST system selected 11 and 12 variables for F-BMD and LS-BMD prediction, which discriminated between high and low BMD with 67% and 66% accuracy respectively. The connectivity map showed that low BMD at both sites was indirectly connected with HbA1c through chronic diabetes complications. So they concluded that low BMD is associated with low BMI and low ClCr and high DID. Chronic complications negatively influence BMD (1) .

J. De Scheppera et al. in their study have also highlighted the BMD DXA of around 20 eugonadal adolescent male and females of T1DM. Fifteen male and 8 female children and adolescents (mean age B SD: 12.5 ± 3.7 years), 1–5 years after the clinical onset of their diabetes, were studied. Measurements of the lumbar spine (L1–L4) BMD, expressed in gHA/cm2 and as a z-score for age, were performed with a commercial DEXA apparatus (Hologic QDR 1000 W, Hologic Inc., Waltham, USA). Calcium-phosphorus metabolism was studied by measuring the circulating levels of calcium, phosphorus, alkaline phosphatase, osteocalcin, 25-OH-vitamin D and parathyroid hormone and the urinary excretion of calcium and phosphorus. The mean BMD of the studied group was 0.75 (0.16) gHA/cm2 giving a mean z-score of –0.31B0.95. Only 1 of the patients had a BMD lower than –2 SD. No sex difference in BMD z-score existed. BMD SD was positively correlated with height SD ($R = 0.56$, $p < 0.005$), but not with the age of the patients, the duration of the disease, the degree of metabolic

control or the studied parameters of the calcium-phosphorus metabolism. So this was one of the study published which had pointed out that initial years of BMD was not been affected by poor glycemic control in T1DM patients (150).

Peter Gunczler et al in their study evaluated total bone mineral content (TBMC) and bone mineral density (BMD) at the lumbar spine and femoral neck in 26 T1DM children with a mean chronological age of 12.1 ± 3.1 yr with average 4 yr long diabetes with a mean HbA1c of 9.2 ± 0.4%. BMD and TBMC standard deviation scores (Z-scores) were determined by comparing results to controls matched for age, sex and pubertal status. BMD, bone formation and resorption markers were determined at the beginning of the study and after one year of follow up. Mean lumbar spine Z-score was -1.06 ± 0.2, with negative values in 24 of 26 children (92.6%); 14/26 patients (53.8%) had a lumbar spine Z-score >1.0 SD below the mean. Mean lumbar spine Z-score remained unchanged after one year of follow up (-1.02 ± 0.3). No significant differences were obtained in femoral neck BMD or TBMC between groups. No correlation was observed between lumbar spine BMD Z-scores and duration of IDDM or degree of diabetes control, as assessed HbA1C. Daily urinary calcium excretion was elevated in patients initially and after one year of follow up; however, no correlation was obtained between lumbar spine BMD and 24 h urinary calcium excretion. Carboxy-terminal propeptide of type 1 collagen values and levels of urinary cross-linked N-telopeptides of type 1 collagen in the diabetic children were significantly lower than those of the matched controls. Osteoblastic activity as assessed by serum osteocalcin and carboxy terminal propeptide of type I collagen and bone resorption as measured by crosslinked N-telopeptides of type 1 collagen did not correlate with the lumbar spine Z-scores. When T1DM patients were subdivided into males and females and into children with more than or less than 2 yr duration of diabetes since diagnosis, no differences between groups were found. These results suggested that T1DM-children were having a low bone turnover resulting in osteopenia in the growing bone. This defect was already present in trabecular bone early on in the disease and seems not to be related to glycemic control (152).

Lucy D. Mastrandrea et al. in their study of 63 patients of type 1 DM, they have measured BMD in DEXA and compared to similar proportion of age & sex matched individual. They have followed all patients for about a year and measured various non-glycemic parameters like

IGF-1, IGFBP-3 and bone-formation & resorption markers like Osteocalcin and Urine N-telopeptide. They had found out that after adjusting for age, BMI, and oral contraceptive use, BMD at year 2 continued to be lower in women 20 years of age with T1DM compared with control subjects at the total hip, femoral neck, and whole body. Lower BMD values were observed in cases <20 years of age compared with control subjects; however, the differences were not statistically significant. As expected, lower BMD did not correlate with diabetes control, growth factors, or metabolic bone markers (153).

Mahshid mohsoni did a very interesting study on the effect of severe neuropathy on BMD and fracture risks in T1DM patients. The objective of that review was to analyse available literature on the effect of peripheral neuropathy on BMD of the foot, spine, or hip. They hypothesize that the presence of diabetic neuropathy leads to lower BMD in adults with diabetes. They included 5 studies for this evaluation among them 4 of them did not find any significant association between neuropathy and BMD. One study showed a significant negative impact of neuropathy on calcaneal BMD in patients with type 1 diabetes. The meta-analysis did not show a significant effect of peripheral neuropathy on BMDs of proximal femur, spine, and calcaneus in diabetic adults (154).

T1DM studies on HrpQCT:

Two studies examining T1D bone microarchitecture have used HR-pQCT. These studies reported trends of higher cortical porosity among those with T1DM than in those with diabetes, however, the most largest difference was seen in trabecular features: trabeculae thickness, trabeculae spacing and trabecular bone volume/total volume ratio (18) (91). Shanbhogue et al. showed a very interesting pattern in their study; T1DM patients with microvascular complications had poor bony architecture than patients without the complications.

T1DM studies on TBS:

In a 2016 paper, ***Neumann et al*** demonstrated that TBS of the lumbar spine was more effective in identifying fracture risk than BMD alone. TBS assesses trabecular bone texture of the spine using DXA scans, a measure which correlates with trabecular microarchitecture. The biggest advantage of TBS measurement is the simultaneous measurement of BMD and TBS in a single

DXA machine. So a composite measurement of TBS and BMD in a DXA machine is warranted (155).

This study aimed at investigating the TBS in T1D patients and healthy controls. Associations with prevalent fractures were tested. One hundred nineteen T1D patients (59 males, 60 premenopausal females; mean age 43.4±8.9 years) and 68 healthy controls matched for gender, age, and body mass index (BMI) were analysed. The TBS was calculated in the lumbar region, based on two-dimensional (2D) projections of DXA assessments. TBS was 1.357±0.129 in T1D patients and 1.389± 0.085 in controls (p=0.075). T1D patients with prevalent fractures (n=24) had a significantly lower TBS than T1D patients without fractures (1.309±0.125 versus 1.370± 0.127, p=0.04). The presence of fractures in T1D was associated with lower TBS (odds ratio=0.024, 95 % confidence interval (CI)=0.001–0.875; p=0.042) but not with age or BMI. TBS and HbA1c were independently associated with fractures. The area-under-the curve (AUC) of TBS was similar to that of total hip BMD in discriminating T1D patients with or without prevalent fractures. In this set-up, a TBS cutoff <1.42 discriminated the presence of fractures with a sensitivity of 91.7 % and a specificity of 43.2 %. Conclusions TBS values are lower in T1D patients with prevalent fractures, suggesting an alteration of bone strength in this subgroup of patients. Reliable TBS cutoffs for the prediction of fracture risk in T1D need to be determined in larger prospective studies.

Aditi Wagh et al. in their recently published study in evaluated and compared the TBS and volumetric BMD measured by HRpQCT in Indian T1DM patients. This is one of the first kind of study which has measured vBMD in Indian T1DM patients. A total of 205 children were assessed for their LS bone mineral content (BMC) and LS aBMD by dual energy x-ray absorptiometry (DXA) and Trab vBMD at distal radius by peripheral quantitative computed tomography (pQCT). Machine generated Z-scores for both LS aBMD and Trab vBMD were used. The retrospective DXA LS scans in children with T1DM (n=137, age 13.1 § 3.2 years) and controls (n = 68, age 13.0 § 2.7 years) were analysed with a research trial version of TBS iNsight software (Medimaps Group). The established TBS cut-offs were used to categorize TBS. All the BMD parameters including vBMD were lower in T1DM children. Distribution of T1DM and control children was similar in the TBS categories. Over a fourth of the T1DM children with low Trab vBMD (below -2 Z score) had normal TBS, while, in children with LS aBMD Z-score > -2 from both groups, >50% had degraded or partially degraded TBS. Degraded TBS was seen in half the control children although none of them had low Trab

vBMD. So this study confidently concluded that TBS and vBMD do not co relate in Indian T1DM patients (156).

Micro-Ct studies in T1Dm:

Armas et al, in biopsies from the iliac crest using micro-CT, compared individuals with T1D with and without fracture, but no vascular complications, and those without diabetes (5). Abdalrahaman et al found differences between those with T1DM and without diabetes in the trabecular bone of the lumbar spine, including apparent BV/TV, a lower number of trabeculae, as well as larger spacing between trabeculae. This was on a backdrop of no difference in BMD. This supports the idea that examination of the microarchitecture of the bone gives a better sense of the integrity of the bone.

Studies encompassing the relationship between vitamin d status, calcium intake and BMD in T1DM:

María Cristina Gil-Díaz et al. have evaluated this topic extensively in their publication. The objective of their study was to systematically review the literature for evidence of associations between calcium intake, vitamin D intake, and physical activity and skeletal outcomes in T1D. The prevalence of calcium deficiency was high and encompassed greater than 50% of participants in the majority of studies. Despite this finding, there was no clear association between calcium intake and bone density in any study. Calcitriol use was associated with gains in bone density in one study but was not associated with changes in bone turnover markers in a second report. No studies specifically investigated the impact of vitamin D2 or D3 supplementation on bone health. These results were based on the analysis of 10 studies carried on children and 4 studies caried out on adults.

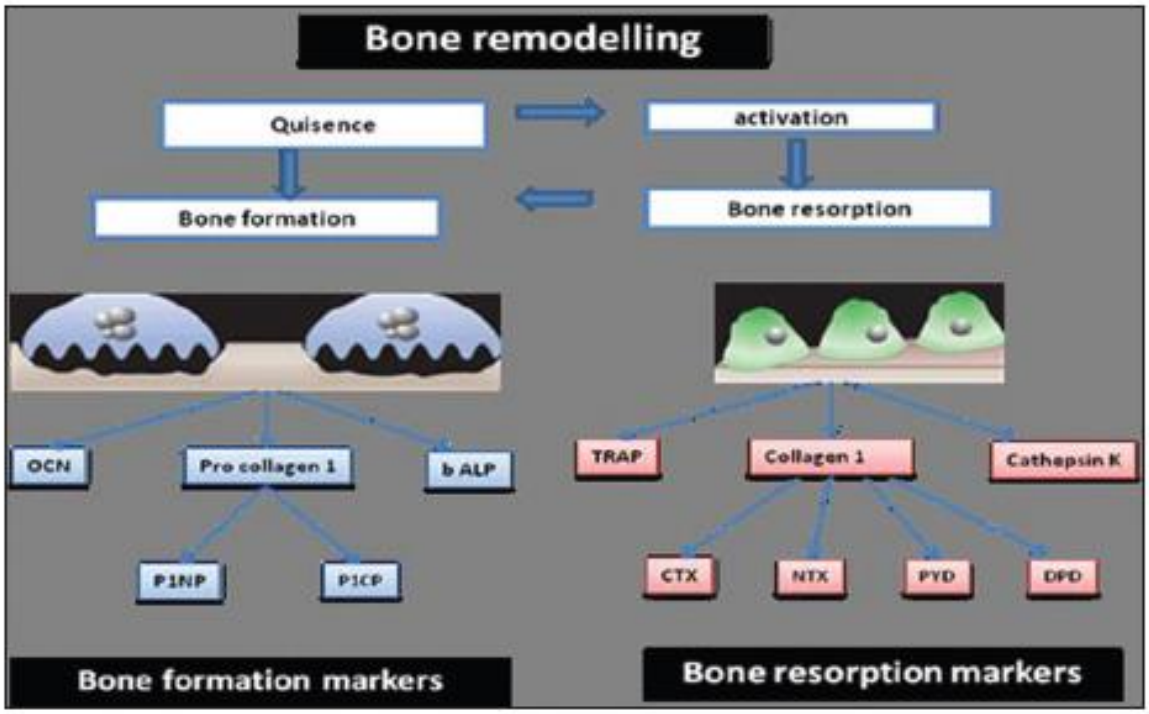
Studies on Bone turnover markers and T1DM and association with BMD:

Various biomolecules released into the circulation during bone resorption and formation are called Bone Turnover markers (BTM). Assessment of the BTM's is an indirect or surrogate marker of bone turnover status. We have already discussed about the low bone turnover status in T1DM. Under optimal physiological conditions, bone resorption takes place in around 10 days and bone formation takes about 3 months. Up to 20% of the skeleton, it may be replaced

by remodelling every year. BTM's are generally of two types; formation and resorption markers. International Osteoporosis Foundation (IOF) and International Federation of Clinical Chemistry and Laboratory Medicine has proposed serum CTX-1 (sCTX) and serum P1NP to be used as reference markers of bone resorption and formation, respectively, for the assessment of fracture risk and monitoring therapy in clinical settings (2).

PINP is primarily secreted from osteoblasts and fibroblasts with having minor contributions from dermis, tendon, cartilage. P1NP exists in serum as trimeric or monomeric form. Immunoassays detect either trimeric (automated IDS ISYSS assays) or both forms which are otherwise called as total P1NP assays. P1NP is proposed as a reference bone formation marker by IOF in view of its predictable response to treatment and the reliability of P1NP assays as evidenced by low intra-individual variability, smaller circadian variation, stability at room temperature, and a good assay precision. CTX are degradation products of Type 1 collagen of bone generated by the activity of the enzyme cathepsin K. The sCTX1 has been recommended as reference bone resorption marker by IOF. Its prominent circadian rhythm, peaking in the late night and nadir in the afternoon has make assay a bit difficult in routine laboratory settings (157) (158).

Figure 5. Schematic diagram of bone remodelling with involvement of various bone turnover markers



Another important pre analytical aspect of CTX is its dependence on food intake. Post prandial state reduces it by around 20%. So it's always recommended to collect the sample at fasting state only. P1NP is more stable compared to CTX with stability of at least 24 hr at RT and 5 days at 4°C in both EDTA plasma and serum. For long term storage, stability for 3 months for CTX and 6 months for P1NP at ≤-20°C is ensured for all methods (159).

Factors determining the pre-analytical variability of various Bone-turnover markers:

Source	Importance	Nature of effect
Uncontrollable sources		
Age	Very important	BTM increase with age in men and women
Menopausal status	Very important	BTM increase within a few months after the last menstrual period
Gender	Very important	BTM are higher in older women than older men
Fractures	Important—limits evaluation of case control studies	BTM increase after a fracture (maximal at 2 to 12 weeks, but effect lasts for up to 52 weeks)
Pregnancy and lactation	Important	BTM are increased during pregnancy; highest levels during third trimester, even higher postpartum
Drugs	Important: corticosteroids, anticonvulsants, heparin, GnRH agonists	BTM may be decreased (glucocorticoids) or increased (anticonvulsants)
Disease	Important: thyroid disease, diabetes, renal impairment, liver disease	BTM often increased (thyrotoxicosis, chronic kidney disease)
Bed rest/immobility	Important	Bone formation markers decrease and resorption markers increase
Geography	Somewhat important	Small changes amongst countries, usually explained by differences in lifestyle
Ethnicity	Not important	Small changes, such as lower OC in African Americans vs. Caucasians
Oral contraception	Not important, except in women over 35 years	Lower values for BTM
Controllable sources		
Circadian	Extremely important	Most striking for bone resorption markers; highest values in second half of night and on waking; lowest values in afternoon and evening
Fasting status	Important for specific markers	Feeding results in a decrease in BTM; for example, s-CTX decreases by 20% after breakfast
Exercise	Important—chronic and acute effects	Changes occur but depend on type of exercise and age of subjects
Menstrual	Not important	Small decreases in bone resorption and increases in bone formation during luteal phase
Seasonal	Not important for individual, but maybe for longitudinal studies	Small decreases in BTM over winter
Diet	Not important	Small reduction in BTM immediately following calcium supplementation

Changes in the BTMs must be large to monitor clinical response in view of biological and analytical variations. While interpreting the BTM response, "least significant change" (LSC) for each BTM must be utilized which is derived by multiplying the each BTMs precision error provided by the laboratory by 2.77 (95% confidence interval) (160). BTM gives an pharmacodynamic response in a patient on anti-osteoporotic therapy. However, their

usefulness in predicting adequate response is doubted in various literatures. Their vast pre analytical variability has questioned its usefulness.

The study on BTM monitoring in predicting osteoporosis treatment response have been studied extensively in post-menopausal women. But till date no studies have evaluated this point in T1DM or any other types of diabetes. There is definitely an academic lacuna in this point. However periodic BTM do not substitute the need of BMD measurements in any of the patients, it can act just as a surrogate marker.

Physical activity and relationship with BMD in T1DM:

The quantifying index for physical activity is MET minutes per week. It is calculated from days of activity multiplied by its intensity. Physical inactivity is a modifiable risk factor of low bone health and physical activity has been unequivocally proven to be increasing bone health. The American College of Sports Medicine recommends weight-bearing endurance activities, including those that involve jumping (such as tennis) and jogging, three to fi ve times per week and resistance exercise two to three times per week to preserve bone health during adulthood. During physical activity, bone is subjected to mechanical forces exerted by muscle contraction and gravitational loading. At the cellular level, bone cells (osteocytes) perceive these mechanical forces as cell deformation, changes in extracellular fl uid shear stress, pressure gradients and electric fi elds. The osteocytes communicate with osteoblasts and osteoclasts to modulate bone formation and resorption thereby changing bone geometry and material properties (161).

Methodology

Aims & Objectives

Title

Assessment of Bone Mineral Density (BMD) by DXA (Dual Energy Xray-Absorptiometry) in Type 1 Diabetes Mellitus patients and Co-relation with Glycemic and Non-glycemic parameters; An Observational study

Aim of the study- To assess the BMD of Type 1 DM patients by Horizon-A DXA scanner and to co-relate with various glycemic as-well-as non-glycemic clinical and biochemical markers

Primary Objective:

To evaluate the Bone-mineral Density in patients with Type 1 Diabetes Mellitus who presents to department of Endocrinology & Metabolism in All India Institute of Medical Sciences, Jodhpur

Secondary Objectives:

1. To compare and correlate the BMD findings with Glycemic status

2. To evaluate and compare Trabecular Bone Score (TBS) in study subjects with Healthy controls

3. To evaluate and compare lean body mass and fat body mass in study subjects by anthropometry and DXA VAT (Visceral Adipose Tissue) assessment

4. To compare and correlate the BMD findings with pubertal staging & Physical activity

5. To co-relate the BMD findings with Vitamin-D status & serum iPTH in T1DM patients

6. Evaluation of the baseline bone turnover markers (BTM) in cases

Materials & Methods

Study Setting: This is a single-time case control observational study among type 1 DM patients in Department of Endocrinology & Metabolism at AIIMS, Jodhpur

Study Type: Observational Case-Control Study

Study Participants: Patients with type 1 diabetes presenting to Endocrinology & Metabolism OPD at AIIMS Jodhpur

Inclusion Criteria:

Patients presenting with type 1 Diabetes mellitus above 12 years of age

Exclusion Criteria:

1. Type 1 Diabetes Mellitus patients with less than 12 years of age.

2. Type 1 Diabetes Mellitus with known Bone-Mineral diseases like Rickets or Osteogenesis Imperfecta

3. Type 1 Diabetes Mellitus patients with known traumatic injury causing permanent bony abnormalities

4. All other types of Diabetes including suspected Maturity Onset Diabetes of the Young or Secondary Diabetes due to pancreatic cause

5. Type 1 DM patients with known chronic organ failure like Chronic Liver disease, chronic kidney disease or Chronic Obstructive Respiratory Diseases.

6. Type 1 DM patients taking drugs which can affect the Bone metabolism like Steroids and other Immuno-suppressants

7. Not willing to consent

Sampling:

Type 1 DM Patients presenting to the departments of Endocrinology and Metabolism at AIIMS Jodhpur, fulfilling the inclusion criteria will be consecutively enrolled into the study in the study duration, after being explained about the study and obtaining due informed consent for performing DXA scan for assessing BMD in these patients.

Sample Size:

The sample size was calculated from the study done by Joshi et al. It was a comprehensive study designed to measure the prevalence of Osteoporosis in Type 1 DM patients compared with controls done in Indian populations. So, from the calculation of mean+/-SD values of whole body BMD in case & control groups, the sample size for our study comes as 60 patients per group (case/control) using the prevalence data and power of 80% with alpha value 0.05. Precision of individual technologist for total body and lumbar spine neck was 1% and 0.9% respectively. Formula used here- $Z^2 \times SD^2 \div d^2$ (Z= Confidence Interval; SD= Standard Deviation found from the previous score; d= Difference between the mean found from the reference study); sample size came out as sample size = 59.33 with the mean difference of 0.3

Study Duration:

18 months (From January 2021 to June'2022).

Data Collection:

After assessing the inclusion and Exclusion criterions of patients with Type 1 DM, they will be included in the study. At first, all the clinical parameters pertaining to type 1 DM shall be noted including all Anthropometric measurements. Then clinical history including the duration of the diabetes, history of severe hypoglycemic episodes & Diabetic-ketoacidosis requiring hospital admission, Physical activity history (MET minutes/week), family history shall be obtained.

Biochemical battery of tests including Complete Hemogram, HbA1C, Lipid Profile, LFT, KFT, Bone-Mineral Panel {Ca/Po4/ALP/25 (OH)D}, Thyroid profile, Anti-TTG, Testosterone/Estradiol (according to sex), spot urine albumin creatinine ratio (ACR), Fasting Insulin & C-peptide, IGF-1, Cortisol shall be sent afterwards.

Then after formal & written consent, patient shall be evaluated for Bone Mineral Density in Hologic-A DXA scanner in both Axial (AP Lumbar spine and femoral neck) and Peripheral Skeleton (Non-dominant arm anterior Radius) and results shall be interpreted with similar age & sex-matched population. Associated TBF (Total body fat percentages), VAT (Visceral Adipose Tissue), LBM (Lean Body Mass), TBS (Trabecular Bone Score) shall also be measured.

Controls recruitment and matching:

Controls were recruited in age, sex, BMI and geographical location matched manner. One control of similar age, sex, BMI and same geographical region corresponding a to a particular case were recruited. These controls were taken histories of any known co morbidity which can affect the bone health. Essentially all controls need to be non-diabetic. Control from the same family of a case were also encouraged to get recruited for doing BMD DXA. For the controls, after ruling out diabetes, BMD DXA was performed after informed consent.

Though the controls were not getting active treatment for any of the indications, they were under our follow up for BMD DXA related repots and in any abnormality, they had undergone detailed evaluation.

Details and technicalities of the BMD DXA machine:

The APEX^{TM} for QDR^{TM} Xray bone densitometers is indicated for the estimation of bone mineral density (BMD), comparison of measured variables obtained from a given QDR scan to a database of reference values.

Parameters which are calculated in BMD DXA:

The hologic whole body DXA reference database software used on Hologic QDR bone densitometers measures the:

- Regional and whole-body bone mineral density
- Lean and fat tissue mass
- Calculate derivate values of:
- Bone mineral content

- Area
- Soft tissue mass
- Regional soft tissue mass
- Total soft tissue mass
- Fat free mass
- Regional and total soft tissue mass ratios
- Percentage regional fat
- Percentage total body fat
- Percentage android fat
- Percentage gynoid fat
- Percentage fat. Android/gynoid ratio
- BMI

Visceral fat calculation:

The hologic visceral fat software used in Hologic horizon bone densitometer total body scans estimate the visceral adipose tissue content within the android region. The content that is estimated is the visceral fat area, visceral fat mass and visceral fat volume.

10-year fracture risk indications:

Femoral neck BMD and clinical risk factors are used to estimate 10-year risk of hip fracture and 10 year risk of major osteoporotic fracture using the world health organization (WHO) algorithm (FRAX) in adults. It is used as a fracture risk where therapeutic judgement can be undertaken.

Country specific FRAX:

This FRAX tool has been developed by WHO to evaluate fracture risk of patients. It is based on individual patient models that integrate the risks associated with clinical risk factors as well as BMD at the femoral neck.

These models have been developed from studying population based cohorts from Europe, North America, Asia and Australia. These algorithms give the 10-year probability of fracture. The output is a 10-year probability of hip fracture and the 10-year probability of a major osteoporotic fracture (clinical spine, forearm, hip or shoulder fracture).

Hip structure analysis:

HAS for QDR Xray bone densitometers uses data from conventional dual energy absorptiometry scans to measure the distribution of bone mineral mass at specific cross sections of the hip and allows to understand the structural properties.

Pediatric calculation of BMD and related parameters:

The measurements are plotted on a gender and ethnicity matched reference curve. Corresponding results are based upon the available measures selected in the system configuration for this report.

Each DXA measure is plotted on a percentile scale and the z-score and centile for the subject's measurement relative to gender and ethnicity-matched peers is provided at the far right of the scale. Reference data from Hologic, the bone mineral density in childhood study, and NHANES is used for Z scores and percentiles.

Precautions before undertaking a scan:

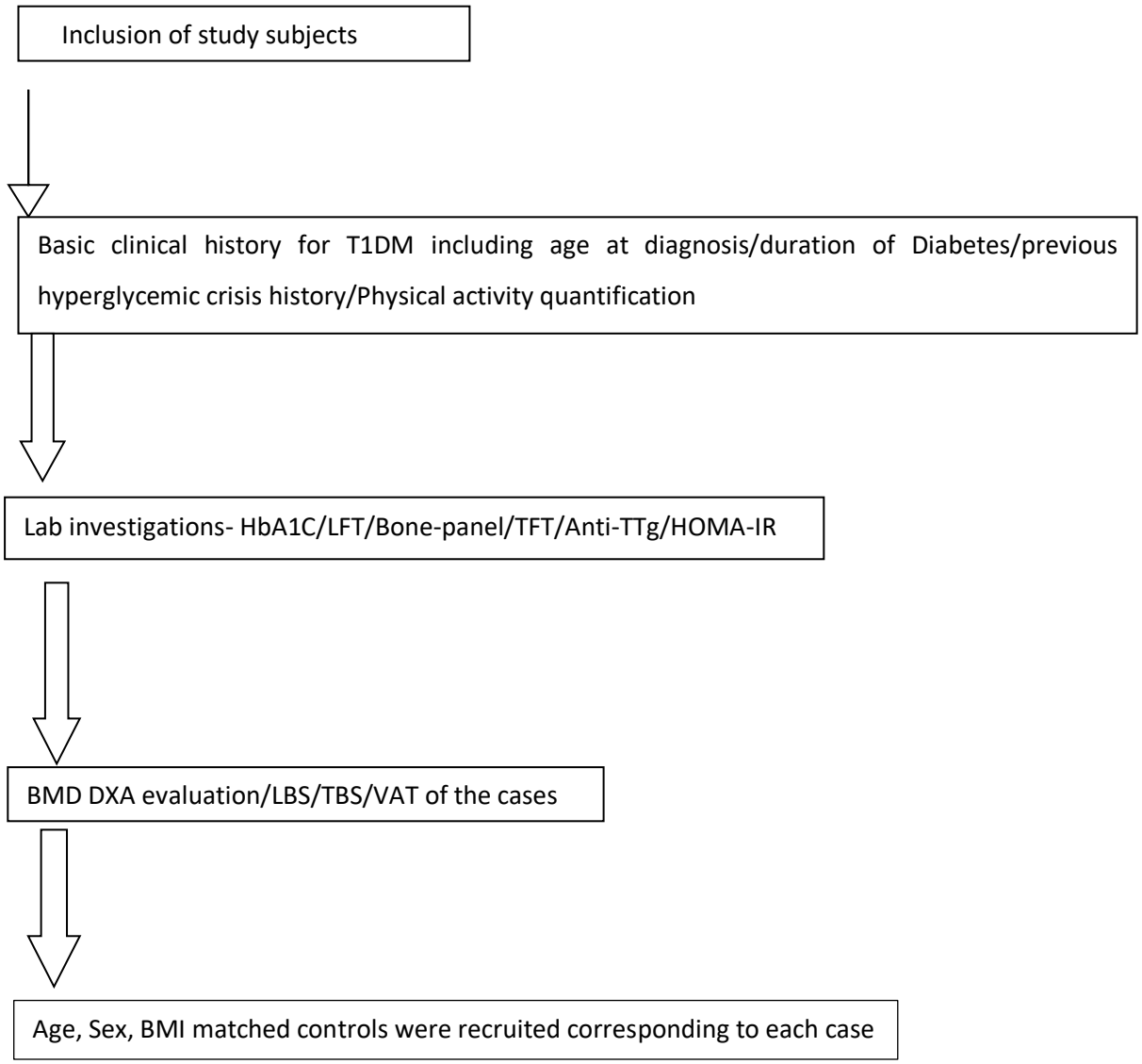
- Not to take any pregnant patients for scanning; need to postpone the scan until pregnancy is ruled out.
- If the patient has undergone any radiological procedure using the following contrast agents such as Iodine and barium, we need to wait for 7 days for scanning. Radiological contrast agents used for Xray and Ct can interfere with the DXA scans. In particular oral contrasts can remain in the GI tract for several days affecting DXA results. Intravenous iodine normally clears within 72 hours for those patients with normal kidney function.
- Hologic DXA measurements have been shown in several studies to be unaffected by nuclear isotope studies, so DXA measurements can be done immediately after nuclear isotope studies as long as the studies do not also include radiological contrast agents (such as iodine and barium).
- Patient wearing any objects such as ostomy device, metal buttons or snaps or jewelry can interfere with the results. Patients need to remove all these materials before undertaking the scan.
- If the patient had surgery on a hip or forearm, then the uninjured hip or forearm should be scanned.

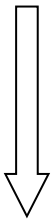
Patient preparation:

To prepare the patient for the examination:

- We need to ensure there is no metal (zipper, snap, belt etc) in the scan field. If necessary, have the patient change into a gown for the examination.
- For AP lumbar spine, hip or whole-body examinations, we need to instruct the patient to remove their shoes.
- The subject weight limit is 227 kg, over this we need to scan the forearm only.
- To stop any calcium supplementation for at least 1 day before the scan.

Study Flow:





Matched controls underwent BMD DXA, calculation of the BMD parameters, LBS/VAT/TBS

Bone-Turnover Marker assay:

Bone Turnover markers (BTM) provide a complimentary assessment to BMD evaluation in such studies. After thorough literature searching, we have opted for P1NP & CTX assessment as a part of our BTM analysis as per the recommendation of International Osteoporosis Foundation. However due to a technical snag, P1NP assessment could not be done. However additionally serum sclerostin was measured.

Statistical analysis

Data was expressed as mean ± standard deviation for most of the parameters but some of the biochemical indices have been expressed as median (range).

Independent Student t-test and ANOVA have been used for comparison of numerical variables between two groups. Mann-Whitney U and Wilcoxon W test was applied in a non-normally distributed variable. Spearman's rank correlation coefficient was used to understand the statistical dependence between two variables. Pearson’s corelation coefficient was also used in understanding statistical dependence between normally distributed variables.

Analysis was done using SPSS version 23.

Results

Basic epidemiological data:

Table 4. Sex Distribution of the study population:

Sex	**Number (percentages)**
Male	36 (60)
Female	24 (40)

Table 5. District wise distribution of the study population:

District	**Number**
Jodhpur	30
Nagaur	14
Barmer	6
Pali	4
Jalore	3
Jaisalmer	1
Ajmer	1
Churu	1

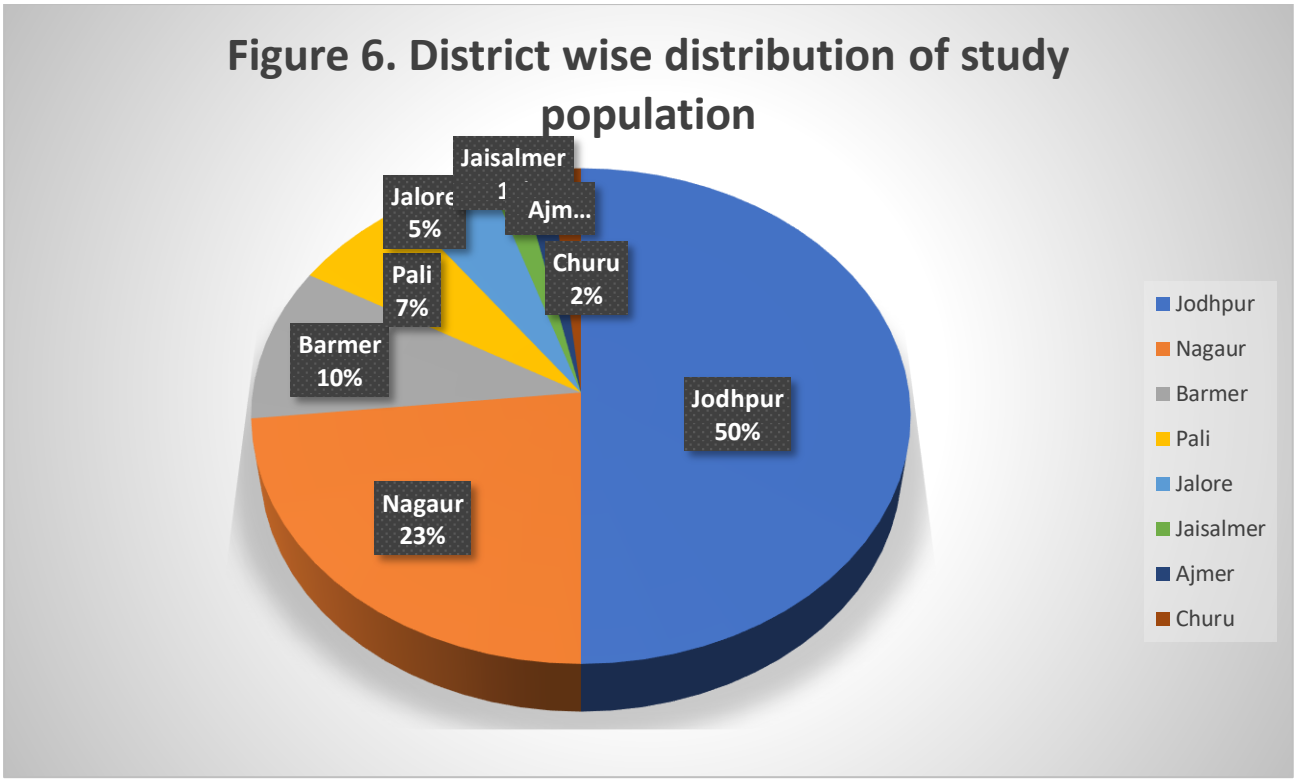


Table 6. Distribution of the study population according to occupation:

Occupation	**Numbers (percentages)**
School student	27 (45)
Home maker	10 (16.6)
Graduation student	8 (13.3)
Unemployed	2 (3.3)
Farmer	5 (8.3)
Nursing officer	1 (1.6)
Tailor	1 (1.6)
Teacher	1 (1.6)
Carpenter	2 (3.3)

Daily worker	2 (3.3)
Indian Army	1 (1.6)

Our study population was predominated by school students who were 45% among all study cases. Home makers and graduation students were next highest populations with 16.6% and 13.3% respectively. Our study population had also covered population spectrum of farmer, nursing officer, tailor, teacher, carpentor and indian army officials.

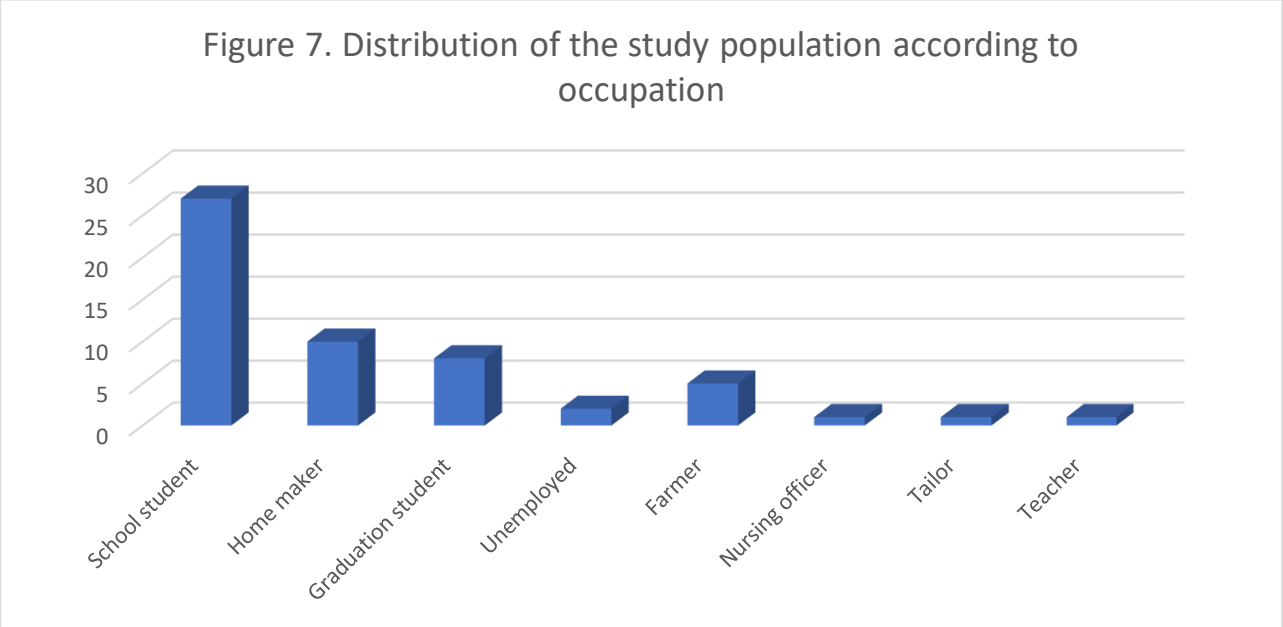


Table 7. Religion wise distribution of the study population:

Religions	**Number (percentage)**
Hinduism	56 (93.3)
Islam	3 (5)
Jainism	1 (1.7)

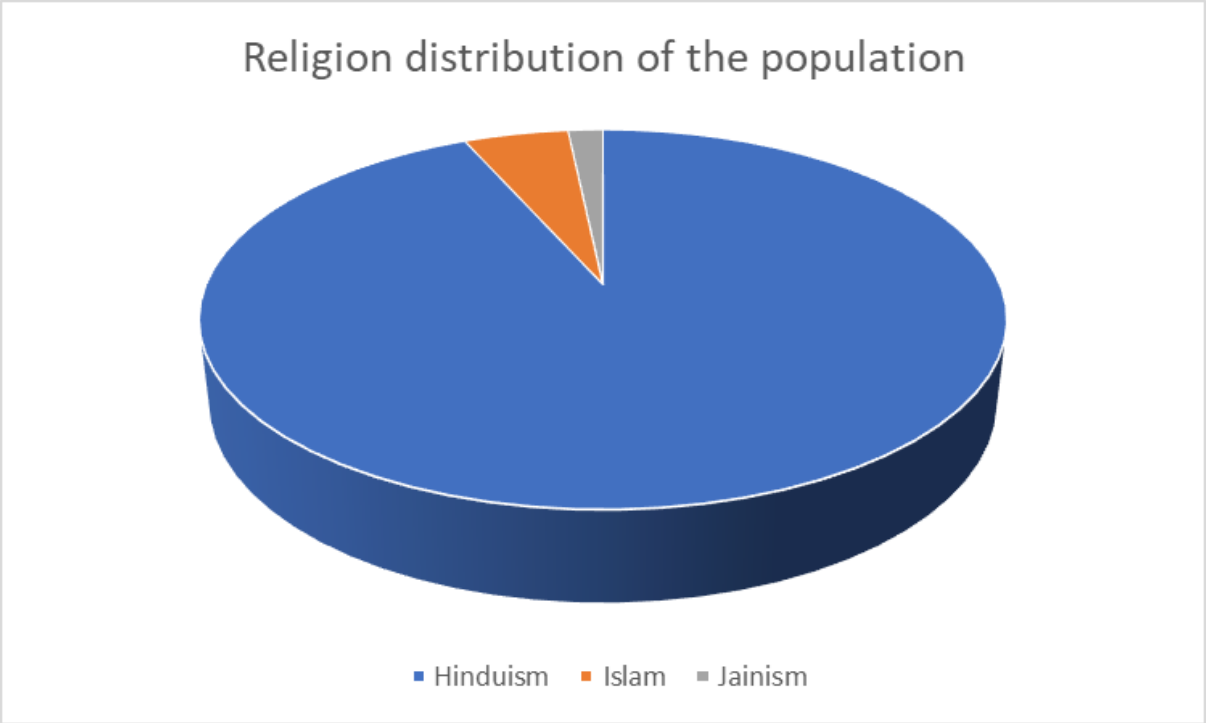


Table 8. Presence of Family history of Diabetes (both T1DM and T2DM):

Family history of diabetes	**Number (percentage)**
Yes	22 (36.66)
No	38 (63.33)

Table 9. Smoking status of the study population:

Smoking status	**Number (percentage)**
Yes	2 (3)
No	58 (97)

Table 10. Status of oral anti diabetic usage in study population:

Usage of OAD	**Number (percentage)**

Yes	1 (1.6)
No	59 (98.4)

Table 11. Distribution of autoimmune hypothyroidism in the study population:

Hypothyroidism	**Number (percentage)**
Yes	4 (6.6%)
No	56 (93.4%)

Other autoimmune disease presence:

No patients of Rheumatoid arthritis, vitiligo, or graves' disease were found in our study subjects.

Table 12. Other diseases:

Among known co-morbidity following diseases were found in study populations:

Disease	**Numbers**
Celiac	2
Essential HTN	2
B/L SNHL (sensory neural hearing loss)	1
Iron deficiency anemia	2
Benign breast nodule	1
B/L renal calculi	1

Table 13. Distribution of study population according to daily insulin need:

Daily insulin need (U/kg)	**Mean ± SD**
Basal insulin	0.35 ± 0.15
Bolus insulin	0.51 ± 0.30

Table 14. Annual hypoglycemic and DKA episodes in study population:

Clinical parameters	**Median values (range)**
DKA episodes (Annual)	1 (0-10)
All hypoglycemic episodes (Annual)	4.5 (0-75)
Severe hypoglycemic episodes (Annual)	0 (0-10)

Table 15. Distribution of the physical exercise among cases (presented as MET minutes/week):

Exercise	**Numbers**	**MET minutes/week (median and range)**
Yes	33	550 (120- 10080)
No	27	

Table 16. Distribution of diabetic complications among study population:

Complication	**Numbers (percentages)**	**Mean duration (years)**	**On treatment (percentages)**
Neuropathy (DSPN)	18 (30)	2	5 (27.7)
Kidney	3 (5)	1.67	3 (100)
Gastroparesis	14 (23.3)	1.64	14 (100)
Dermatological	8 (13.3)	1.75	1 (12.5)
Autonomic	12 (20)	1.91	0 (0)
CAD	1 (1.6)	1	1 (100)
LJM	5 (8.3)	3.66	2 (40)

Recurrent abortions	1 (1.6)	1	1 (100)

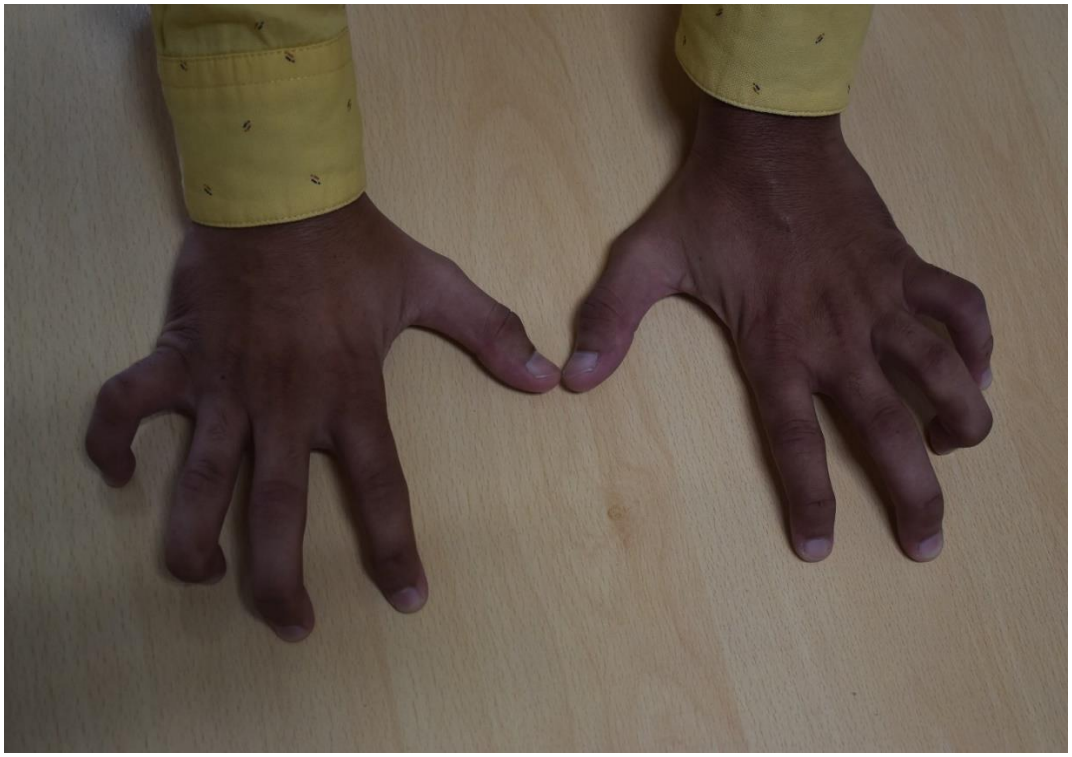
30 patients (18%) were suffering from neuropathy predominantly distal symmetric polyneuropathy. Among them only 5 patients were undergoing treatment. Mostly were taking pregabalin and methylcobalamin combination. The average duration of such symptoms were 2 years.

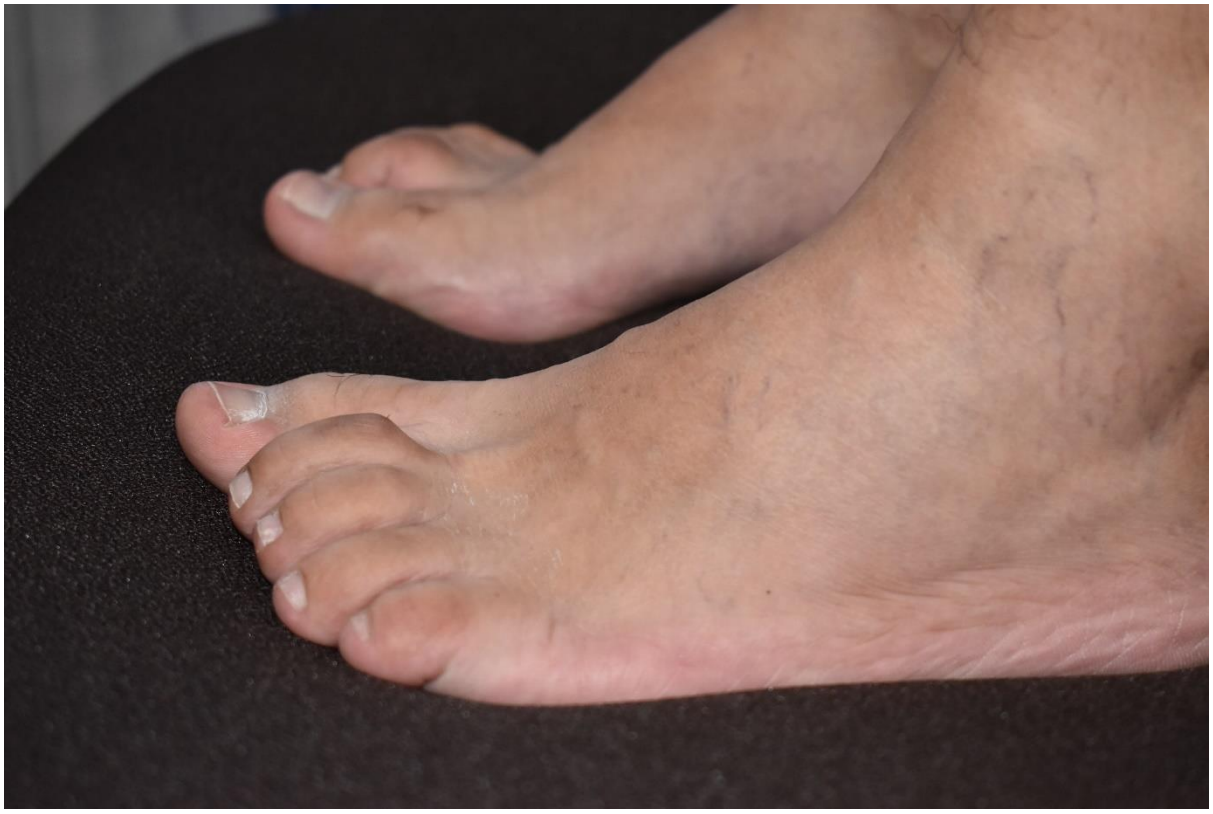
Diabetic gastroparesis was found in 23.3% of our study population among all patients were put on prokinetic agents before our contact. Dermatological complications of diabetes was 3rd most common complicating features in our study group with an prevalence of 13.3%. Most of the skin lesions were diabetes skin spots and one each case of diabetes xerosis and tinea cruris was found. Autonomic neuropathy was found in 20% of our cases where most of them were suffering from sudomotor dysfunction. Orthostatic hypotension, hypoglycemia associated autonomic failure and erectile dysfunction was also present in one each patient respectively.

5 of our study subjects were suffering from limited joint mobility due to long term diabetes. Interestingly one of our patient had significant and symmetrical upper and lower limb LJM.

Figure 8: Advanced LJM in one of our study patient

Pictures are suggestive of symmetrical fixed contractures in both upper and lower limbs (Brink-Starkman LJM type III) along with thickened skin in dorsum of the hand. Patient is found to have positive ‘Table Top sign’ and ‘Prayer sign’.





Diabetic kidney disease was found in only 5% of our cases and one patient had previous history of ischemic heart disease.

Table 17. Vitals measurements of the study population:

Parameters	**Values (mean ± SD)**
Systolic BP	111.016 ± 14.22
Diastolic BP	69.28 ± 7.72
Pulse	80.05 ± 9.97

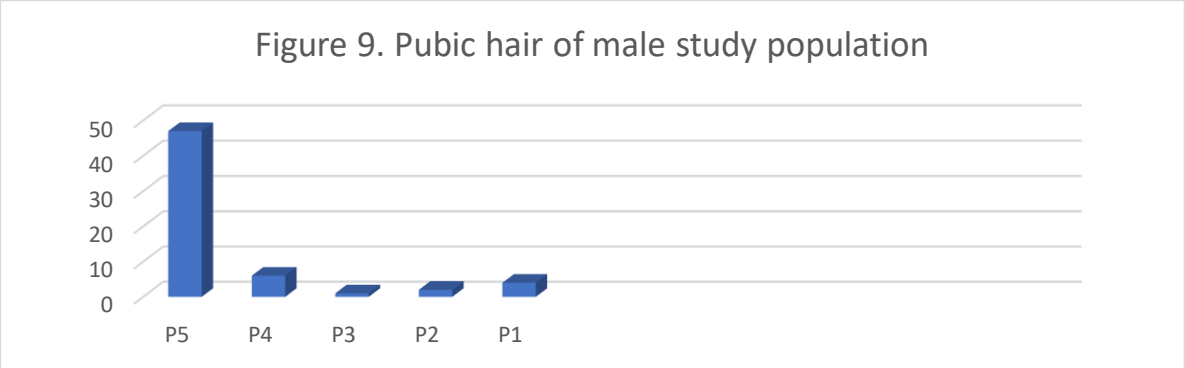
Table 18. Classifications of the pubertal status among study population:

Presence of axillary hair:

Yes	56 (94)
No (scanty)	4 (6)

Pubic hair status:

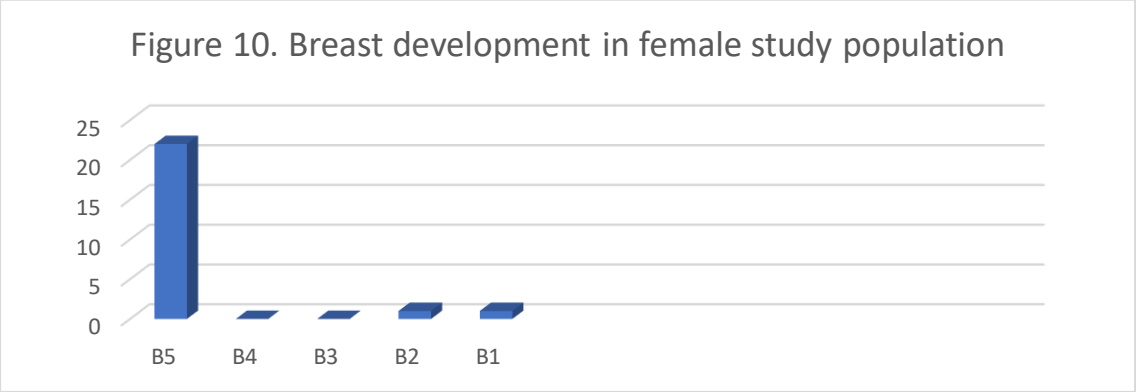
P5	47
P4	6
P3	1
P2	2
P1	4



Breast development status (among 24 females):

B5	22
B4	0
B3	0

B2	1
B1	1



Among Male patients (genital status) (36 patients):

Normal genitalia	32 (88.8%)
Pre-pubertal	4 (11.2%)

Among female patients (genital status) (24 patients):

Normal genitalia	22 (91.6%)
Pre-pubertal	2 (8.4%)

Table 19. Biochemical characteristics of the study patients:

Parameters	**Mean ± SD/ median (range)**
Hba1c (%)	10.34 ± 2.44
C-peptide (ng/ml)	0.01 (0.01-2.22)
TG (ng/dl)	95.08 ± 46.81
LDL (ng/dl)	110.91 ± 37.73

TSH (mIU/L)	1.86 (0.51-23.2)
Creatinine (mg/dl)	0.8 (0.4-6)
SGOT/PT (IU/L)	21.05 ± 8.71
Testosterone (ng/dl)	375 (5- 909.4)
IGF-1 (ng/ml)	222.9 (84.42-661.1)
Calcium (mg/dl)	9.54 ± 0.8
Phosphorus (mg/dl)	4.05 ± 0.8
ALP (IU/L)	128 (53-623)
25 (OH) D (ng/ml)	16.81 (4-42.5)
iPTH (pg/ml)	45.9 (16.2-140)
CTX (pg/ml)	480.85 (53.95-1037.40)
Sclerostin (ng/ml)	1.32 (0.047-580)
24 hours urinary calcium (mg/24 hours)	91.13 (13.3-260.8)
24 hours urinary phosphorus (mg/24 hours)	470 (190-3600)
24 hours urinary creatinine (mg/24 hours)	831 (161-4650)
Urinary microalbumin (mg/g of creatinine)	14.2 (0.3-3600)

ECG

It was found normal in 59 cases and sinus tachycardia was found in 1 case only.

Table 20. Fundus abnormality among study cases (n=54) :

Status of DR	**Numbers (percentage)**
No DR	47 (87.03)
Mild NPDR	2 (3.7)

Moderate NPDR	3 (5.5)
Severe NPDR	0
PDR	2 (3.7)

Figure 11. Distribution of fundus abnormality among cases

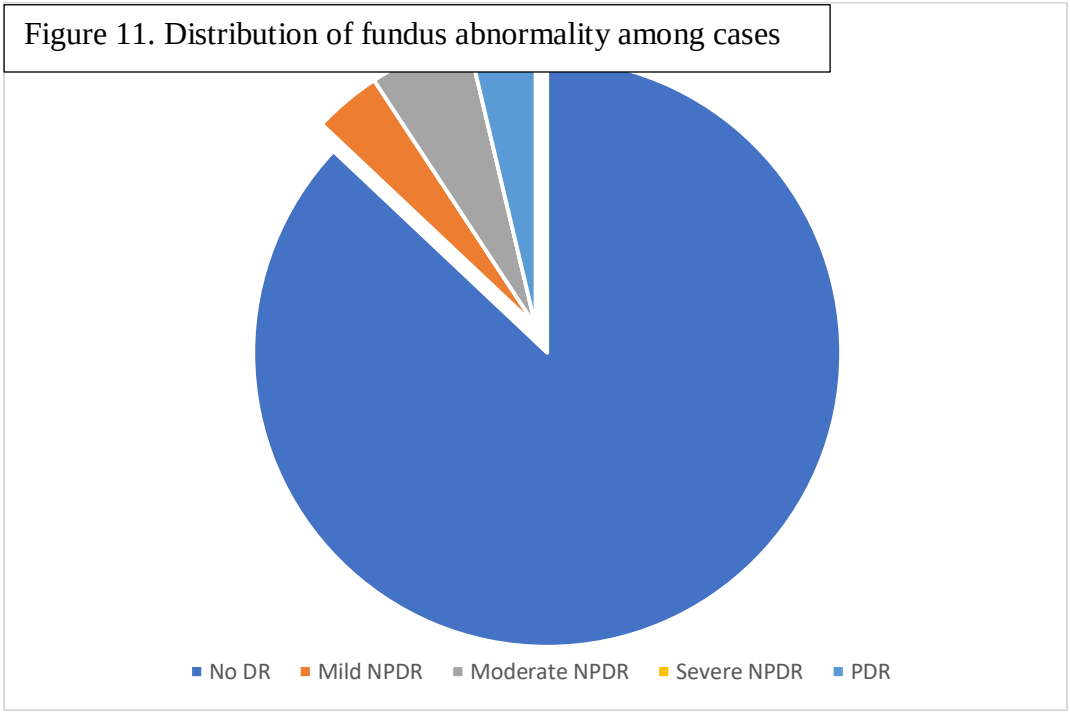


Table 21. BMD and related DXA parameters comparisons between cases and controls:

Parameters	**Cases (mean ± SD)**	**Control (mean ± SD)**	**p-value**
BMI	19 ± 3.25	19.14 ± 2.71	0.261
LS BMC (g/cm2)	0.86 ± 0.14	0.96 ± 0.15	**0.036**
LS BMD t score	-1.2 ± 1.15	-0.6 ± 0.95	**0.025**
LS BMD z score	-1.22 ± 1.27	-0.42 ± 1.02	0.056
FN BMC (g/cm2)	0.74 ± 0.129	0.81 ± 0.139	0.132
FN BMD t score	-1.09 ± 1.03	-0.69 ± 0.93	0.139
FN BMD z score	-1.23 ± 1.07	-0.75 ± 0.97	**0.003**
Wrist BMC	0.69 ± 0.14	0.70 ± 0.10	**0.015**
Wrist BMD t score	-0.504 ± 1.44	0.613 ± 1.05	0.052
Wrist BMD z score	-0.852 ± 1.34	-0.422 ± 1.14	0.095
TBS	1.306 ± 0.113	1.376 ± 0.083	**0.001**
TBS t score	-1.029 ± 0.82	-0.653 ± 0.798	0.081
VAT	15.66 ± 8.94	18.59 ± 7.82	**0.000**
TBF percentage	27.66 ± 7.26	31.94 ± 9.55	**0.000**
TLBM	37.10 ± 8.36	39.40 ± 10.05	**0.000**
TLBM percentage	70.65 ± 8.32	68.11 ± 9.60	**0.000**
WB t score	-1.025 ± 1.06	-0.692 ± 0.92	0.166
WB z score	-1.13 ± 1.196	-0.64 ± 0.89	0.018
WB BMC	0.91 ± 0.14	1.07 ± 0.11	**0.000**

Co-relation analysis of clinical parameters:

Table 22. Co -relation analysis of duration of diabetes with DXA parameters

Parameters	**Co-relation co-efficient**	**p value**
LS BMC	0.234	0.072
FN BMC	0.055	0.678
TBS	-0.115	0.380
VAT	0.208	0.111
TLBM	0.167	0.201
WB BMC	0.119	0.363

Table 23. Co relation analysis of duration of diabetes with biochemical parameters:

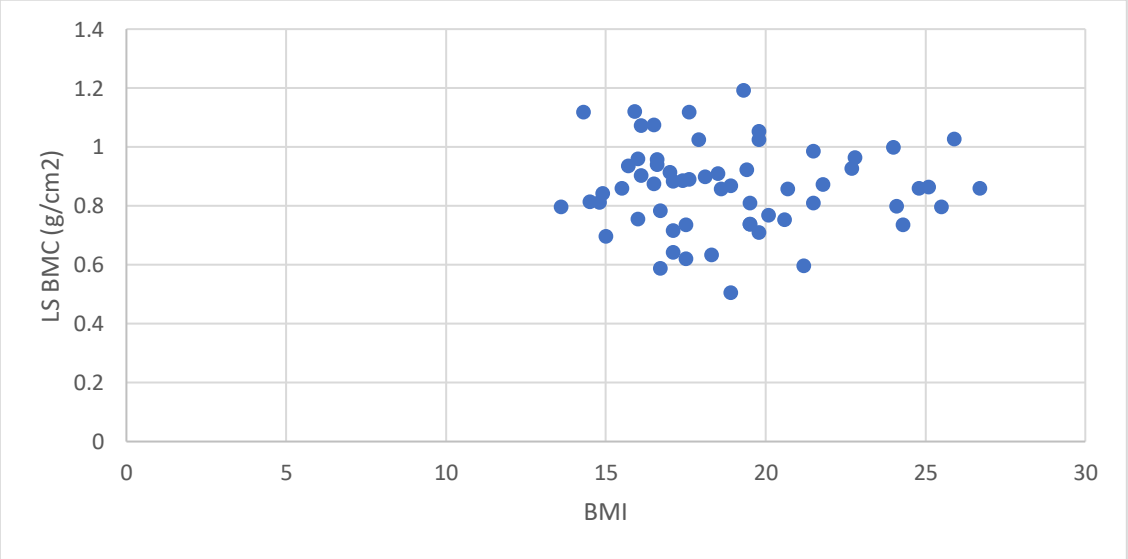
Parameters	**Co-relation co-efficient**	**p value**
Age	0.620	**0.000**
CTX	0.058	0.695
Hba1c	-0.290	**0.026**
C-peptide	-0.439	**0.001**
TG	0.168	0.260
LDL	0.083	0.577
Testosterone	0.315	0.144
IGF-1	-0.425	0.019
iPTH	0.447	**0.004**
25 (OH) D	0.080	0.635
ALP	-0.183	0.293

24 hours urinary Ca	-0.252	0.235
24 hours urinary PO4	-0.065	0.763

Table 24. Co-relation analysis of BMI with DXA parameters

Parameters	**Co relation co-efficient**	**P value**
LS BMC	0.324	**0.000**
FN BMC	0.220	**0.016**
TBS	0.175	0.056
VAT	0.160	0.082
TLBM	0.144	0.116
WB BMC	0.195	**0.033**

Figure 12. Co-relation diagram between LS BMC and BMI



Co-relation analysis of all BMD parameters:

Table 25. Co-relation of LS BMC with rest DXA parameters:

Parameters	**Co relation co-efficient**	**P value**
FN BMC	0.666	**0.000**
TBS	0.574	**0.000**
VAT	0.304	**0.001**
TLBM	0.332	**0.000**
WB BMC	0.707	**0.000**

Figure 13. Co-relation diagram between LS BMC and FN BMC

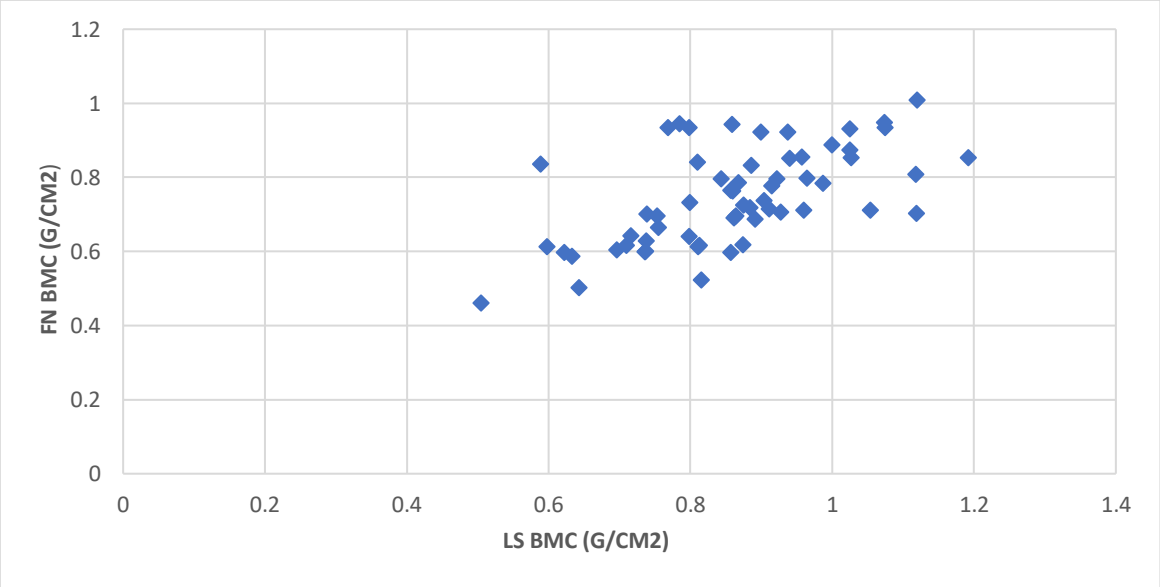


Table 26. Co-relation of FN BMC with rest DXA parameters:

Parameters	**Co relation co-efficient**	**P value**
LS BMC	0.666	**0.000**
TBS	0.479	**0.000**

VAT	0.160	0.080
TLBM	0.364	**0.000**
WB BMC	0.585	**0.000**

Figure 14. Co-relation diagram between FN BMC and WB BMC

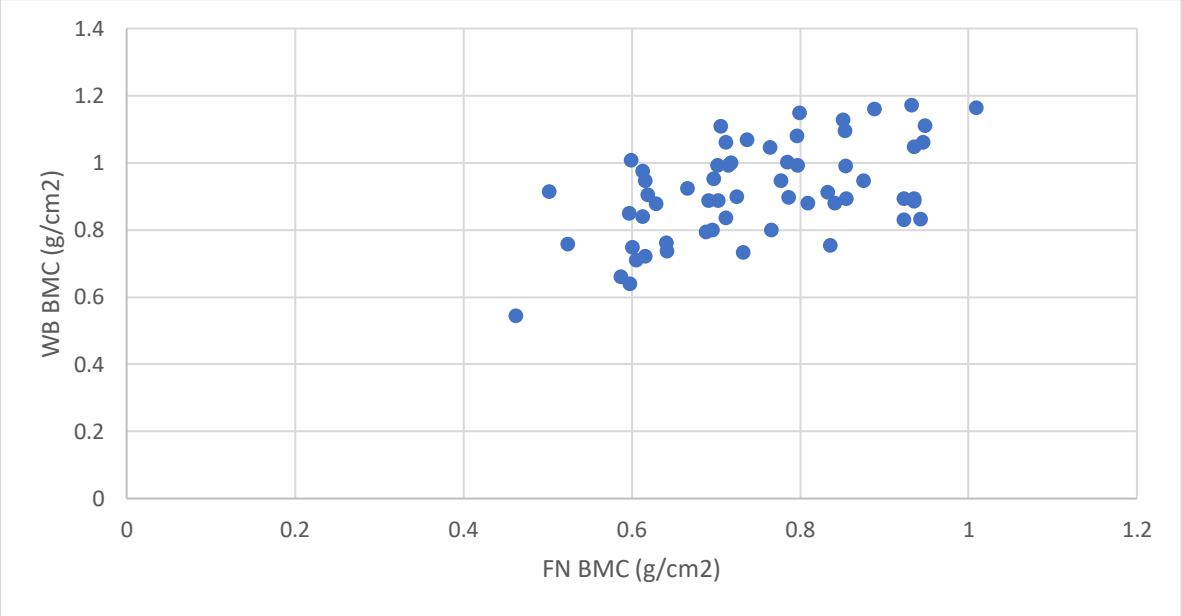


Table 27. Co-relation of WB BMC with rest DXA parameters:

Parameters	**Co relation co-efficient**	**P value**
LS BMC	0.707	**0.000**
FN BMC	0.585	**0.000**
TBS	0.500	**0.000**
VAT	0.155	0.091
TLBM	0.485	**0.000**

Figure 15. Co-relation diagram between LS BMC and WB BMC

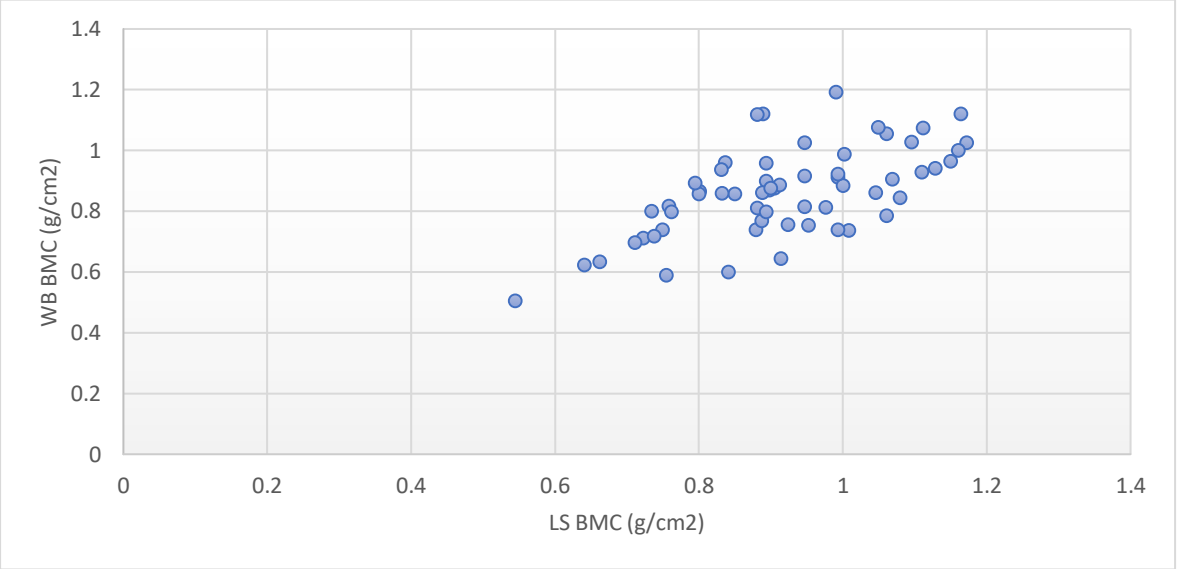


Table 28. Co-relation analysis of WB BMC with biochemical parameters:

Parameters	**Co relation co-efficient**	**P value**
Age	0.233	**0.011**
CTX	-0.031	0.830
Hba1c	-0.149	0.260
C-peptide	-0.129	0.353
TG	-0.159	0.285
LDL	0.159	0.287
Testosterone	-0.518	**0.011**
IGF-1	-0.276	0.140
iPTH	-0.153	0.352
25 (OH) D	-0.348	0.032
ALP	-0.476	**0.004**

24 hours urinary Ca	0.501	**0.013**
24 hours urinary PO4	0.482	**0.017**

Figure 16. Co-relation diagram between serum testosterone and WB BMC

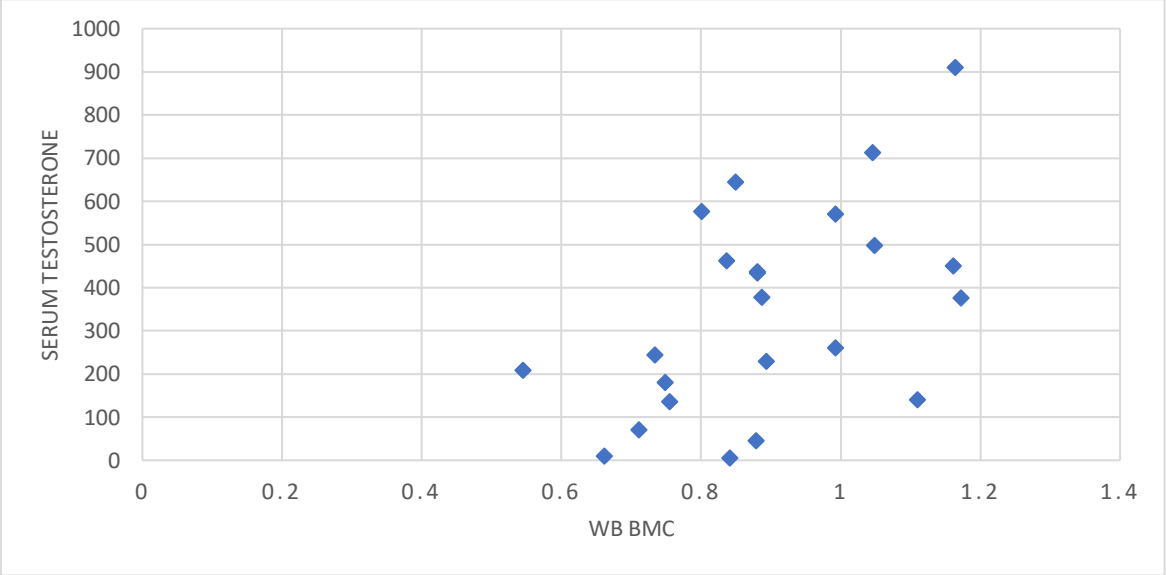


Table 29. Co-relation of TBS with BMD parameters:

Parameters	**Co relation co-efficient**	**P value**
LS BMC	0.574	**0.000**
FN BMC	0.479	**0.000**
VAT	0.025	0.785
TLBM	0.199	**0.029**
WB BMC	0.500	**0.000**

Table 30. Co-relation of VAT with BMD parameters:

Parameters	**Co relation co-efficient**	**P value**
LS BMC	0.304	**0.001**

FN BMC	0.160	**0.001**
TBS	0.025	0.785
TLBM	0.043	0.645
WB BMC	0.155	0.091

Table 31. Co-relation analysis of VAT with biochemical parameters:

Parameters	**Co relation co-efficient**	**P value**
Age	0.139	0.129
CTX	0.015	0.915
Hba1c	0.002	0.985
C-peptide	-0.120	0.387
TG	-0.025	0.870
LDL	-0.051	0.733
Testosterone	-0.124	0.572
IGF-1	-0.052	0.785
iPTH	-0.107	0.519
25 (OH) D	0.280	0.088
ALP	0.140	0.424
24 hours urinary Ca	-0.001	0.996
24 hours urinary PO4	-0.003	0.987

Table 32. Co-relation of TLBM with BMD parameters:

Parameters	**Co relation co-efficient**	**P value**
LS BMC	0.332	**0.000**

FN BMC	0.364	**0.000**
TBS	0.199	0.029
VAT	0.043	0.645
WB BMC	0.485	**0.000**

Co-relation analysis of various biochemical parameters:

Table 33. Co-relation analysis of Hba1c with other biochemical parameters:

Parameters	**Co relation co-efficient**	**P value**
Age	-0.214	0.103
CTX	-0.112	0.447
C-peptide	-0.137	0.324
TG	0.101	0.497
LDL	0.186	0.211
Testosterone	-0.224	0.305
IGF-1	-0.116	0.542
iPTH	-0.446	**0.004**

Table 34. Co-relation of C-peptide with biochemical parameters:

Parameters	**Co relation co-efficient**	**P value**
Age	-0.301	**0.027**
VAT	-0.120	0.380
WB BMD	-0.129	0.353

CTX	-0.019	0.900
TG	-0.099	0.530
LDL	0.044	0.782
Testosterone	-0.175	0.437
IGF-1	0.580	**0.001**
iPTH	0.041	0.810
25 (OH) D	-0.210	0.225
ALP	-0.183	0.316
24 hours urinary Ca	0.242	0.255
24 hours urinary PO4	0.173	0.418

Figure 17. Co-relation diagram between Serum IGF-1 and c-peptide

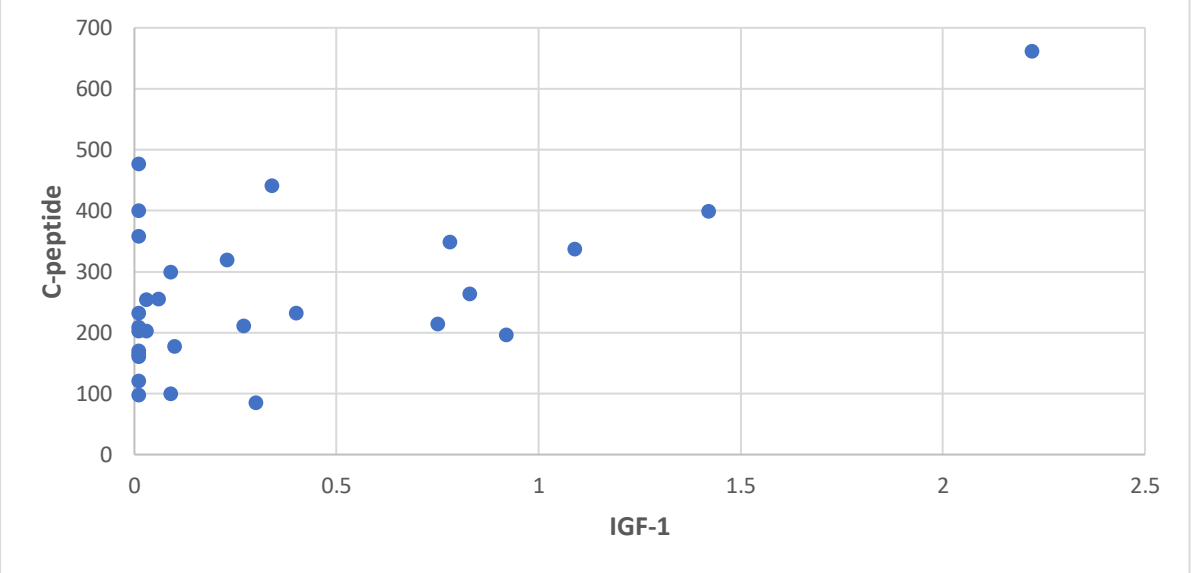


Table 35. Co-relation of CTX with biochemical and DXA parameters:

Parameters	**Co relation co-efficient**	**P value**
Age	0.250	0.074

VAT	0.015	0.915
WB BMC	-0.031	0.830
Duration of diabetes	0.058	0.695
Hba1c	-0.112	0.447
C-peptide	-0.019	0.900
TG	-0.281	0.083
LDL	-0.336	**0.037**
Testosterone	0.217	0.358
IGF-1	0.000	0.999
iPTH	-0.041	0.828

Table 36. Co-relation of serum Testosterone with other biochemical parameters:

Parameters	**Co relation co-efficient**	**P value**
Age	0.426	**0.043**
CTX	0.217	0.358
VAT	-0.124	0.572
WB BMC	0.518	**0.011**
Duration of diabetes	0.315	0.144
Hba1c	-0.224	0.305
C-peptide	-0.175	0.437
TG	-0.085	0.728
LDL	0.061	0.804
IGF-1	-0.270	0.312

iPTH	0.406	0.095
25 (OH) D	0.151	0.564
ALP	-0.566	**0.022**
24 hours urinary Ca	0.445	0.127
24 hours urinary PO4	0.276	0.362

Table 37. Co-relation of IGF-1 with various biochemical parameters:

Parameters	**Co relation co-efficient**	**P value**
Age	-0.393	**0.032**
CTX	0.000	0.999
VAT	-0.052	0.785
WB BMC	-0.276	0.140
Duration of diabetes	-0.425	**0.019**
Hba1c	-0.116	0.542
C-peptide	0.580	0.001
TG	-0.112	0.587
LDL	-0.177	0.387
Testosterone	-0.270	0.312
iPTH	0.394	0.063
25 (OH) D	-0.042	0.849
ALP	0.515	**0.017**
24 hours urinary Ca	0.055	0.858
24 hours urinary PO4	-0.119	0.698

Table 38. Co-relation of 25 (OH) D with various biochemical parameters:

Parameters	**Co relation co-efficient**	**P value**
Age	0.030	0.857
CTX	0.294	0.114
VAT	0.280	0.088
WB BMC	0.348	**0.032**
Duration of diabetes	0.080	0.635
Hba1c	-0.192	0.249
C-peptide	-0.210	0.225
TG	-0.414	**0.021**
LDL	-0.387	**0.032**
Testosterone	0.151	0.564
IGF-1	-0.042	0.849
iPTH	-0.062	0.737

Table 39. Co-relation of serum ALP with various biochemical parameters:

Parameters	**Co relation co-efficient**	**P value**
Age	-0.473	**0.004**
CTX	-0.233	0.241
VAT	0.140	0.424
WB BMC	-0.476	**0.004**
Duration of diabetes	-0.183	0.293

Hba1c	0.267	0.121
C-peptide	-0.183	0.293
TG	-0.043	0.829
LDL	-0.107	0.587
Testosterone	-0.566	**0.022**
IGF-1	0.515	**0.017**
iPTH	0.136	0.483

Table 40. Co-relation analysis of Urine microalbumin with various biochemical parameters:

Parameters	**Co relation co-efficient**	**P value**
Age	-0.047	0.781
CTX	-0.242	0.190
VAT	0.016	0.926
WB BMC	0.087	0.610
Duration of diabetes	-0.038	0.821
Hba1c	-0.079	0.643
C-peptide	0.148	0.383
TG	0.418	**0.027**
LDL	0.534	**0.003**
Testosterone	0.242	0.384
IGF-1	-0.158	0.531
iPTH	0.221	0.250

Discussion

This study was conducted in 120 T1D patients and age, sex, and BMI matched controls from north west India in our tertiary care centre of All India Institute of Medical Sciences Jodhpur. From the above extensive discussions on bone health in type 1 diabetes, we can chalk out some important points which we have tried to address in this study.

Bone loss generalized or localized?

T1D patients suffers from low bone mass which have been established in various literatures for last 2 decades, but is this bone loss generalized or localized to some compartments? Various studies have used some specific sites to measure the BMD, so it is a very important unmet need to address bone density in all parts of the body. In our study we have evaluated BMD in lumbar spine, femoral neck, distal 1/3rd of radius and whole body to provide a wholesome and robust bone mineral density data. This includes the major axial and appendicular skeleton structures. This evaluation has given us the information about the involvement of both cortical and trabecular involvement in T1D which is not very consistent across the literatures.

Analysis of the BMD data's:

Various studies have evaluated this bone mineral data in terms of either bone mineral concentration (expressed in g/cm2) or in "z" score which compares this bone concentration with the age and sex matched individuals. Most of the literatures have used "z" score as the main parameter for comparison between cases and controls. Here we have tried to explore both in our study. "Z' score has been obtained from comparing age and sex matched individuals which generally uses Caucasians data in this Hologic discovery DXA machines. We have simultaneously analysed the "t" and "z" score for comparisons.

Analysing TBS and body fat analysis in our study:

The various literatures have pointed out histomorphometric changes in T1D patients which accounts for higher fragility fractures even in presence of normal bone mineral mass. Studies of Neumann et al., Aditi Wagh et al. have pointed out low TBS in T1DM patients which accounts for higher fragility fractures disproportionate to BMD (155,156). But very few studies have evaluated both BMD and TBS on a single patient cohort. As an Indian study, we can say this study is first of such studies where we have looked upon in both parameters. Importantly another knowledge gap which has persisted is that of a co relation between these parameters

and relationship with various clinical and biochemical parameters. In our study we have tried to explore all these things

Discussions regarding the distribution of the population:

We have enrolled a total 60 T1D patients and matched with 60 age, sex and BMI matched healthy controls. Among 60 cases, males constitute 36 among them, making them 60% of the case population. This is in concordance with most of the literatures as there has been a slight male preponderance on most of the studies (137,139,140,142). Various studies have found out comparatively lower bone mineral density in either sex due to various factors but we could not find any differences between male and female BMD among cases. As discussed in review of literature section, males can have a slightly lower bone mineral density due to more prevalence of hypogonadism. Our study did not have any disproportionate incidence of hypogonadism between males and females.

Regarding the sample size, we had calculated this sample size from Joshi et al. study where they had taken 75 T1D patients for evaluation. Our sample size of 60 patients and 60 controls is one of the largest compared to various case control studies. This is only the 2nd Indian case control study to look at this topic. Eller-Vainicher et al. had conducted similar case control study on T1D in bone mineral study on 175 patients thus making it one of the largest sample sizes till date. It was published in 2011 from Belarus. Rest most of the western studies have taken a sample size between 30 to 50. But our study stands unique among all these due to simultaneous measurements of BMD, TBS, VAT and TLBM which none of the previously mentioned studies have done (140,141,143,145–149). Munoz-Torres et al., Lunt et al., Rozadilla et al. conducted their case control study in about 90 patients from Spain, New Zealand, and Spain respectively. These are the only few larger published studies apart from us which have looked at the bone health in T1DM (24,65).

Distribution of the population according to the places:

The only previous study from Indian subset was published from Mumbai, where Joshi et al. have mostly concentrated patients from Maharashtra state and western India. Our study is based on north west India where our institutions draining population hails from western Rajasthan as well as northern and southern parts. Our study has got around 50% population from Jodhpur

district with significant contribution from Nagaur, Jalore and Pali districts. Some other districts like Churu, Ajmer, Jaisalmer and Barmer have also contributed to our cases numbers. Rajasthan's T1DM incidence, prevalence, their clinical and biochemical characteristics have also not been explored in any of the previous larger studies from India (162). So, our study stands out as a maiden representation of the T1D population from this state.

Distribution of the population by occupation:

Our study population maximally constitutes school students. 45% of the study population are school student where as 3.3% students were pursuing their graduation. 2nd highest population is consisted of female home makers. This occupation part has not been well discussed in previous literatures. The Indian study Joshi et al. has not mentioned about the occupations of the study subjects. Most of the western studies have taken a mean age of around 30-40 years of age of presentation which makes school student very unlikely. What we can infer from our data is that this age group can be an ideal time for insulin education as well as bone health education in this group of children. The mean age of diagnosis in T1D have been described to be around 10 years in world and slightly later in Indian contexts (around 12-14 years) (3,113). Interestingly the incidence of T1D in below 5 years have been increasing steadfastly in last 2 decades and India is certainly not an exception. As our study have concentrated more on the school going group, the concept of low bone mass and awareness among them can be grown from this age only. The diabetes educators, endocrinologists, nurses, administration invest innumerable economy and workforce behind educating a single T1D patient. These students can get a definite bone mineral health awareness in their school life along with insulin education so that they can prevent the further bone loss in future.

The mean age of case population and duration of diabetes:

The mean age of our case population at presentation was 22.4 ± 8.6 with mean age of diagnosis of diabetes of 15.86 ± 6.6 years. Our mean age of population surpasses the completion of pubertal age. Physiologically the peak bone mass accrual completes almost 90% by at the age of 18 and remaining 10% achieved in 3-4 years later than that (7). So, any low bone mass findings at this stage can unequivocally confirms the presence of disease related effect on the bony accrual. The disease process has halted the peak bone accrual in our group of patients. Females generally attain peak bone mass 2 years earlier than males which almost corroborates

with their onset of menarche. Our male dominant case population also can explain a comparatively lower bone mineral density as males had more severe bone effect with onset of diabetes. Our mean duration of disease was 5 years.

The other studies which we have discussed in review section, have mostly taken mean age of 4th or 5th decade as a patient population. Munoz-Torres et al., Lunt et al., Rozadilla et al., Miazgowski et al., Eller-Vainicher et al., Zhukouskaya et al. in their studies have taken mean age of 30, 43, 31, 43, 33 and 31 respectively (24,65,138,140,141,152). All the studies haven't compared with healthy controls; still whoever have compared have got a difference of around 30% from controls. Some of the notable studies have a mean age in 1st or 2nd decade in the study population. Those study findings can have a limitation as those patients haven't achieved peak bone mass at that age. Gunczler et al., Leger et al., Parthasarathy et al., De Schepper et al., Pascual et al. have included T1DM patients of mean age of 9.5, 13,11, 12.7, 9 respectively (131,133,137,142,150). Hamilton et al. in their study have included a mean age of 45 years of T1DM for analysis which looks by far the highest mean age among all the study groups. Joshi et al in their study have got a mean age of 27.2 years which closely resembles us.

The duration of diabetes has also been variable in all studies. As our mean duration of diabetes was 5 years, similar diabetes duration was found in Roe et al, Lettgen et al., Gunczler et al., De Schepper et al., Pascual et al studies (131,148–150,152). Finding low bone mass in patients of less than 5 years of diabetes can indicate severe bone losing property of T1DM itself but later in our corelation analysis, duration of diabetes didn't find to be associated with low bone mass.

Discussion regarding primary objective:

Our primary objective of this study was to evaluate and analyse bone mineral density in T1DM patients and to compare with healthy controls. We had matched age, sex and BMI during recruitment of controls. At the outset BMI was confirmed to match well (19 ± 3.25 vs 19.14 ± 2.71; p=0.261).

Lumbar spine analysis:

In lumbar spine analysis bone mineral concentration (LS BMC) found to be significantly low compared to controls (0.86 ± 0.14 vs 0.96 ± 0.15; p=0.036) whereas the LS BMD "t" score was also significantly lower in cases (-1.2 ± 1.15 vs -0.6 ± 0.95; p=0.025). The LS BMD z score found to non-significantly lower than controls (-1.22 ± 1.27 vs -0.42 ± 1.02; p=0.056). As we have previously described, calculating both BMC and BMD are more acceptable and holistic

approach to determine bone loss. The dubiosity in calculating "t" and "z" score remains in our Hologic machines where Asian databases are used. This finding is very much like most of the larger studies which we have quoted in previous sections. Most of the studies have reported LS BMD z scores of around -0.4 to -0.7 in contrast to our studies which has got LS BMD z score of -1.22. This difference may be explained by the racial differences as our database resembles Caucasians data. Comparatively poor HbA1c (mean 10.34% in our study), frequent ketoacidosis, hypoglycemic episodes additionally can explain this difference compared to western counterparts. Our LS BMC was 12% less in cases compared to controls whereas almost 30% less in terms of LS BMD z score. Most of the studies quoted previously have documented around 20 to 35% less BMD in lumbar spine. One notable study here to mention is Gunczler et al. study where this difference was around 45% (152). Very low mean age of study population (12 years), poor glycemic control (mean Hba1c > 9%), chronic acidotic state and significant hypercalciuria accounted for this significant bone loss in this study. Indian study of Joshi et al. did found a similar low BMD in lumbar spine as in our case (17). Low lumbar spine bone density establishes trabecular bone loss in T1DM.

BMD in femoral neck and wrist:

These two sites represent two very important sites of axial and appendicular skeleton as well as cortical sites for bone density measurement. In our study both femoral neck and wrist had low bone mass compared to controls. When we see the femoral neck data; z score was significantly low in cases compared to controls (-1.23 ± 1.07 vs -0.75 ± 0.97; p=0.003). Rest FN BMC and t score was not significantly lower than controls (0.74 ± 0.129 vs 0.81 ± 0.139; p=0.132). This finding also mostly corroborates with the various other studies where the average difference between FN BMD has been around 30%. Eller-Vainicher et al. and Zhukouskaya et al. have reported 33.4% and 30% low BMD in femoral neck (140,141). Our mean FN BMD z score was -1.23 ± 1.07 which is again quite contrasting to other studies. The large cohort studies have documented z scores of -0.4 to -0.7 (133,136,139). Again, the possible explanation could be our dataset and poor glycemic status.

Regarding the wrist joint, Wrist BMC is significantly lower in cases compared to controls (0.69 ± 0.14 vs 0.70 ± 0.10; p=0.015) whereas t and z scores were nonsignificant low in our study. Wrist BMD has not been much evaluated in other T1DM studies. Levin et al., Wiske et al., Leon et al. have reported low bone mineral density in wrist BMD (143–145). These studies were done previously before the regular advent of DXA technology. Pascual et al. had

evaluated wrist BMD by DXA technology but did not find any significance difference compared to controls (131). So, our study stood fairly as a unique study which has found low BMD in wrist by DXA technology. This has got clinical implications as well. Generally, all data's in T1DM bone health have concentrated on lumbar spine and femoral neck only (evaluating trabecular bones) but data on cortical bone are rare. Our study has important insights to this.

Whole body bone health:

In concordance with most of the studies, whole body BMC was found to be lower in cases compared to controls (0.91 ± 0.14 vs 1.07 ± 0.11; p=0.000). The difference is almost 19% compared to 45% and 22% in Gunczler et al. and Parthasarathy et al. studies (133,142). Interestingly the WB z score (-1.13 ± 1.196) was quite similar to various studies (Ersoy et al., Lettgen et al., Joshi et al.) (17,147,149). Very importantly Joshi et al. had very similar whole body z scores compared to our study.

So, to summarize the primary objective part, we have found out low bone mass in all possible bony parts which were analysed in DXA. Most of the studies which we are discussing have not used all 4 of these parameters, making our study robust and comprehensive. Besides poor glycemic control, low total body fat and insufficient 25 (OH) D levels found to be the underlying factors in our study which we will be discussing in further paragraphs.

Discussion regarding secondary objectives:

Evaluation and comparison of TBS in cases and controls:

TBS assesses trabecular bone texture of the spine using DXA scans, a measure which correlates with bony microarchitecture. The biggest advantage of TBS measurement is the simultaneous measurement of BMD and TBS in a single DXA machine. Extensive evaluation of BMD in all bony parts along with TBS has not been done by most of the studies. No Indian studies have till now have made such comprehensive effort. The TBS acts as a perfect surrogate marker for bone histomorphometry. Till the regular availability of HRpQCT in our set up, TBS can act very well as the marker for bony architectural deterioration. So a composite measurement of TBS and BMD in a DXA machine is warranted (155).

Our trabecular bone score analysis showed significantly lower values in cases compared to controls (1.306 ± 0.113 vs 1.376 ± 0.083; p=0.001). Regarding TBS measurement, studies are scarce. Neumann et al. had conducted a large cohort study. This study aimed at investigating the TBS in T1D patients and healthy controls. Associations with prevalent fractures were tested. 119 T1D patients (59 males, 60 premenopausal females; mean age 43.4±8.9 years) and 68 healthy controls matched for gender, age, and body mass index (BMI) were analysed. TBS was 1.357±0.129 in T1D patients and 1.389± 0.085 in controls (p=0.075). Findings were very similar to our study. The presence of fractures in T1D was associated with lower TBS (odds ratio=0.024, 95 % confidence interval (CI)=0.001–0.875; p=0.042) but not with age or BMI (155). The authors had suggested a cut-off of <1.42 as a discriminatory tool for predicting fractures. Though this cut off has been mostly arbitrary and needs to be evaluated in large prospective studies. Another similar study was carried out by V N shah et al. who have looked upon TBS in T1DM (13). Their sample size was 45 each in cases and control arm. TBS was significantly lower in adults with T1D compared to controls (1.42 ± 0.12 vs 1.44 ± 0.08, p = 0.02) after adjusting for age, sex, current smoking status, and lumbar spine BMD, despite no difference in lumbar spine BMD between the groups. Interestingly their TBS value was far higher compared to our values (1.42 ± 0.12 vs 1.306 ± 0.113). Insulin resistance and metabolic syndrome corelated negatively with TBS in their study.

Unni Syversen et al. have also evaluated TBS as well as BMD in their cohort of 33 Norwegian T1DM men. Subjects with T1D exhibited lower whole-body BMD than controls (P = 0.04). TBS and BMSi were attenuated in men with T1D vs controls (P = 0.016 and P = 0.004, respectively), and T1D subjects also had a lower bone turnover (163). Nadzeya Karytska et al. in their abstract presented similar data in European Society of Endocrinology (ESE) congress in 2019. Aditi Wagh et al. in their recently published study compared the TBS and volumetric BMD measured by HRpQCT in Indian T1DM patients. This is one of the first kind of study which has measured vBMD in Indian T1DM patients. The retrospective DXA LS scans in children with T1DM (n=137, age 13.1 ± 3.2 years) and controls (n = 68, age 13.0 ± 2.7 years) were analysed with a research trial version of TBS iNsight software (Medimaps Group). Over a fourth of the T1DM children with low Trab vBMD (below -2 Z score) had normal TBS, while, in children with LS aBMD Z-score > -2 from both groups, >50% had degraded or partially degraded TBS. Degraded TBS was seen in half the control children although none of

them had low Trab vBMD. So they concluded that TBS and vBMD did not co related well in Indian T1DM patients (156).

Evaluation and analysis of VAT and TLBM in cases compared to controls:

We have evaluated the total body fat and its percentages (subcutaneous fat), visceral adipose tissue and total lean body mass in all cases and controls by DXA. The results are presented as visceral adipose tissue in kgs (absolute value) and total body fat percentage (relative value). Similarly, the total lean body mass is also expressed. Our study reveals significant low VAT in cases compared to controls (27.66 ± 7.26 vs 31.94 ± 9.55; p=0.000). Expectedly the TLBM is found to be more in T1DM cases compared to controls (70.65 ± 8.32 vs 68.11 ± 9.60; p=0.000). These results are expected in line of comparison between BMI matched population. The evaluation of body fat analysis in T1DM subjects and corelation with BMD has not been discussed in most of the literatures. Ingberg et al. have evaluated both BMD and body fat analysis in 38 T1DM cases and compared with similarly age and sex matched controls. However this study did not show any statistical difference between body fats between two groups (19.6 ± 8.3 vs 18.5 ± 5.7 in males; 34.6 ± 7.9 vs 32.7 ± 7.3 in females) (136). This study cannot be directly compared to our study as their mean age of presentation was 43.1 and 41 in males and females respectively. As our study population's mean age is only 22 years, the age related sarcopenic changes and fat mass changes are unlikely to be a confounding factor in our study which possibly had interfered in that study. Ingberg et al. did not find any significant difference in BMD making this study unique and contrasting compared to various other studies. Joshi et al. in their study had found out females have higher BMD due to their higher visceral adiposity (17).

Soha M. et al in their study had evaluated body composition and bone density in T1DM patients (164). The study included 47 patients with T1DM and 30 age- and sex-matched controls. However, they did not match for BMI in their cohort. Lean body mass and lean fat ratio were lower, while, total fat mass, abdominal fat%, soft tissue fat mass%, and fat/lean ratio were higher in diabetics compared to controls. This coincides with Dub é et al., who reported that daily insulin dose and strict HbA1c related to deleterious body composition such as increased visceral adipose tissue. Various other studies in long term T1DM have also depicted higher visceral fat percentage compared to controls. This higher visceral fat contributes to abdominal obesity and resultant insulin resistance. Higher visceral adiposity and total body fat percentages can preserve the bone density in this group of patients. Higher adipocytokines and higher

estrogen status can prevent from low bone mass in overweight T1DM (17). But our Indian T1DM counterparts are phenotypically very different. Indian T1DM are comparatively thinner with very few obese T1DM cases. These patients still can have significant insulin resistance (10,11). Poor glycemic control, recurrent ketoacidosis, inadequate diabetic education along with subclinical malnutrition can also contributes to lean habitus.

Anna R. Kahkoska et al. in their study have mentioned about 30% prevalence of obesity and overweight in T1DM individuals which is stark contrast to Indian population. The authors have attributed to longer diabetes duration and higher insulin doses to increasing obesity in T1DM patients (165). Thus, our population group forms a unique cohort of lean T1DM which has found to have lower VAT and TBF compared to BMI matched controls. This finding also additionally explains the low bone mass in T1DM which most of the European and American studies have failed to do so.

Co-relation analysis:

As we have evaluated all relevant clinical and biochemical profiles in all patients of T1DM, we have tried to corelate their inter-relationships. Results were obtained by Spearman's rank corelation test and Pearson's corelation formula. For systematic discussions, we have divided this into 3 categories:

- Co-relation analysis of various clinical parameters
- Co-relation analysis of BMD related parameters
- Co-relation analysis of various biochemical parameters

Co-relation analysis of various clinical parameters:

At the outset we have corelated duration of diabetes with various BMD parameters and biochemical parameters. Duration of diabetes have not corelated with any of the BMD parameters (LS BMC, FN BMC, WB BMC, TBS, VAT, TLBM). Many of the previously quoted studies which have found variable corelation of BMD parameters with duration of diabetes. De Schepper et al. in their study have evaluated BMD in T1DM patients of less than 5 years of diabetes onset where the authors had shown these patient's BMD remain unaltered within first 5 years of diabetes (150). This study had also failed to show any association of BMD with the onset of disease. Similarly Ponder SW. et al., and Slemenda SW et al. have also shown that BMD did not reduce in T1DM patients of recent onset diabetes (15,166). The

authors had postulated that long term diabetes can affect the height and weight of a respective patient thus making them prone to low BMD due to stunted anthropometry which is certainly not found in our case. We have already discussed in our review of literature section that most of the studies have shown that T1DM patients are not shorter than their target height. Napoli et al, Hamilton et al., Lettgen et al., Hough FS et al. have all documented that BMD parameters had worsen with longer duration of diabetes (19,139,149,167). However most these studies were cross-sectional study thus limiting their clinical utility. Hamilton et al. did extend their diabetic cohort follow up to 10 years and shown longer duration of diabetes does affect the bony parameters greatly. However, the authors had failed to show any definite diabetes duration cut off above which bone loss greatly increases. The authors had also shown the bone loss is symmetrical in each passing decade of diabetes.

Though the duration of diabetes did not corelate with the bony parameters in our study, it had corelated negatively with Hba1c and c-peptide expectedly {co-relation co-efficient of (-0.290) (-0.4390) respectively, p=.026, p=0.001}. It had co related positively with iPTH also.

BMI has been found to be associated positively with the BMD parameters (LS BMC, FN BMC, WB BMC with corelation co-efficient being 0.324, 0.220, 0.195 respectively). This is in line with almost all of the studies which have evaluated BMD in T1DM (17,133,139,143,147). Higher BMI leads to adequate visceral adipose tissue content leading to increased BMD in such patients. As we have previously described, Indian T1DM patients are typically lean habitus thus having comparatively lower BMD compared to western counterparts.

Co-relation analysis of BMD related parameters:

We have analysed all the BMD data's and co related with each other. LS BMC strongly corelated positively with FN BMC, WB BMC, TBS, VAT and TLBM (co relation co efficient being (0.666, 0.574, 0.304, 0.332, 0.707). FN BMC also co related with all above mentioned parameters except VAT. WB BMC have also corelated with LS BMC, FN BMC, TBS and TLBM leaving aside VAT. TBS also co related with all bony parameters except VAT.

We have analysed the associations of WB BMC with all biochemical parameters also where we had found it is associated with age, 24 hours urinary calcium and phosphorus excretion positively whereas negatively with testosterone and alkaline phosphatase.

VAT had corelated with LS BMC and FN BMC whereas TLBM had co related with LS BMC, FN BMC and WB BMC. Additionally, we had checked for association between VAT and various biochemical parameters which did not have any significant associations. So to summarize this corelation analysis, most of the bone mineral density parameters have co related with each other suggesting intricate relationship between all these parameters. Indirectly it tells us about the wholesome bony deterioration taking place in T1DM. This findings are in concordance to the largest network analysis study done by Eller-Vainicher et al. (140). This correlation analysis between BMD parameters had not been undertaken by most of the above-mentioned studies. It is also not mentioned in larger meta-analysis which we have described in our review of literature section (3,168).

Co relation analysis of Hba1c:

Hba1c is regarded as the most suitable indicator for glycemic control in diabetes. Though it has got many limitations, no other biochemical test had replaced it till now. In our study Hba1c has been seen to co relate negatively with LS BMC, FN BMC and WB BMC. It also negatively co related with VAT. This finding is also very classical of T1DM where poor glycemic control has been found to be the most important underlying factor for early bone loss. This has been consistently across all case control studies including Indian counterpart study of Joshi et al. (17,29,146,153,167). Poor glycemic control leading to further insulinopenia, low body weight, low visceral adiposity, increased inflammatory markers, low IGF-1, amylin deficiency, diabetic kidney disease and other microvascular changes leading to poor bone mass. In our study Hba1c co related negatively with TBS also suggesting bony histomorphometric changes in poor glycemic control. One of the largest meta-analysis published in 2021 in Diabetes Care by Phoebe Loxton et al. had also evaluated Hba1c's co relation with various bone density parameters. Surprisingly they could not found out Hba1c relationship with any of the LS or FN BMD (169).

Co-relation analysis of C-peptide:

Fasting C-peptide has been regarded as a convenient tool for assessing beta cell reserve in T1DM (167). We had evaluated fasting c-peptide in all our cases. In our Pearson's corelation analysis, c-peptide is found to negatively co-relate with age and positively co-relating with IGF-1 (corelation co-efficient of -0.301 and 0.580 respectively). Both are having very important clinical implications. With increasing age, c-peptide is expected to decrease in

T1DM patients. We had already discussed in our previous sections that IGF-1 values decrease with advancing T1DM, which here seems to co relate positively with fasting c-peptide. Long standing diabetes with poor beta cell reserve diminishes total IGF-1, causing defect in the collagen cross linking and poor bone mass. We have discussed in our review of literature section how IGF-1 negatively co-relates with glycemic status. IGF-1R is present in the osteoblasts which are responsible for the osteoblastic cell production from mesenchymal stem cells. IR knock out mice have shown to be associated with poor trabecular bone status, poor post-natal growth and low bone density (73). However fasting c-peptide had not co related with any of our bone density parameters. Our fasting c-peptide level was 0.01 (0.01-2.22) (median and range). None of the studies had evaluated c-peptide with bone density parameters earlier making this co-relation analysis unique compared to previous case-control studies as well as large network analyses.

Co-relation analysis of CTX with bone density related and biochemical parameters:

We have measured CTX as a bone turnover marker in our study. Not many studies have evaluated the relationship of CTX and BMD parameters. We had also planned to perform P1NP as a bone formation marker but we could not perform due to some technical glitches. Our median CTX level was 480.85 pg/ml (range 53.95-1037.40). Most of the literatures have advocated to use CTX normal limits as 150-450 pg/ml; in that aspect our median value surpasses it (133). Increased CTX indirectly confirms the high bone resorption state as we had described in our review of literature section. Increased RANK-L activity along with suppressed OPG is one of the hallmarks of T1DM. Ideally bone turnover markers (BTM) need to be followed as a surrogate marker for anti-osteoporotic management monitoring. Generally, BMD needs to be repeated after 1-3 years of anti-osteoporotic management but meanwhile the monitoring of BTM can give us a good idea about bone turnover status. Our baseline CTX value did not corelated with any of the BMD parameters. It did not corelated with prominent biochemical indices of diabetes also like Hba1c, c-peptide or IGF-1. Our finding has been quite unique compared to various other western literatures. Peter Gunczler et al evaluated P1NP and CTX in their BMD evaluation studies among 26 T1DM children where they have found out lower baseline values in study population (152). This finding substantiated low bone turnover state in those patients which is a very important mechanism for low bone mass in T1DM. Inadequate osteoblast synthesis, poor mechanosensory stimulation perception of osteocytes and increased apoptosis of both these cell line leads to poor bone turnover state in T1DM (85).

Gunczler et al. also didn't find any associations between BTM and bone density parameters. No Indian studies have previously assayed BTM in bone density studies of T1DM which in fact was highlighted as a major limitation to Joshi et al. study (17). However, a recent meta-analysis found an increase rather than a reduction in NTX. In both T1D and T2D, GLP1-related insulin release may be impaired. This may be associated with a decreased formation of crosslinks (including, among others, CTX) and thus possibly with the accumulation of fragile bone matrix (36,37). Practically our knowledge in BTM status in T1DM is very inadequate and our study surely adds to the existing knowledge.

Co-relation analysis of IGF-1 with BMD parameters and other biochemical parameters:

Unlike BTM's, the finding of low IGF-1 in poorly controlled diabetes is an established finding. Our case population median IGF-1 was 222.9 ng/ml. IGF-1 is an age and sex dependant parameter where the females have comparatively higher values than males. The IGF-1 normative data's trend shows increasing values after 8-9 years of age. The proposed normal IGF-1 values range from 146-541 ng/ml (males), 178-636 ng/ml (females) to 207-576 ng/ml (males), 185-551 ng/ml (females) with increasing ages of puberty (69). Among our study population only 5 patients had lower IGF-1 values compared to age and sex related standard cut-offs. This is in contrast with most of the studies. Ingberg et al. had observed significantly low IGF-1 compared to controls (136). Lucy D. Mastrandrea et al had followed 63 patients for about a year and measured various non-glycemic parameters like IGF-1, IGFBP-3 in their study where they had also found out low IGF-1 in their study population. Joshi et al. in their study attributed low BMD due to low IGF-1 (17). Interestingly in our corelation analysis IGF-1 negatively correlated with age and duration of diabetes (co-relation co-efficient of -0.393 and-0.425 respectively) but it didn't correlate with bone density parameters. This is in line with the normal median IGF-1 levels.

Co-relation analysis of testosterone with BMD and biochemical parameters:

In our study testosterone levels co-related positively with WB BMC. We had already described in detail how the hypogonadism can affect the bone health in T1DM and males are commonly affected in this regard. None of our male patients had hypogonadal or delayed pubertal features and all had appropriate pubertal features according to age. Among 36 male study subjects, only 4 patients were in pre-pubertal stage. The median testosterone level among our study population was 375 ng/dl. Testosterone did not co-related with age, duration of diabetes or

hba1c and c-peptide. Previous discussed studies had not evaluated testosterone levels with BMD parameters. Our's study had added another significant knowledge in terms of hypogonadism and bone loss in T1DM.

Co-relation analysis of 25 (OH)D with BMD and biochemical parameters:

Very similar and classical to other case-control studies, 25(OH)D had co-related positively with WB BMC. Hypovitaminosis state can affect the bone mass and it is one of the reversible causes of low bone mass. The median value of 25 (OH) D in our study was 16.81 ng/dl (range 4-42.5 ng/dl). This median value falls in the insufficiency range and warrants for supplementation and thus contributes to low bone density in our study population. Eller-Vainicher et al. in their network analysis of low bone mass in T1DM have proven the abovesaid association (140). That registry in US children found that 36 % of T1D participants were deficient in vitamin D; however, the prevalence was like what was reported in similarly aged children from the nationally representative National Health and Nutrition Examination Survey. Tsentidis C et al. in their study had looked for RANKL and OPG interaction in lead upto the low bone mass in T1DM where the authors haven't found any association between 25 (OH) D and T1DM patients bone density. We have described how poor sunlight exposure, nutritional deficiency and increased excretion of vitamin D binding proteins can lead to deficient vitamin D status in Indian T1D population. Riyaz Ahmad Daga et al. had found out 92% of T1DM children from north India are vitamin D deficient. The authors had also matched with same aged controls where the prevalence rate was close to 60%. Our study had 25 (OH) D deficiency prevalence of 85%. That study could not find any association between 25 (OH) D and any diabetes related biochemical parameters but we have found out that 25 (OH) D negatively corelated with serum TG and LDL (170). This is another very clinically important associations. Though a bit outward from our main study objectives, this finding holds key in treating cardiovascular morbidity and mortality in T1DM patients. Charles J. Glueck et al. in their study evaluated 1534 patients and found out that 25 (OH) D was inversely associated with serum triglyceride, LDL. Serum vitamin D was a significant independent inverse explanatory variable for total cholesterol, triglyceride, and LDL cholesterol, and accounted for the largest amount of variance in serum total cholesterol (partial R2 = 3.6%), triglyceride (partial R2 = 3.1%), and LDLC (partial R2 = 2.9%) ($P < 0.0001$ for all) (171). AlJohara M AlQuaiz et al. also evaluated 1717 Saudi patients and found out similar findings (172).

Co-relation analysis of microvascular complications with BMD and biochemical parameters:

In our analysis, microalbuminuria did not co related with any of the BMD parameters. It had co-related with serum TG and LDL as like 25 (OH) D. Microvascular complications can be an underlying factor for developing low bone mass in T1DM. Campos-Pastor et al. in their study showed that development of microvascular complications can induce 2nd phase of bone loss after achieving plateau following bone loss due to poor glycemic control (18). Microvascular complications leads to defective osteoblastogenesis and loss of vascular pericytes which in turn causes low bone mass (114). Presence of persistent microalbuminuria confirms diabetic kidney disease (173). Only one of our study populations had decreased creatinine clearance and Nine patients (among 37 cases who are having urinary ACR data) (24%) had significant macroalbuminuria (nephritic range proteinuria) and among them one patient had nephrotic range proteinuria. Three out of those nine patients did not have any evidence of retinopathy whereas rest 6 patients had moderate to severe NPDR and even PDR also. None of these patients were on ACE inhibitor or ARB's. Renal osteodystrophy related adynamic bone loss is generally found in patients with decreased eGFR which was certainly not present in our case. Secondary hyperparathyroidism was also not present in our study (median iPTH 40.9 pg/ml). Joshi et al. in their study did not find any association between BMD parameters and any microvascular complications as in our case. Eller-Vainicher et al. in their study found poor bone mass in decreased renal function patients of T1DM. They had also documented retinopathy and microalbuminuria as independent factor for low bone mass in femoral neck and lumbar spine (1). 30% of our patients had documented neuropathic symptoms. Neuropathy has also been linked to poor bone mass as an independent factor as found in Eskildsen et al. study. We couldn't find this association in our study (174). Possibly a larger sample size could have been ideal to prove this associations.

Other corelation analyses of our study:

We had also co-related serum ALP with various BMD related and biochemical parameters. Here in our study sALP had co-related negatively with age and positively with testosterone and IGF-1. Though this analysis was not undertaken in bone specific ALP subunit, still it holds some value as a bone formation marker. It did not co relate with BMD parameters.

Other salient features for brief discussion:

Osteocytes secrete sclerostin, an inhibitor of the Wnt pathway. Various mouse-based studies have evaluated this as a marker for abnormal Wnt pathway mechanism. Mechanical unloading, advancing age, detrimental bone quality all increases sclerostin levels (87). It impedes the osteoblast synthesis and thus acts as a negative regulator of bone synthesis. The reasons behind increased sclerostin are not clear in T1DM however advancing age in puberty, high glycated hemoglobin, associated insulin resistance can give rise to exaggerated sclerostin response. Our median sclerostin level was 1.32 ng/ml (range 0.047-580) which was within normal range. Only 2 patients among case population had high sclerostin levels. Luigi Gennari et al. in their study of 43 T1DM patients had shown sclerostin levels were higher in T1DM patients, and this difference persisted when adjustments were made for age and BMI (175). Sclerostin levels had negatively associated with estrogen but not androgen levels in that study. We did not make any co-relation analysis of sclerostin as a part of our study.

Our study population did not have in any abnormality in serum calcium or serum phosphorus levels neither they had any abnormality in 24 hours urinary calcium or phosphorus excretion. This is in line with most of the studies like Joshi et al (17). Our case population had normal lipid profile which is not concordant with all Indian T1DM lipid profile studies. Selvaraj M et al., Shah N et al, Billow A et al. in their study had found significant LDL elevation in their cohort of T1DM patients (176–178).

Conclusion

The bone mineral density studies in T1DM are plenty from various parts of the world. Most of the studies have found low bone mass in T1DM which have been described separately in lumbar spine or femoral neck. Some of the studies have evaluated in whole body and very few studies have measured BMD in wrist by DXA. The scarcity of literature increases when TBS and body fat analysis in T1DM are concerned. Very few studies had evaluated the simultaneous co relation of TBS, VAT, HbA1c, IGF-1, Testosterone, 25 (OH) D, urinary microalbumin with BMD parameters.

Our study findings can be summarized in a few below said points:

- We had taken a sample size of 120 with case control recruitment in 1:1 ratio making one of the largest studies ever conducted on BMD parameters among T1DM patients. This study had also provided maiden epidemiological, clinical and biochemical cross-sectional data's on T1DM patients from north-west India.

- We had evaluated and analysed all site BMD including wrist and whole body making our study inclusive and comprehensive. Our study had able to find bone loss in both cortical and trabecular compartments in T1DM patients.

- Our mean age of the case population was 22.4 ± 8.6. It surpasses complete bone accrual physiologically. Our mean diabetes duration was also 5 years. So, our patient's low bone mass was completely attributed to disease process per se without any physiological interference. Our case population was predominantly school and graduate students giving us the opportunity to educate them about preventing bone loss along with proper diabetic education.

- All BMD parameters including lumbar spine, femoral neck, whole body and wrist in cases were significantly lower than the age, sex and BMI matched controls.

- Trabecular bone score which is a surrogate marker for bone histomorphometry was found to be significantly low in T1DM patients compared to controls. It had confirmed additional bone quality deterioration along with low bone mineral density in such patients.

- T1DM patients found to have low visceral adipose tissue as well as total body fat percentages which is in sharp contrast to various previous studies. Low total body fat percentages can account for the low bone mass in T1DM patients. Our case population was predominantly having lean habitus making our population unique compared to western counterparts.

- Duration of diabetes did not corelate with various BMD parameters in our study in contrast to most of the existing literatures. Various notable studies have also shown normal bone density within 5 years of diabetes contrary to our findings. All bone density parameters have co-related with each other confirming simultaneous and generalized bone loss in T1DM.

- Hba1c had co-related negatively with most of the BMD parameters here. Poor glycemic control and recurrent ketoacidosis caused bone loss in our subset of patients.

- Our's study population had normal median IGF-1 with very few subnormal values according to age and sex related reference values. Most of the studies had found low IGF-1 in long standing T1DM along with negative co-relation with BMD parameters unlike our study.

- Serum testosterone had positively co-related with WB BMC in our analysis which has substantiated the association between hypogonadism and low bone mass in t1DM.

However, none of our patient had features of delayed or absent puberty according to chronological age.

- In our study, serum 25 (OH) D had positively co-related with WB BMC which is very similar to all studies across the globe. Our study population had low median 25 (OH) D levels which had accounted for low bone mass. It had negatively co-related with serum triglyceride and LDL which had re-enforced our knowledge of keeping normal vitamin D level to prevent CV events in T1DM.

- Very few patients in our study had significant proteinuria and retinal changes for diabetes (corelates with mean shorter duration of diabetes in case cohort). These complications did not co-relate with BMD parameters.

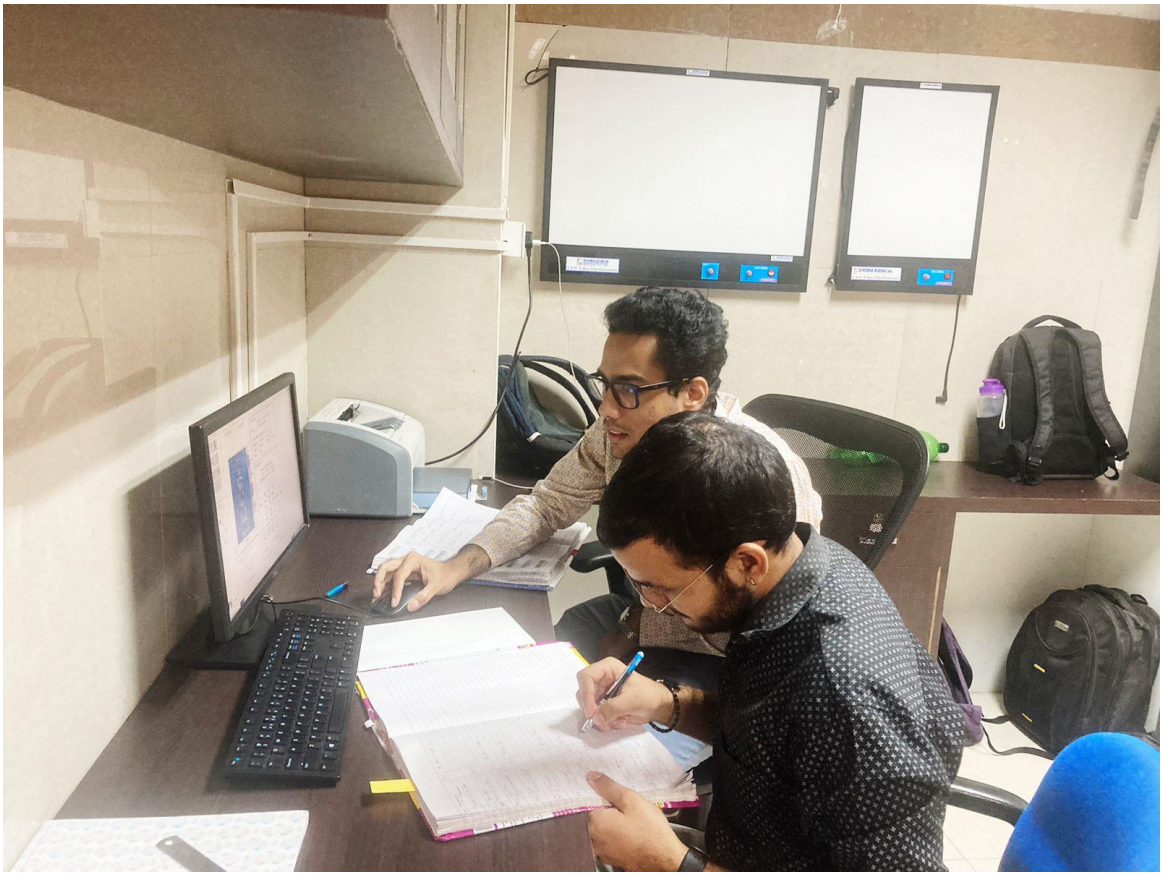
- We have evaluated baseline sclerostin which came to be normal contrary to various mouse model study which have established higher sclerostin values. Our baseline CTX values had come to be in the higher side indicating more bone resorption. Though this baseline evaluation holds very little value in understanding bone remodelling.

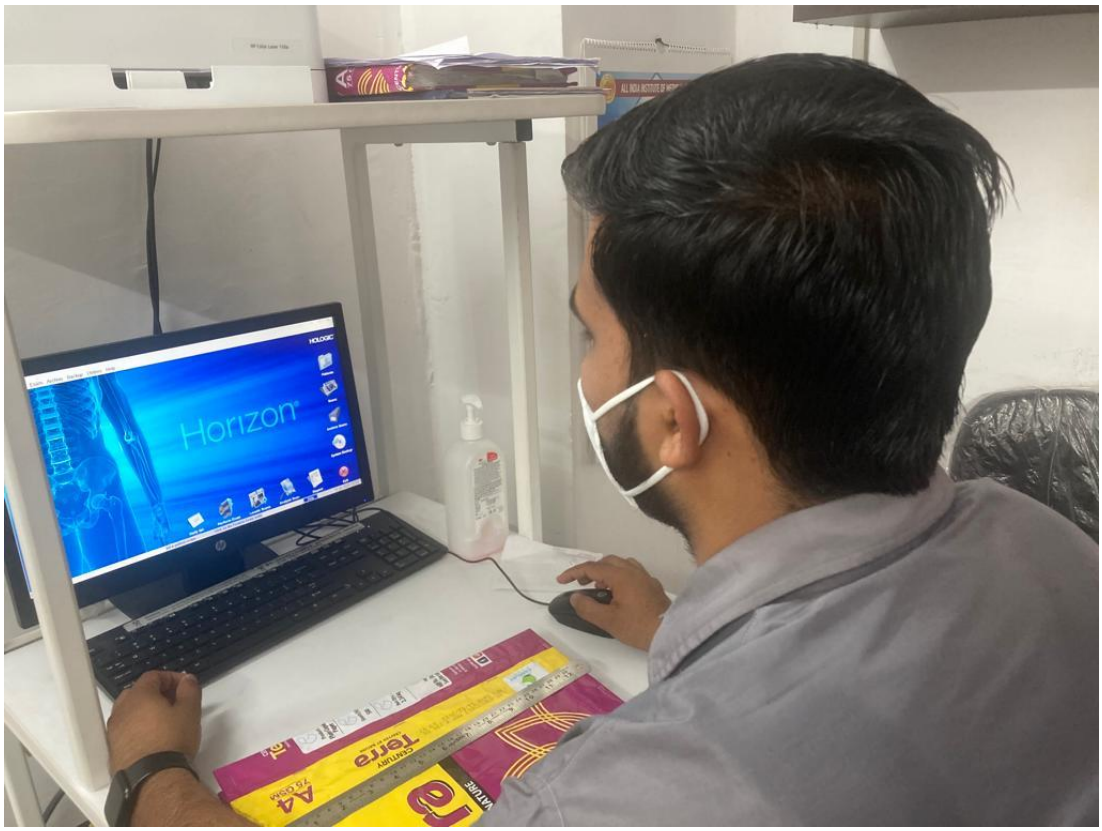
Final take away message:

Our study on 120 subjects of T1DM patients and age, sex and BMI matched controls confirmed low bone mass in cases compared to controls. All BMD parameters were involved suggesting cortical and trabecular bone loss. Low TBS confirmed bone quality deterioration also. The chief causes behind this bone loss seems to be:

- Poor glycemic control and resultant various mechanisms which we had discussed in literature section.
- Lower total body fat percentages
- Low 25 (OH) D levels

Gallaries




Horizon

Bibliography

1. CRISTINA ELLER-VAINICHER. Low BoneMineral Density and ItsPredictors in Type 1 Diabetic PatientsEvaluated by the Classic Statistics andArtificial Neural Network Analysis. DIABETES CARE, VOLUME 34, OCTOBER 2011.

2. Shetty S, Kapoor N, Bondu JD, Thomas N, Paul TV. Bone turnover markers: Emerging tool in the management of osteoporosis. Indian J Endocr Metab 2016;20:846-52.

3. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes – a meta-analysis. Osteoporosis International 2007 18 427–444.

4. Shah VN, Shah CS, Snell-Bergeon JK (2015) Type 1 diabetes and risk of fracture: meta-analysis and review of the literature. Diabet Med 32:1134–1142.

5. Armas LA, Akhter MP, Drincic A & Recker RR. Trabecular bone histomorphometry in humans with type 1 diabetes mellitus. Bone 2012 50 91–96.

6. Abdalrahaman N, McComb C, Foster J, McLean J, Lindsay R, McClure J, McMillan M, Drummond R, Gordon D, McKay G et al. Deficits in trabecular bone microarchitecture in young women with type 1 diabetes mellitus. Journal of Bone and Mineral Research 2015 30 1386–1393.

7. Bailey DA, Mckay HA, Mirwald RL, Crocker PRE, Faulkner RA. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the University of Saskatchewan bone mineral accrual study. J Bone Miner Res. 1999; 14(10): 1672–9.

8. Bechtold S, Putzker S, Bonfig W, Fuchs O, Dirlenbach I, Schwarz HP. Bone size normalizes with age in children and adolescents with type 1 diabetes. Diabetes Care. 2007; 30:2046–2050.

9. Kalra S, Kalra B, Sharma A. Prevalence of type 1 diabetes mellitus in Karnal district, Haryana state, India. Diabetol Metab Syndr 2010;2:14.

10. Prasanna Kumar KM, Krishna P, Reddy SC, Gurrappa M, Aravind SR, Munichoodappa C. Incidence of type 1 diabetes mellitus and associated complications among children and young adults: results from Karnataka Diabetes Registry 1995-2008. J Indian Med Assoc 2008;106:708-11.

11. Ramachandran A, Snehalatha C, Krishnaswamy CV. The Incidence of IDDM in children in urban population in southern India. Madras South India. Diabetes Res Clin Pract 1996;34:79-82.

12. Ramachandran A, Snehalatha C, Khader O, Joseph TA, Visvanathan M. Prevalence of childhood diabetes in urban population in South India. Diabetes Res Clin Pract 1992;17:227-31.

13. Shah VN, Moser EG, Blau A, Dhingra M, Garg SK. The future of Basal insulin. Diabetes Technol Ther 2013;15:727-32.

14. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet 2013.

15. Ponder SW, McCormick DP, Fawcett HD, Tran AD, Ogelsby GW, Brouhard BH, et al. Bone mineral density of the lumbar vertebrae in children and adolescents with insulin-dependent diabetes mellitus. J Pediatr 1992;120:541-5.

16. Olmos JM, Pérez-Castrillón JL, García MT, Garrido JC, Amado JA, González-Macías J. Bone densitometry and biochemical bone remodeling markers in type 1 diabetes mellitus. Bone Miner 1994;26:1-8.

17. Joshi A, Varthakavi P, Chadha M, Bhagwat N. A study of bone mineral density and its determinants in type 1 diabetes. J Osteoporos 2013;2013:397814.

18. Shanbhogue VV, Hansen S, Frost M, Jørgensen NR, Hermann AP, Henriksen JE, et al. Bone geometry, volumetric density, microarchitecture, and estimated bone strength assessed by HR-pQCT in adult patients with type 1 diabetes mellitus. J Bone Miner Res. 2015; 30(12): 2188–99.

19. Napoli N, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL. Mechanisms of diabetes mellitus-induced bone fragility. Nat Rev Endocrinol. 2017; 13(4): 208– 19.

20. Janghorbani M, Feskanich D, Willett WC, Hu F. Prospective study of diabetes and risk of hip fracture: the Nurses' Health Study. Diabetes Care. 2006; 29(7): 1573–8.

21. Eknoyan G, Lewin A & Levin N. National Kidney Foundation. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. American Journal of Kidney Diseases 2003 42 S1–S202.

22. Vestergaard P, Rejnmark L & Mosekilde L. Diabetes and its complications and their relationship with risk of fractures in type 1 and 2 diabetes. Calcified Tissue International 2009 84 45–55.

23. Weber DR, Haynes K, Leonard MB, Willi SM, Denburg MR (2015) Type 1 diabetes is associated with an increased risk of fracture across the life span: a population-based cohort study using The Health Improvement Network (THIN). Diabetes Care 38: 1913–1920.

24. Lunt H, Florkowski CM, Cundy T, Kendall D, Brown LJ, Elliot JR, et al. A population-based study of bone mineral density in women with longstanding type 1 (insulin dependent) diabetes. Diabetes Res Clin Pract 1998;40:31-8.

25. Domargard A, Sarnblad S, Kroon M, Karlsson I, Skeppner G, Aman J. Increased prevalence of overweight in adolescent girls with type 1 diabetes mellitus. Acta paediatrica. 1999; 88:1223–1228.

26. Hypponen E, Virtanen SM, Kenward MG, Knip M, Akerblom HK. Obesity, increased linear growth, and risk of type 1 diabetes in children. Diabetes Care. 2000; 23:1755–1760.

27. Songer TJ, LaPorte RE, Tajima N, Orchard TJ, Rabin BS, Eberhardt MS, Dorman JS, Cruickshanks KJ, Cavender DE, Becker DJ, et al. Height at diagnosis of insulin dependent diabetes in patients and their non-diabetic family members. Br Med J (Clin Res Ed). 1986; 292:1419–1422.

28. Massa G, Dooms L, Bouillon R, Vanderschueren-Lodeweyckx M. Serum levels of growth hormone-binding protein and insulin-like growth factor I in children and adolescents with type 1 (insulin-dependent) diabetes mellitus. Diabetologia. 1993; 36:239–243.

29. Vestergaard P, Rejnmark L & Mosekilde L. Osteoporosis is markedly underdiagnosed – a nationwide study from Denmark. Osteoporosis International 2005 16 134–141.

30. Abbassy MA, Watari I & Soma K. The effect of diabetes mellitus on rat mandibular bone formation and microarchitecture. European Journal of Oral Sciences 2010 118 364–369.

31. Follak N, Klo¨ting I, Wolf E & Merk H. Improving metabolic control reverses the histomorphometric and biomechanical abnormalities of an experimentally induced bone deficit in spontaneously diabetic rats. Calcified Tissue International 2004 74 551–560.

32. Fujii H, Hamada Y & Fukagawa M. Bone formation in spontaneously diabetic Torii-newly established model of non-obese type 2 diabetes rats. Bone 2008 42 372–379.

33. Hamada Y, Kitazawa S, Kitazawa R, Fujii H, Kasuga M & Fukagawa M. Histomorphometric analysis of diabetic osteopenia in streptozotocininduced diabetic mice: a possible role of oxidative stress. Bone 2007 40 1408–1414.

34. Manavalan JS, Cremers S, Dempster DW, Zhou H, Dworakowski E, Kode A, Kousteni S & Rubin MR. Circulating osteogenic precursor cells in type 2 diabetes mellitus. Journal of Clinical Endocrinology and Metabolism 2012 97 3240–3250.

35. Starup-Linde J, Eriksen SA, Lykkeboe S, Handberg A & Vestergaard P. Biochemical markers of bone turnover in diabetes patients – a metaanalysis, and a methodological study on the effects of glucose on bone markers. Osteoporosis International 2014 25 1697–1708.

36. Stark A & Mentlein R. Somatostatin inhibits glucagon-like peptide-1- induced insulin secretion and proliferation of RINm5F insulinoma cells. Regulatory Peptides 2002 108 97–102.

37. Osei K, Falko JM, O'Dorisio TM, Fields PG & Bossetti B. Gastric inhibitory polypeptide responses and glucose turnover rates after naturalmeals in type II diabetic patients. Journal of Clinical Endocrinology and Metabolism 1986 62 325–330.

38. Henriksen DB, Alexandersen P, Bjarnason NH, Vilsbøll T, Hartmann B, Henriksen EE, Byrjalsen I, Krarup T, Holst JJ & Christiansen C. Role of gastrointestinal hormones in postprandial reduction of bone resorption. Journal of Bone and Mineral Research 2003 18 2180–2189.

39. Gangoiti MV, Cortizo AM, Arnol V, Felice JI & McCarthy AD. Opposing effects of bisphosphonates and advanced glycation end-products on osteoblastic cells. European Journal of Pharmacology 2008 600 140–147.

40. Adams JE, Engelke K, Zemel BS, Ward KA, Densitometryof ISC. Quantitative computer tomography in children and adolescents: the 2013 ISCD pediatric official positions. J Clin Densitom. 2014; 17(2): 258–74.

41. Leslie WD, Aubry-Rozier B, Lamy O, Hans D & Manitoba Bone Density Program . TBS (trabecular bone score) and diabetes-related fracture risk. Journal of Clinical Endocrinology and Metabolism 2013 98 602–609.

42. Kim JH, Choi HJ, Ku EJ, Kim KM, Kim SW, Cho NH & Shin CS. Trabecular bone score as an indicator for skeletal deterioration in diabetes. Journal of Clinical Endocrinology and Metabolism 2015 100 475–482.

43. Heilmeier U, Carpenter DR, Patsch JM, Harnish R, Joseph GB, Burghardt AJ, Baum T, Schwartz AV, Lang TF & Link TM. Volumetric femoral BMD, bone geometry, and serum sclerostin levels differ between type 2 diabetic postmenopausal women with and without fragility fractures. Osteoporosis International 2015 26 1283–1293.

44. Patsch JM, Burghardt AJ, Yap SP, Baum T, Schwartz AV, Joseph GB & Link TM. Increased cortical porosity in type 2 diabetic postmenopausal women with fragility fractures. Journal of Bone and Mineral Research 2013 28 313–324.

45. Gordon CM, Ackerman KE, Berga SL, Kaplan JR, Mastorakos G, Misra M, Murad MH, Santoro NF & Warren MP. Functional hypothalamic amenorrhea: an Endocrine Society clinical practice guideline. Journal of Clinical Endocrinology & Metabolism 2017 102 1413–1439.

46. Burr D & Turner C. Biomechanics of bone 2003 In Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, pp 58–64. ASBMR: Washington, USA.

47. Saito M, Fujii K, Mori Y & Marumo K. Role of collagen enzymatic and glycation induced cross-links as a determinant of bone quality in spontaneously diabetic WBN/Kob rats. Osteoporosis International 2006 17 1514–1523.

48. Einhorn TA, Boskey AL, Gundberg CM, Vigorita VJ, Devlin VJ & Beyer MM. The mineral and mechanical properties of bone in chronic experimental diabetes. Journal of Orthopaedic Research 1988 6 317–323.

49. Xu F, Ye Y, Dong Y, Guo FJ, Chen AM & Huang SL. Inhibitory effects of high glucose/insulin environment on osteoclast formation and resorption in vitro. Journal of Huazhong University of Science and Technology. Medical Sciences 2013 33 244–249.

50. Clemens TL & Karsenty G. The osteoblast: an insulin target cell controlling glucose homeostasis. Journal of Bone and Mineral Research 2011 26 677–680.

51. McNair P, Madsbad S, Christiansen C, Christensen MS, Faber OK, Binder C & Transbøl I. Bone loss in diabetes: effects of metabolic state. Diabetologia 1979 17 283–286.

52. Meier C, Kraenzlin ME, Bodmer M, Jick SS, Jick H & Meier CR. Use of thiazolidinediones and fracture risk. Archives of Internal Medicine 2008 168 820–825.

53. Botolin S & McCabe LR. Chronic hyperglycemia modulates osteoblast gene expression through osmotic and non-osmotic pathways. Journal of Cellular Biochemistry 2006 99 411–424.

54. Silva JC, Sampaio P, Fernandes MH & Gomes PS. The osteogenic priming of mesenchymal stem cells is impaired in experimental diabetes. Journal of Cellular Biochemistry 2015 116 1658–1667.

55. Xu Y, Gu Z, Shen B, Xu G, Zhou T, Jiang J, Xing J, Liu S, Li M, Tan W et al. Roles of Wnt/b-catenin signaling in retinal neuron-like differentiation of bone marrow mesenchymal stem cells from nonobese diabetic mice. Journal of Molecular Neuroscience 2013 49 250–261.

56. Beisiegel U, Weber W, Ihrke G, Herz J & Stanley KK. The LDL-receptorrelated protein, LRP, is an apolipoprotein E-binding protein. Nature 1989 341 162–164.

57. De Laet C, Kanis J, Oden A, Johanson H, Johnell O, Delmas P, Eisman JA, Kroger H, Fujiwara S, Garnero P et al. Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporosis International 2005 16 1330–1338.

58. Hofbauer LC, Brueck CC, Singh SK, Dobnig H. Osteoporosis in patients with diabetes mellitus. J Bone Miner Res 2007;22:1317-28.

59. Lombardi F, Franzese A, Iafusco D, del Puente A, Esposito A, Prisco F, et al. Bone involvement in clusters of autoimmune diseases: Just a complication. Bone 2010;46:551-5.

60. Khan TS, Fraser LA. Type 1 diabetes and osteoporosis: from molecular pathways to bone phenotype. 2015; 2015:174186. doi:10.1155/2015/174186.

61. Komori T. Regulation of osteoblast differentiation by transcription factors. Journal of cellular biochemistry. 2006; 99(5):1233–9. doi:10.1002/jcb.20958.

62. Wu H, Whitfield TW, Gordon JA, Dobson JR, Tai PW, van Wijnen AJ, et al. Genomic occupancy of Runx2 with global expression profiling identifies a novel dimension to control of osteoblastogenesis. Genome biology. 2014; 15(3):R52.

63. Fowlkes JL, Bunn RC, Liu L, Wahl EC, Coleman HN, Cockrell GE, et al. Runt-related transcription factor 2 (RUNX2) and RUNX2-related osteogenic genes are down-regulated throughout osteogenesis in type 1 diabetes mellitus. Endocrinology. 2008; 149(4):1697–704.

64. Hie M, Iitsuka N, Otsuka T, Tsukamoto I. Insulin-dependent diabetes mellitus decreases osteoblastogenesis associated with the inhibition of Wnt signaling through increased expression of Sost and Dkk1 and inhibition of Akt activation. International journal of molecular medicine. 2011; 28(3):455–62.

65. Munoz-Torres M, Jodar E, Escobar-Jimenez F, Lopez-Ibarra PJ, Luna JD. Bone mineral density measured by dual X-ray absorptiometry in Spanish patients with insulin-dependent diabetes mellitus. Calcif Tissue Int. 1996; 58(5):316–9.

66. Bloch CA, Clemons P, Sperling MA. Puberty decreases insulin sensitivity. J Pediatr. 1987; 110:481–487.

67. Daughaday WH, Phillips LS, Mueller MC. The effects of insulin and growth hormone on the release of somatomedin by the isolated rat liver. Endocrinology. 1976; 98:1214–1219.

68. Rogers DG, Sherman LD, Gabbay KH. Effect of puberty on insulinlike growth factor I and HbA1 in type I diabetes. Diabetes Care. 1991; 14:1031–1035.

69. Radetti G, Paganini C, Antoniazzi F, Pasquino B, Valentini R, Gentili L, Tato L. Growth hormone-binding proteins, IGF-I and IGF-binding proteins in children and adolescents with type 1 diabetes mellitus. Horm Res. 1997; 47:110–115.

70. Bereket A, Lang CH, Blethen SL, Ng LC, Wilson TA. Insulin treatment normalizes reduced free insulin-like growth factor-I concentrations in diabetic children. Clinical endocrinology. 1996; 45:321–326.

71. Mahesh S, Karp RJ, Castells S, Quintos JB. Mauriac syndrome in a 3-year-old boy. Endocr Pract. 2007; 13:63–66.

72. Xian L, Wu X, Pang L, Lou M, Rosen CJ, Qiu T, et al. Matrix IGF-1 maintains bone mass by activation of mTOR in mesenchymal stem cells. Nature medicine. 2012; 18(7):1095–101.

73. Fulzele K, Riddle RC, DiGirolamo DJ, Cao X, Wan C, Chen D, et al. Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. Cell. 2010; 142(2):309–19.

74. Kream BE, Smith MD, Canalis E, Raisz LG. Characterization of the effect of insulin on collagen synthesis in fetal rat bone. Endocrinology. 1985; 116(1):296–302.

75. Chen SC, Shepherd S, McMillan M, et al. Skeletal Fragility and Its Clinical Determinants in Children With Type 1 Diabetes. J Clin Endocrinol Metab. ; 2019;104:3585–3594.

76. Horcajada-Molteni MN, Chanteranne B, Lebecque P, Davicco MJ, Coxam V, Young A, et al. Amylin and bone metabolism in streptozotocin-induced diabetic rats. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2001; 16(5):958–65.

77. Peng J, Hui K, Hao C, Peng Z, Gao QX, Jin Q, et al. Low bone turnover and reduced angiogenesis in streptozotocin-induced osteoporotic mice. Connective tissue research. 2016:1–13.

78. Motyl KJ, McCauley LK, McCabe LR. Amelioration of type I diabetes-induced osteoporosis by parathyroid hormone is associated with improved osteoblast survival. Journal of cellular physiology. 2012; 227(4):1326–34. doi:10.1002/jcp.22844.

79. Verhaeghe J, Van Herck E, van Bree R, Moermans K, Bouillon R. Decreased osteoblast activity in spontaneously diabetic rats. In vivo studies on the pathogenesis. Endocrine. 1997; 7(2):165–75.

80. Weinberg E, Maymon T, Weinreb M. AGEs induce caspase-mediated apoptosis of rat BMSCs via TNFalpha production and oxidative stress. Journal of molecular endocrinology. 2014; 52(1):67–76.

81. Gilbert LC, Chen H, Lu X, Nanes MS. Chronic low dose tumor necrosis factor-alpha (TNF) suppresses early bone accrual in young mice by inhibiting osteoblasts without affecting osteoclasts. Bone. 2013; 56(1):174–83. doi:10.1016/j.bone.2013.06.002.

82. Perrien DS, Liu Z, Wahl EC, Bunn RC, Skinner RA, Aronson J, et al. Chronic ethanol exposure is associated with a local increase in TNF-alpha and decreased proliferation in the rat distraction gap. Cytokine. 2003; 23(6):179–89.

83. Kirel B, Dogruel N, Korkmaz U, Kilic FS, Ozdamar K, Ucar B. Serum leptin levels in type 1 diabetic and obese children: relation to insulin levels. Clinical biochemistry. 2000; 33:475–480.

84. Ahmed ML, Ong KK, Watts AP, Morrell DJ, Preece MA, Dunger DB. Elevated leptin levels are associated with excess gains in fat mass in girls, but not boys, with type 1 diabetes: longitudinal study during adolescence. J Clin Endocrinol Metab. 2001; 86:1188–1193.

85. Clarke B. Normal bone anatomy and physiology. Clinical journal of the American Society of Nephrology : CJASN. 2008; 3(Suppl 3):S131–9.

86. Bellido T, Ali AA, Gubrij I, Plotkin LI, Fu Q, O'Brien CA, et al. Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. Endocrinology. 2005; 146(11):4577–83.

87. Kim JH, Lee DE, Woo GH, Cha JH, Bak EJ, Yoo YJ. Osteocytic Sclerostin Expression in Alveolar Bone in Rats With Diabetes Mellitus and Ligature-Induced Periodontitis. Journal of periodontology. 2015; 86(8):1005–11.

88. Tanaka K, Yamaguchi T, Kanazawa I, Sugimoto T. Effects of high glucose and advanced glycation end products on the expressions of sclerostin and RANKL as well as apoptosis in osteocyte-like MLO-Y4-A2 cells. Biochemical and biophysical research communications. 2015; 461(2):193–9.

89. Daniele G, Winnier D, Mari A, Bruder J, Fourcaudot M, Pengou Z, et al. Sclerostin and Insulin Resistance in Prediabetes: Evidence of a Cross Talk Between Bone and Glucose Metabolism. Diabetes care. 2015; 38(8):1509–17.

90. Zhou YJ, Li A, Song YL, Zhou H, Li Y, Tang YS. Role of sclerostin in the bone loss of postmenopausal chinese women with type 2 diabetes. Chinese medical sciences journal = Chung-kuo i hsueh k'o hsueh tsa chih / Chinese Academy of Medical Sciences. 2013; 28(3):135–9.

91. Starup-Linde J, Lykkeboe S, Gregersen S, Hauge EM, Langdahl BL, Handberg A, et al. Bone Structure and Predictors of Fracture in Type 1 and Type 2 Diabetes. The Journal of clinical endocrinology and metabolism. 2016; 101(3):928–36.

92. Ito N, Wijenayaka AR, Prideaux M, Kogawa M, Ormsby RT, Evdokiou A, et al. Regulation of FGF23 expression in IDG-SW3 osteocytes and human bone by pro-inflammatory stimuli. Molecular and cellular endocrinology. 2015; 399:208–18.

93. Lai X, Price C, Modla S, Thompson WR, Caplan J, Kirn-Safran CB, et al. The dependences of osteocyte network on bone compartment, age, and disease. Bone research. 2015:3.

94. Tolosa MJ, Chuguransky SR, Sedlinsky C, Schurman L, McCarthy AD, Molinuevo MS, et al. Insulin-deficient diabetes-induced bone microarchitecture alterations are associated with a decrease in the osteogenic potential of bone marrow progenitor cells: preventive effects of metformin. Diabetes research and clinical practice. 2013; 101(2):177–86.

95. Parajuli A, Liu C, Li W, Gu X, Lai X, Pei S, et al. Bone's responses to mechanical loading are impaired in type 1 diabetes. Bone. 2015; 81:152–60.

96. Krakauer JC, McKenna MJ, Buderer NF, Rao DS, Whitehouse FW, Parfitt AM. Bone loss and bone turnover in diabetes. Diabetes. 1995; 44(7):775–82.

97. Horowitz MC, Xi Y, Wilson K, Kacena MA. Control of osteoclastogenesis and bone resorption by members of the TNF family of receptors and ligands. Cytokine & growth factor reviews. 2001.

98. Wittrant Y, Gorin Y, Woodruff K, Horn D, Abboud HE, Mohan S, et al. High d(+)glucose concentration inhibits RANKL-induced osteoclastogenesis. Bone. 2008; 42(6):1122–30.

99. Catalfamo DL, Calderon NL, Harden SW, Sorenson HL, Neiva KG, Wallet SM. Augmented LPS responsiveness in type 1 diabetes-derived osteoclasts. Journal of cellular physiology. 2013; 228(2):349–61.

100. Roszer T. Inflammation as death or life signal in diabetic fracture healing. Inflammation research : official journal of the European Histamine Research Society [et al]. 2011; 60(1):3–10.

101. A. Goldin, J. A. Beckman, A. M. Schmidt, and M. A. Creager, "Advanced glycation end products: sparking the development of diabetic vascular injury," Circulation, vol. 114, no. 6, pp. 597– 605, 2006.

102. Viguet-Carrin S, Roux JP, Arlot ME, Merabet Z, Leeming DJ, Byrjalsen I, Delmas PD, Bouxsein ML (2006) Contribution of the advanced glycation end product pentosidine and of maturation of type I collagen to compressive biomechanical properties of human lumbar vertebrae. Bone 39:1073–1079.

103. Thangavelu T, Silverman E, Akhter MP, et al. Trabecular bone score and transilial bone trabecular histomorphometry in type 1 diabetes and healthy controls. Bone;2020;137:115451.

104. Thalassinos NC, Hadjiyanni P, Tzanela M, Alevizaki C, Philokiprou D. Calcium metabolism in diabetes mellitus: effect of improved blood glucose control. Diabet Med. 1993 Jun; 10(4): 341–4.

105. Schwarz P, Sorensen HA, Momsen G, Friis T, Transbol I, McNair P. Hypocalcemia and parathyroid hormone responsiveness in diabetes mellitus: a tri-sodium-citrate clamp study. Acta Endocrinol (Copenh). 1992; 126(3):260–3.

106. Greer RM, Portelli SL, Hung BS, Cleghorn GJ, McMahon SK, Batch JA, et al. Serum vitamin D levels are lower in Australian children and adolescents with type 1 diabetes than in children without diabetes. Pediatr Diabetes. 2013; 14(1): 31–41.

107. Janner M, Ballinari P, Mullis PE, Flück CE. High prevalence of vitamin D deficiency in children and adolescents with type 1 diabetes. Swiss Med Wkly. 2010; 140(3536): w13091.

108. Winzenberg T, Powell S, Shaw KA, Jones G. Effects of vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis. BMJ. 2011 Jan; 342: c7254.

109. Winzenberg TM, Powell S, Shaw KA, Jones G. Vitamin D supplementation for improving bone mineral density in children. Cochrane Database Syst Rev. 2010; 10: CD006944.

110. Wood JR, Connor CG, Cheng P, Ruedy KJ, Tamborlane WV, Klingensmith G, et al. Vitamin D status in youth with type 1 and type 2 diabetes enrolled in the Pediatric Diabetes Consortium (PDC) is not worse than in youth without diabetes. Pediatr Diabetes. 2015 doi:10.1111/pedi.12340.

111. Thorsen SU, Mortensen HB, Carstensen B, Fenger M, Thuesen BH, Husemoen LL, et al. No difference in vitamin D levels between children newly diagnosed with type 1 diabetes and their healthy siblings: a 13-year nationwide Danish study. Diabetes Care. 2013; 36(9):e157–8.

112. Tsentidis C, Gourgiotis D, Kossiva L, Doulgeraki A, Marmarinos A, Galli-Tsinopoulou A, et al. Higher levels of s-RANKL and osteoprotegerin in children and adolescents with type

1 diabetes mellitus may indicate increased osteoclast signaling and predisposition to lower bone mass: a multivariate cross-sectional analysis. Osteoporos Int. 2016; 27(4):1631–43.

113. Campos-Pastor MM, López-Ibarra PJ, Escobar- Jiménez F, Serrano Pardo MD, García-Cervigón A. Intensive insulin therapy and bone mineral density in type 1 diabetes mellitus: a prospective study. Osteoporos Int. 2000; 11(5): 455–9.

114. Shanbhogue VV, Hansen S, Frost M, Brixen K, Hermann AP. Bone disease in diabetes: another manifestation of microvascular disease? Lancet Diabetes Endocrinol. 2017; 5(10): 827– 38.

115. Aiello LP, Wong JS. Role of vascular endothelial growth factor in diabetic vascular complications. Kidney Int. Suppl. 2000; 77:S113–S119.

116. Kiel DP, Kauppila LI, Cupples LA, Hannan MT, O'Donnell CJ, Wilson PW. Bone loss and the progression of abdominal aortic calcification over a 25 year period: the Framingham Heart Study. Calcif. Tissue Int. 2001; 68:271–276.

117. de Oliveira RA, Barreto FC, Mendes M, dos Reis LM, Castro JH, Britto ZML, et al. Peritoneal dialysis per se is a risk factor for sclerostin-associated adynamic bone disease. Kidney Int. 2015; 87:1039–1045.

118. Lima EM, Goodman WG, Kuizon BD, Gales B, Emerick A, Goldin J, et al. Bone density measurements in pediatric patients with renal osteodystrophy. Pediatr. Nephrol. 2003; 18:554–559.

119. Lachin JM, White NH, Hainsworth DP, Sun W, Cleary PA, et al. Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group. Effect of intensive diabetes therapy on the progression of diabetic retinopathy in patients with type 1 diabetes: 18 years of follow-up in the DCCT/EDIC. Diabetes. 2015; 64:631–642.

120. Chakravarthy H, Beli E, Navitskaya S, O'Reilly S, Wang Q, Kady N, et al. Imbalances in Mobilization and Activation of Pro-Inflammatory and Vascular Reparative Bone Marrow-Derived Cells in Diabetic Retinopathy. PLoS One. 2016; 11:e0146829.

121. Lim Y, Chun S, Lee JH, Baek KH, Lee WK, Yim H-W, et al. Association of bone mineral density and diabetic retinopathy in diabetic subjects: the 2008–2011 Korea National Health and Nutrition Examination Survey. Osteoporos. Int. 2016 Feb.

122. Bonnet N, Brunet-Imbault B, Arlettaz A, Horcajada MN, Collomp K, Benhamou CL, et al. Alteration of trabecular bone under chronic beta2 agonists treatment. Med. Sci. Sports Exerc. 2005; 37:1493–1501.

123. Swift JM, Swift SN, Allen MR, Bloomfield SA. Beta-1 adrenergic agonist treatment mitigates negative changes in cancellous bone microarchitecture and inhibits osteocyte apoptosis during disuse. PLoS One. 2014; 9:e106904.

124. de Ferranti SD, de Boer IH, Fonseca V, Fox CS, Golden SH, Lavie CJ, et al. Type 1 Diabetes Mellitus and Cardiovascular Disease: A Scientific Statement From the American Heart Association and American Diabetes Association. Diabetes Care. 2014; 37:2843–2863.

125. Bagger YZ, Tankó LB, Alexandersen P, Qin G, Christiansen C. Radiographic measure of aorta calcification is a site-specific predictor of bone loss and fracture risk at the hip. J. Intern. Med. 2006; 259:598–605.

126. Collins TC, Ewing SK, Diem SJ, Taylor BC, Orwoll ES, Cummings SR, et al. Peripheral arterial disease is associated with higher rates of hip bone loss and increased fracture risk in older men. Circulation. 2009; 119:2305–2312.

127. Schwartz AV, Vittinghoff E, Sellmeyer DE, et al. Diabetes-related complications, glycemic control, and falls in older adults. Diabetes Care. 2008; 31:391–6.

128. Greenspan SL, Myers ER, Kiel DP, et al. Fall direction, bone mineral density, and function: risk factors for hip fracture in frail nursing home elderly. Am J Med. 1998; 104:539–45.

129. Ottenbacher KJ, Ostir GV, Peek MK, et al. Diabetes mellitus as a risk factor for hip fracture in mexican american older adults. J Gerontol A Biol Sci Med Sci. 2002; 57:M648–53.

130. Maggio AB, Rizzoli RR, Marchand LM, Ferrari S, Beghetti M, Farpour-Lambert NJ. Physical activity increases bone mineral density in children with type 1 diabetes. Med Sci Sports Exerc 2012;44:1206-11.

131. Pascual J, Argente J, Lopez MB, Munoz M, Martinez G, Vazquez MA, et al. Bone mineral density in children and adolescents with diabetes mellitus type 1 of recent onset. Calcif Tissue Int. 1998; 62(1):31–5.

132. Tuominen JT, Impivaara O, Puukka P, Ronnemaa T. Bone mineral density in patients with type 1 and type 2 diabetes. Diabetes Care. 1999; 22(7):1196–200.

133. Gunczler P, Lanes R, Paoli M, Martinis R, Villaroel O, Weisinger JR. Decreased bone mineral density and bone formation markers shortly after diagnosis of clinical type 1 diabetes mellitus. J Pediatr Endocrinol Metab. 2001; 14(5):525–8.

134. López-Alvarenga JC, Zariñán T, Olivares A, González-Barranco J, Veldhuis JD, Ulloa-Aguirre A. Poorly controlled type I diabetes mellitus in young men selectively suppresses luteinizing hormone secretory burst mass. J Clin Endocrinol Metab 2002;87:5507–15.

135. Valerio G, del Puente A, Esposito-del Puente A, Buono P, Mozzillo E, Franzese A. The lumbar bone mineral density is affected by long-term poor metabolic control in adolescents with type 1 diabetes mellitus. Horm Res. 2002; 58(6):266–72.

136. Ingberg CM, Palmer M, Aman J, Arvidsson B, Schvarcz E, Berne C. Body composition and bone mineral density in long-standing type 1 diabetes. J Intern Med. 2004; 255(3):392–8.

137. Leger J, Marinovic D, Alberti C, Dorgeret S, Chevenne D, Marchal CL, et al. Lower bone mineral content in children with type 1 diabetes mellitus is linked to female sex, low insulin-like growth factor type I levels, and high insulin requirement. J Clin Endocrinol Metab. 2006; 91(10):3947–53.

138. Miazgowski T, Pynka S, Noworyta-Zietara M, Krzyzanowska-Swiniarska B, Pikul R. Bone mineral density and hip structural analysis in type 1 diabetic men. Eur J Endocrinol. 2007; 156(1):123–7.

139. Hamilton EJ, Rakic V, Davis WA, Chubb SA, Kamber N, Prince RL, et al. Prevalence and predictors of osteopenia and osteoporosis in adults with Type 1 diabetes. Diabet Med. 2009; 26(1):45–52.

140. Eller-Vainicher C, Zhukouskaya VV, Tolkachev YV, Koritko SS, Cairoli E, Grossi E, et al. Low bone mineral density and its predictors in type 1 diabetic patients evaluated by the classic statistics and artificial neural network analysis. Diabetes Care. 2011; 34(10):2186–91.

141. Zhukouskaya VV, Eller-Vainicher C, Vadzianava VV, Shepelkevich AP, Zhurava IV, Korolenko GG, et al. Prevalence of morphometric vertebral fractures in patients with type 1 diabetes. Diabetes Care. 2013; 36(6):1635–40.

142. Parthasarathy LS, Khadilkar VV, Chiplonkar SA, Zulf Mughal M, Khadilkar AV. Bone status of Indian children and adolescents with type 1 diabetes mellitus. Bone. 2016; 82:16–20.

143. Levin ME, Boisseau VC, Avioli LV: Effects of diabetes mellitus on bone mass in juvenile and adult-onset diabetes. N Engl J Med 1976;294: 241–245.

144. Wiske PS, Wentworth SM, Norton JA Jr, Epstein S, Johnston CC Jr: Evaluation of bone mass and growth in young diabetics. Metabolism 1982;31:848–854.

145. Leon M, Larrodera L, Lledo G, Hawkins F: Study of bone loss in diabetes mellitus type 1. Diabetes Res Clin Pract 1989;6:237–242.

146. Weber G, Beccaria L, de Angelis M, Mora S, Galli L, Cazzuffi MA, Turba F, Frisone F, Guarneri MP, Chiumello G: Bone mass in young patients with type 1 diabetes. Bone Miner 1990;8:23–30.

147. Ersoy B, Göksen D, Darcan S, Mavi E, Öztürk C: Evaluation of bone mineral density in children with diabetes mellitus. Indian J Pediatr 1999;66:375–379.

148. Roe TF, Mora S, Costin G, Kaufman F, Carlson ME, Gilsanz V: Vertebral bone density in insulin-dependent diabetic children. Metabolism 1991;40:967–971.

149. Lettgen B, Hauffa B, Mohlmann C, Jeken C, Reiners C: Bone mineral density in children and adolescents with juvenile diabetes: Selective measurement of bone mineral density of trabecular and cortical bone using peripheral quantitative computed tomography. Horm Res 1995;43:173–175.

150. De Schepper J, Smitz J, Rosseneu S, Bollen P, Louis O: Lumbar spine bone mineral density in diabetic children with recent onset. Horm Res 1998;50:193–196.

151. Daniel Novak, Gun Forsander 1,2, Eva Kristiansen1,2, Anna Svedlund 1,2, Per Magnusson 3* & Diana Swolin-Eide. Altered cortical bone strength and lean mass in young women with long-duration (19 years) type 1 diabetes. Scientific Reports | (2020) 10:22367.

152. Peter Gunczler1 et al. Decreased Lumbar Spine Bone Mass and Low Bone Turnover in Children and Adolescents with Insulin Dependent Diabetes Mellitus Followed Longitudinally. Journal of Pediatric Endocrinology & Metabolism, 11,413-419 (1998).

153. Mastrandrea and Associates. Young Women With Type 1 Diabetes Have Lower Bone Mineral Density That Persists Over Time. DIABETES CARE, VOLUME 31, NUMBER 9, SEPTEMBER 2008.

154. Mahshid Mohseni, Pooya Hosseinzadeh, Roberto Civitelli, Seth Eisen. Effect of Peripheral Neuropathy on Bone Mineral Density in Adults with Diabetes: A Systematic Review of the Literature and Meta-analysis. Bone. 2021 June ; 147: 115932.

155. Neumann T, Lodes S, Kästner B, Lehmann T, Hans D, Lamy O, et al. Trabecular bone score in type 1 diabetes—a cross-sectional study. Osteoporos. Int. 2016; 27:127–133.

156. Aditi Wagh,1 Veena Ekbote,1 Vaman Khadilkar,1,2 and Anuradha Khadilkar. Trabecular Bone Score has Poor Association With pQCT Derived Trabecular Bone Density in Indian Children With Type 1 Diabetes and Healthy Controls. Journal of Clinical Densitometry: Assessment & Management of Musculoskeletal Health, vol. 24, no. 2, 268_274, 2021.

157. Hannon R, Eastell R. Preanalytical variability of biochemical markers of bone turnover. Osteoporos Int 2000;11 Suppl 6:S30-44.

158. Qvist P, Christgau S, Pedersen BJ, Schlemmer A, Christiansen C. Circadian variation in the serum concentration of C-terminal telopeptide of type I collagen (serum CTx): Effects of gender, age, menopausal status, posture, daylight, serum cortisol, and fasting. Bone 2002;31:57-61.

159. Roche Diagnostics. Elecsys β-Crosslaps/serum (B-CTX in serum) immunoassay cobas package insert (V 14.1). Manheim, DE: Roche Diagnostics; 2014.

160. Dawson-Hughes B, Lindsay R, Khosla S, Melton LJ, Tosteson AN, Favus MJ, et al. Clinician's Guide to Prevention and Treatment of Osteoporosis. Washington DC: National Osteoporosis Foundation; 2008.

161. Turner, C.H. and A.G. Robling, Mechanisms by which exercise improves bone strength. J Bone Miner Metab, 2005. 23 Suppl: p. 16-22.

162. Prasanna Kumar KM. Incidence trends for childhood type 1 diabetes in India. Indian J Endocr Metab 2015;19:34-5.

163. Unni Syversen1,2, Mats Peder Mosti1,3, Ida Maria Mynarek1, Trude Seselie Jahr Vedal1, Kristin Aasarød1,4, Trude Basso5, Janne E Reseland6, Per Medbøe Thorsby7, Bjorn O Asvold2,8, Erik Fink Eriksen6 and Astrid Kamilla Stunes1,3. Evidence of impaired bone quality in men with type 1 diabetes: a cross-sectional study. Endocrine Connections (2021) 10, 955–964.

164. SOHA M. ABD EL DAYEM 1 , AMAL M. EL-SHEHABY 2 , ASMAT ABD EL GAFAR 3 , ASHRAF FAWZY 1 & HASSAN SALAMA. Bone density, body composition, and markers of bone remodeling in type 1 diabetic patients. Scandinavian Journal of Clinical & Laboratory Investigation, 2011; 71: 387–393.

165. Anna R. Kahkoska et al. Sociodemographic Associations of Longitudinal Adiposity in Youth with Type 1 Diabetes. Pediatr Diabetes. 2018 December ; 19(8): 1429–1440.

166. Slemenda CW, Reister TK, Hui SL, Miller JZ, Christian JC, Johnston CC: Influences on skeletal mineralization in children and adolescents: Evidence for varying effects of sexual maturation and physical activity. J Pediatr 1994;125: 201–207.

167. Hough FS, Pierroz DD, Cooper C, Ferrari SL; IOF CSA Bone and Diabetes Working Group. Mechanisms in endocrinology: mechanisms and evaluation of bone fragility in type 1 diabetes mellitus. Eur J Endocrinol. 2016;174(4):R127–R138.

168. E. Strotmeyer and J. Cauler, "Diabetes mellitus, bone mineral density, and fracture risk," Current Opinion in Endocrinology, Diabetes and Obesity, vol. 14, no. 6, pp. 429–435, 2007.

169. Phoebe Loxton, Kruthika Narayan, Craig F. Munns, and Maria E. Craig. Bone Mineral Density and Type 1 Diabetes in Children and Adolescents: A Meta-analysis. Diabetes Care Volume 44, August 2021.

170. Riyaz Ahmad Daga et al. High prevalence of vitamin D deficiency among newly diagnosed youth-onset diabetes mellitus in north India., Arq Bras Endocrinol Metab. 2012;56/7.

171. Glueck CJ, Jetty V, Rothschild M, Duhon G, Shah P, Prince M, et al. Associations between serum 25-hydroxyvitamin D and lipids, lipoprotein cholesterols, and homocysteine. North Am J Med Sci 2016;8:284-90.

172. AlJohara M AlQuaiz et al. Association between standardized vitamin 25(OH)D and dyslipidemia: a community-based study in Riyadh, Saudi Arabia. l. Environmental Health and Preventive Medicine (2020) 25:4.

173. de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney disease in the United States. JAMA 2011;305:2532–2539.

174. P. Eskildsen, H. Andreassen, and M. Rix, “Impact of peripheral neuropathy on bone density in patients with type 1 diabetes,” Diabetes Care, vol. 22, no. 5, pp. 827–831, 1999.

175. Luigi Gennari et al. Circulating Sclerostin Levels and Bone Turnover in Type 1 and Type 2 Diabetes. Clin Endocrinol Metab, May 2012, 97(5):1737–1744.

176. Selvaraj M, Prasad HK, White S, Prasanna B, Sangaralingam T. Prevalence and determinants of occurrence of dyslipidemia in subjects with type 1 diabetes mellitus. Indian J Pediatr. 2022.

177. Shah N, Khadilkar A, Gondhalekar K, Khadilkar V. Prevalence of dyslipidemia in Indian children with poorly controlled type 1 diabetes mellitus. Pediatr Diabetes. 2020;21:987–94.

178. Billow A, Anjana RM, Ngai M, et al. Prevalence and clinical profile of metabolic syndrome among type 1 diabetes mellitus patients in southern India. J Diabet Complications. 2015;29:659–64.

Annexures

Annexure 1

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2021/3498 **Date: 12/03/2021**

ETHICAL CLEARANCE CERTIFICATE

Member Secretary

Certificate Reference Number: **AIIMS/IEC/2021/3333**

Project title: **"Assessment of bone mineral density (BMD) by DEXA (Dual energy Xray-absorbiometry) in type 1 diabetes mellitus patients and Co-relation with glycemic and non-glycemic parameters: An observational study"**

Nature of Project:	**Research Project Submitted for Expedited Review**
Submitted as:	**D.M. Dissertation**
Student Name:	**Dr. Shinjan Patra**
Guide:	**Dr. Ravindra Shukla**
Co-Guide:	**Dr. M.K.Garg, Dr. Madhukar Mittal & Dr. Mithu Banerjee**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation.

If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary

Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Basni Phase-2, Jodhpur, Rajasthan-342005; **Website:** www.aiimsjodhpur.edu.in; **Phone:** 0291-2740741 Extn. 3109
E-mail : ethicscommittee@aiimsjodhpur.edu.in; ethicscommitteeaiimsjdh@gmail.com

Annexure 2

Case Record Proforma

Patient Particulars

NAME:

Age	
Sex	
Address	
Religion	
AIIMS Registration Number	
Occupation	

Type 1 DM related Clinical History-

Age of Diagnosis of T1DM	
Duration of Diabetes	
Family History	
Drug Hypersensitivity History	

Addiction History:

	Amount/Frequency	Duration
Smoker		
Others		

Type 1 DM related Treatment History-

	Name	Dosing	U/kg/day
Bolus/Prandial Insulin			
Basal Insulin			
Oral Anti-Diabetic Therapy (if any)			

Other Drug history related to Co-morbidity-

Name of the Drug	Indications	Dosing

Exclusion Criteria's Checklist:

Type 1 DM < 12 years of Age	
T1DM with known Bone diseases like Rickets/Osteomalacia/OI	
T1DM known traumatic injury causing permanent bony abnormalities	
All other types of Diabetes including suspected MODY or Pancreatic Diabetes	
T1DM with CKD, CCF or COPD	
T1DM taking drugs which can affect drug metabolism like Steroids/other immunosuppressant's	
T1DM currently on Diabetic Ketoacidosis/Hyperglycemic Crisis	

Type 1 DM Glycemic control related Adverse Events-

	How many Total Episodes	Episodes per year after Diagnosis
Diabetic Keto-acidodsis		
Hypoglycemic episodes		
Severe Hypoglycemic Episodes		

Type 1 DM related Micro/Macro –Vascular Complications/Suggestive symptoms-

	Yes/No	Type of Involvement	Duration	Medical Therapy
Neuropathy				
Kidney disease				
Retinopathy				
Gastropathy				
Dermopathy				
Autonomic dysfunction				
CAD				
CVD				

Auto-immune Co-morbidities associated with Type 1 DM-

	Yes/NO	Type of Involvement	Duration	Medical therapy

Hypothyroidism				
Graves				
Celiac				
Rheumatoid Arthritis				
Vitiligo				
Others				

Examination Findings

Vitals:

BP (Systolic/Diastolic)	
Pulse	
Height	
Weight	
BMI	
Temperature	

Pubertal Status-

	Tanner Staging
Axillary/Pubic Hair	
Male Genitalia	
Female Genitalia	

Physical Activity Status:

	Exercise Mode	Intensity	Duration	Frequency
Known Athlete/Sportsman				
Non-Athlete/ Non-sportsman				

Investigations

Bio-chemical Parameters-

HbA1C (last 12 months average)	
TG	
LDL	
TSH	
Creatinine	
SGOT/SGPT	
Testosterone (for male)	
Estradiol (for female)	
ECG	
Fundus	
Urine Microalbumin	
IGF1	
Fasting Insulin	
C-Peptide	
HOMA-IR	

Bone-Mineral Panel-

Corrected Ca	
Phosphorus	
25 (OH)D	
ALP	
PTH	
Fractional Excretion of Calcium	
Fractional Excretion of Phosphorus	

BMD parameters-

Site	T-Score	Z-Score
AP Spine		
Total Hip		
Femoral Neck		
Wrist		

Other BMD parameters-

Trabecular Bone Score (TBS)	
Visceral Adipose Tissue content (VAT)	
Total Lean body mass	

Bone- Markers-

Serum Osteocalcin	
Serum CTX	

Annexure 3: Patient informed consent form

All India Institute of Medical Sciences Jodhpur

INFORMED CONSENT FORM

Title of Thesis/Dissertation : **Assessment of Bone Mineral Density (BMD) by DEXA (Dual Energy Xray-Absorbiometry) in Type 1 Diabetes Mellitus patients and Co-relation with Glycemic and Non-glycemic parameters; An Observational study**

Name of DM Senior Resident : Dr. SHINJAN PATRA
Mob No. 9475433534 Patient/Volunteer Identification No.

I, _____ S/o or D/o _____
R/o
_____

give my full, free, voluntary consent to be a part of the study "**Assessment of Bone Mineral Density (BMD) by DEXA (Dual Energy Xray-Absorbiometry) in Type 1 Diabetes Mellitus patients and Co-relation with Glycemic and Non-glycemic parameters;An Observational study** ", the procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions.

I understand that my participation is voluntary.
I understand that the information collected about me and any of my medical records may be looked at by responsible individual from AIIMS Jodhpur or from regulatory authorities. I give permission for these individuals to have access to my records.

Date: _____ _____
Place: _____ Signature/Left thumb impression

This to certify that the above consent has been obtained in my presence.

Date: _____ _____
Place: _____ Signature of DM Resident

1. Witness 1 _____
2. Witness _____

Assessment of Bone Mineral Density (BMD) by DEXA (Dual Energy Xray-Absorbiometry) in Type 1 Diabetes Mellitus patients and Co-relation with Glycemic and Non-glycemic parameters; An Observational study

Annexure 4

Patient Information Sheet

You are being invited to willingfully participate in the study entitled

"Assessment of Bone Mineral Density (BMD) by DEXA (Dual Energy Xray-Absorbiometry) in Type 1 Diabetes Mellitus patients and Co-relation with Glycemic and Non-glycemic parameters; An Observational study"

Diabetes mellitus is characterized by abnormally high levels of glucose (sugar) in the blood, which can be highly variable throughout the day. Current investigations available for assessing blood glucose control are HbA1C, laboratory measured and self monitored blood glucose by glucometers. New technologies like DXA machine effectively determines BMD of those T1DM patients.

Study Design

If you are eligible for the study, you will undergone fix set of questionnaire followed by BMD estimation through BMD-DXA machine along with fix set of investigations.

General instructions:
Radiation reaction are rare & asked to report to us in any problems.

Confidentiality

Your medical records and identity will be treated as confidential documents. They will only be revealed to other doctors/scientists/monitors/auditors of the study if required. The results of the study may be published in a scientific journal but you will not be identified by name.
Ethics committee approval has been obtained for the study.

Your participation and rights

Your participation in the study is fully voluntary and you may withdraw from the study anytime without having to give reasons for the same. In any case, you will receive the appropriate treatment for your condition. You will not be paid any amount for the participation in the study. You will have to pay for the routine investigations that will be done.

For further queries/questions or help in emergency please contact.

1. **Dr. Shinjan Patra- 9475433534**
2. **Dr. Ravindra Shukla**

□□□□ □□□□□ □□□□

□□□□ □□□□□□□□□ □□ □□□□□□ □□ □□□□□ □□□□ □□ □□□

□□□□□□□□ □□□□ □□ □□□ □□

"□□□□ 1 □□□□□□□□ □□□□□□ □□ □□□□□□□ □□□ DEXA (□□□□□ □□□□□ □□□□□□-□□□□□□□□□□□□□□□) □□□□□□ □□□□□ □□□□ □□□□□ (BMD) □□ □□□□ □□ □□□□□□□□□□ □□ □□□-□□□□□□□□□□ □□□□□□□□ □□ □□□ □□-□□□□□; □□ □□□□□□ □□□□□□ "

□□□□□□ □□□□□□ □□ □□□□ □□□ □□□□□□□ (□□□□) □□ □□□□□□□□ □□□ □□ □□□□ □□□□ □□ □□□□□□□ □□, □□ □□□□ □□□ □□□□□□□ □□□□□□□□□□ □□ □□□□ □□□ □□□□ □□□□□□ □□□□□□□□ □□ □□□□ □□□□ □□ □□□ □□□□□□ □□□□□□□ □□□□ □□□ HbA1C, □□□□□□□□□□ □□□□□□ □□□□ □□ □□ □□□□□□□□□□ □□□□□□ □□□□ □□□□□□ □□ □□□□□□□ □□ □□□ □□□□□□□ □□□□ □□□□ □□ □□□□□□□□□□□□□□□ □□ T1DM □□□□□□□ □□ □□□□□□ □□ □□□□□□□ □□□ □□ □□□□□□□□□ □□□□ □□□□ □□□□□□ □□□□□

□□□ □□ □□□□□□ □□ □□□ □□□□□ □□□, □□ □□ □□□□ □□ □□□ □□ □□□-□□□ □□□□□□-□□□□□□□ □□□□ □□ □□□□□□ □□ □□□□□□ □□□□ □□ □□□ □□□□□□□□□□ □□ □□□□□ □□□ □□ □□□□□□□□□

□□□□□□□ □□□□□□□□:

□□□□□□ □□□□□□□□□□□ □□□□□□ □□ □□ □□□□ □□□□ □□ □□□□□□ □□□ □□□□□□□ □□□□ □□ □□□ □□□ □□□□ □□□ □□□□□□□□

□□□□ □□□□□□ □□□□□□□ □□ □□□□□ □□ □□□□□□ □□□□□□□□□□ □□ □□□ □□□ □□□□ □□□□□□ □□□ □□□□□□ □□ □□ □□ □□□□ □□□□□□ □□ □□□□ □□□□□□□□ / □□□□□□□□□□□

/ □□□□□□□ / □□□□□□□□ □□ □□□□□ □□□□□□□ □□□□□□□ □□ □□□□□□□ □□ □□□□□□□□□□ □□□□□□□□ □□□□ □□□□□□□□□ □□ □□□□□ □□□□ □□□□□ □□□□□ □□□□ □□ □□□□□ □□□□□□□ □□□□□□□ □□□□□□□ □□ □□□□ □□□□□ □□□□□ □□ □□□□□□□ □□□□ □□ □□□□ □□□□□ □□□□□□□□□ □□ □□□□□□□

□□□□□□□ □□□□ □□□□□ □□□□□□□□□ □□□□□ □□□□ □□ □□□□□□□□□□ □□ □□ □□ □□ □□□□□ □□□□□ □□□□□ □□□□ □□ □□□□□□□ □□ □□ □□□□□ □□□□□ □□□□□ □□ □□□□□□ □□□□, □□ □□□□□ □□□□□□□ □□ □□□□ □□□□□□□□ □□□□□□ □□□□□□□□ □□□□□□□□ □□□□□□□ □□□□ □□□□ □□□□□ □□ □□□□ □□□□□ □□□□ □□□□□ □□□□□ □□ □□□□□□□ □□□□□ □□□□□□□ □□□□□ □□ □□□□ □□□□□□□ □□□□□ □□□□□□

□□□□ □□ □□□□□□□□□ / □□□□□□□□□ □□ □□□□ □□ □□□□□ □□□□□□□ □□□□ □□□□ □□ □□□□ □□□□□□□ □□□□□□

1. □□□□ □□□□□ □□□□□□□- 9475433534
2. □□□□ □□□□□□□□ □□□□□□