AIIMS/JH/ENDO/2023/530

To,

Dated: 16/01/23

The Dean (Academic)

All India Institute of Medical Sciences

Jodhpur

(Through proper channel)

Sub: Regarding submission of thesis for DM degree

Respected Sir,

With due respect, I, **Dr. Prasoon Rastogi**, D.M. (Endocrinology) Resident of July'2020 Session, am submitting five copies of DM thesis in the Dean academics of this institute.

Title of the thesis- "**Assessment of Bone Mineral Density (BMD) and Trabecular Bone Score (TBS) in Hyperthyroidism".**

Kindly accept & oblige for further proceedings.

Thanking you,

Yours Sincerely,

Dr. Prasoon Rastogi
D.M. (Endocrinology) Senior Resident
July'2020 Session
AIIMS, Jodhpur

Chief Guide:

Dr. Madhukar Mittal
Additional Professor
Department of Endocrinology and Metabolism
AIIMS Jodhpur

Forwarded by:

Dr. Mahendra Kumar Garg
Professor and Head
Department of Endocrinology and Metabolism
AIIMS Jodhpur

ASSESSMENT OF BONE MINERAL DENSITY (BMD) AND TRABECULAR BONE SCORE (TBS) IN PATIENTS OF HYPERTHYROIDISM

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 and Metabolism)

JULY 2020 **DR. PRASOON RASTOGI**

AIIMS, JODHPUR

All India Institute of Medical Sciences, Jodhpur

DECLARATION

I hereby declare that the thesis titled **"Assessment of Bone Mineral Density (BMD) and Trabecular Bone score (TBS) in patients of Hyperthyroidism"** embodies the original work carried out by the undersigned in All India Institute of Medical Sciences, Jodhpur**.**

Dr. Prasoon Rastogi
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) and Trabecular Bone score (TBS) in patients of Hyperthyroidism**" is the bonafide work of **Dr. Prasoon Rastogi** carried out under our guidance and supervision, in the Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, Jodhpur.

Chief Guide

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

Co-Guides:

Dr. Mahendra Kumar Garg
Professor and Head
Department of Endocrinology and Metabolism
AIIMS, Jodhpur

Dr. Rajesh Kumar
Professor and Head
Department of Nuclear Medicine
AIIMS Jodhpur

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

Dr. Mithu Banerjee
Additional Professor and Head
Department of Biochemistry
AIIMS, Jodhpur

All India Institute of Medical Sciences, Jodhpur

CERTIFICATE

This is to certify that the thesis titled "**Assessment of Bone Mineral Density (BMD) and Trabecular Bone score (TBS) in patients of Hyperthyroidism**" is the bonafide work of **Dr. Prasoon Rastogi** carried out under our guidance and supervision, in the Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, Jodhpur.

Dr Mahendra Kumar Garg
Professor & Head
Department of Endocrinology and Metabolism
AIIMS, Jodhpur

Acknowledgment

We usually think that we are the captains of our destiny and that achievements manifest our spirit. But we are shaped and molded by those who cross our lives and touch our souls. Here, I want to acknowledge the presence of such people, and I feel privileged to do so.

I would also like to express my gratitude to Dr. Madhukar Mittal, my chief guide, for his constant guidance and support during this research endeavour. I am incredibly grateful for sir's keen interest, insightful ideas, and meticulous approach in conceiving this idea and supporting me in bringing it to fruition. The lessons learned during this arduous but fulfilling journey will go a long way in academics and beyond.

I want to express my heartfelt gratitude to Dr. Mahendra Kumar Garg, Head of the Department of Endocrinology and Metabolism. Words fall short of the kind of support and inspiration sir has been throughout my time in the department. His wealth of knowledge, clinical acumen and dedication is something I look up to and strive to achieve.

I am also indebted to my co-guide, Dr. Ravindra Shukla, Associate Professor, Department of Endocrinology and Metabolism. Sir has supported me throughout my tenure and enriched our clinical and research work with his valuable insights and experience.

I thank my co-guide, Dr. Rajesh Kumar, Professor and Head Department of Nuclear Medicine, for his amicable guidance and support. His unconditional help had been instrumental in getting the earliest possible dates for the tedious process of radioactive iodine uptake and technechian-99 scans. I take this opportunity to express my immense gratitude to my co-guide, Dr. Mithu Bannerjee, Additional Professor and head of the Department of Biochemistry, for always being accessible, giving opinions, and being instrumental in facilitating the laboratory aspects of the dissertation.

I will fail in my duty if I do not mention the selfless attitude, untiring support, and invaluable suggestions of our beloved seniors, Dr. Vanishree Ganakumar and Dr. Parul Gupta. I would thank my colleague Dr. Shinjan for providing much-needed support in my clinical and research work, and I found a friend forever.

This research work would not have been possible without the support of colleagues in the department. The role of Dr. Parth, Dr. Eram, Dr. Pradakshna, Dr. Anupriya, Dr. Azher, Dr. Saloni, and Dr. Charmi has been crucial in the recruitment of patients. I would also sincerely thank Dr. Rakesh, Dr. Ravichandran, and Dr. Priyank, residents in the nuclear medicine department; without their support, my data collection not have been fulfilled. I cannot thank them enough for their unwavering helping nature and cooperation and for providing a cordial and positive working environment.

I would sincerely and heartfully thank Mr. Girish and Mr. Sunny, our BMD technicians, who also worked tirelessly and sincerely toward data collection for my dissertation. I would also like to express my sincere thanks to the support staff in the Department of Endocrinology and Metabolism, Mr. Subhash, Mr. Shivendra, Mr. Shailesh, Mrs. Roshni, Nursing officer Ramswarup ji for extending their help in conducting this study.

My acknowledgment will be incomplete if I do not express my sincere thanks to all those patients and their families who consented to participate in this study.

Above all I thank Almighty God for the strength he gave me to accomplish such a job and express my deepest regards and gratitude to my parents, my soulmate Dr. Swati, and my dear sister Prachi for their constant encouragement and support.

I acknowledge my gratitude to all the editors of journals and authors of articles and books whose copyright material I have utilized in this work.

Dr. Prasoon Rastogi

INDEX

List of Abbreviations

BMD - Bone mineral density

TBS - Trabecular bone score

BTM - Bone turnover marker

MNG - Multinodular goiter

DXA - Dual-energy X-ray absorptiometry

PINP - Procollagen type I N-propeptide

PICP - Procollagen type I C-propeptide

CTX - C-terminal telopeptide

NTX - N-terminal telopeptide

$^{99m}TcO_4$ -Technetium 99m pertechenate

TRAb - Thyroid receptor antibody

RAI - Radioactive iodine

RAIU - Radioactive iodine uptake

ATD - Anti-thyroid drugs

IQR - Interquartile range

TSH - Thyroid stimulating hormone

TSHR - Thyroid stimulating hormone receptor

TSHR-S- Thyroid stimulating hormone receptor stimulating

TR - Thyroid Receptor

IL - Interleukin

PTH - Parathyroid hormone

CRF - Clinical risk factors

CT - Computed tomography

MRI - Magnetic resonant imaging

HR-pQCT - High-resolution peripheral computed tomography

DNA - Deoxyribonucleic acid

ESR - Erythrocyte sedimentation rate

HAART - Highly active antiretroviral therapy

OPG - Osteoprotegerin

RANKL - Receptor activator of the nuclear factor kappa-B ligand

TD - Time to diagnosis

THS - Time in hyperthyroid state

TNHA - Time to normalization of hyperthyroid activity

TRT - Time to rebound of TSH

CAT - Cumulative ATD to TRT

CAI - Cumulative ATD Index

ULN – Upper limit normal

ALP - Alkaline phosphate

LIST OF TABLES

LIST OF FIGURES

List of Annexures

SUMMARY

Background

Hyperthyroidism, a state of excess thyroid hormone secretion, has myriad manifestations, including deleterious effects on bone health. While its impact on bone mineral density (BMD) has been well-known, evidence of its effect on bone micro-architecture, which can now be assessed non-invasively using the trabecular bone score (TBS), is scarce. Thus, our study aimed to compare BMD, TBS, and bone turnover markers (BTMs) markers in hyperthyroidism patients with matched healthy controls and to ascertain the determinants affecting them.

Methods

This was a case-control study including 103 hyperthyroid patients(cases) with Graves' disease (n=94), toxic multinodular goiter (n=7), and toxic adenoma (n=2) and forty age, BMI, and sex-matched healthy controls. Both cases and controls underwent clinical examination, BMD, and TBS evaluation using Hologic-horizon-DXA, amino-terminal peptide of procollagen type-1 (PINP), and c-terminal telopeptide-X (CTX). Additionally, thyroid function test, thyroid receptor antibody (TRAb), Tc99m pertechnetate scan and /or radioactive iodine uptake study (RAIU), and ultrasonography of thyroid for thyroid volume were done in cases.

Results

The median duration in the hyperthyroid phase and the cumulative ATD index (CAI - dose of ATD times duration in months) for cases were nine months (IQR 3 – 28.5) and 170 (IQR 55 - 400), respectively. As compared with controls, cases had lower BMD (kg/m^2) at all measured sites, including the lumbar spine (0.91 ± 0.17 Vs. 1.01 ± 0.12; p= 0.003), femoral-neck (0.74 ± 0.13 Vs. 0.82 ± 0.1; p<0.001), total hip (0.85 ± 0.15 Vs. 0.97 ± 0.11; p<0.001), distal left radius (mean 0.65 ± 0.13 Vs. 0.73 ± 0.07; p<0.001) and whole-body BMD (mean 1.02 ± 0.13 Vs. 1.10 ± 0.08; p<0.001). TBS was also lower in cases than in controls (1.32 ± 0.11 Vs. 1.38 ± 0.06; p=0.002). Both BTMs, PINP [73.9 ng/ml (IQR 61.3 – 92.3) Vs. 63.1 ng/ml (IQR 49.8 – 73.1); p= 0.003) and CTx (0.2 ng/ml (IQR 0.1 – 0.3) Vs. 0.1 ng/ml (IQR 0.1 – 0.2); p=0.001),

were significantly higher in cases versus controls. Thyroid-hormone levels and TSH had no significant correlation with BMD, whereas TRAb titers had a negative correlation with BMD at distal radius (ρ=-0.338, p = 0.003) and whole-body BMD (ρ = -0.298, p = 0.01). Thyroid gland size negatively correlated with TBS (ρ = -0.296, p <0.01), while RAIU (%) had no significant correlation with BMD or TBS. Bone turnover markers levels did not correlate with BMD or TBS.

Conclusion

Hyperthyroidism is associated with significant implications for bone health, including lower BMD, poorer micro-architecture, and higher BTMs. High autoimmunity and increased gland size are associated with lower BMD and TBS, respectively.

INTRODUCTION

Hyperthyroidism, a subset of thyrotoxicosis (elevated levels of thyroxine (T4) and/or triiodothyronine (T3), is caused by excess synthesis and secretion of thyroid hormone. The most common cause of hyperthyroidism in the young and the middle-aged is Grave's disease, while in the elderly, it is multinodular goiter. (1)

Thyrotoxicosis manifests as a hypermetabolic state which affects every organ system. The presentation of thyrotoxicosis may vary among patients. Younger patients tend to exhibit symptoms of sympathetic activation, such as anxiety, hyperactivity, palpitations, sweating, and tremor, while older patients have more cardiovascular symptoms, including dyspnoea, atrial fibrillation, and unexplained weight loss. (1)

The adverse effects of hyperthyroidism on the skeleton were known before the discovery of a satisfactory treatment for hyperthyroidism. One of the earliest reports of hyperthyroid bone disease was in 1891 when von Recklinghausen described the "worm-eaten" appearance of the long bones of a young woman who died from hyperthyroidism (2).

In hyperthyroidism, osteoclastic resorption is stimulated out of proportion to osteoblastic remineralization. As a result, the average cycle duration of approximately 200 days is halved, and each cycle is associated with a 9.6 percent loss of mineralized bone.(1) These changes in bone metabolism are associated with negative calcium balance, hypercalciuria, and, rarely, hypercalcemia. (3)

Thyroid hormone may affect bone calcium metabolism either by a direct action on osteoclasts or by acting on osteoblasts, mediating osteoclastic bone resorption. Experimental studies by Bassett et al. in mice lacking either the thyroid receptor- α or -β demonstrated that bone loss in thyrotoxicosis is independent of circulating TSH levels and mediated predominantly by TRα (4). Thyroid-stimulating hormone (TSH) may also directly affect bone formation and resorption, mediated via the TSH receptor on osteoblast and osteoclast precursors. Also, TSH seems to be a negative regulator of bone remodeling, inhibiting the formation, the survival of osteoclasts, and the differentiation of osteoblasts (5).

Increased serum interleukin-6 (IL-6) concentrations in hyperthyroid patients may also play a role in thyroid hormone-stimulated bone loss. Interleukin-6 stimulates osteoclast production and may affect the action of parathyroid hormone (PTH) on bone. (6)

Thus, hyperthyroidism is associated with accelerated bone remodeling, reduced bone density, osteoporosis, and increased fracture rate. The extent of reduction in bone density in hyperthyroid patients ranges from 10 to 20 percent. (7) Studies show variable results about the reversibility of bone density changes with therapy. Also the extent of bone loss reversibility with therapy is unclear. (8,9)

Bone density measurements during the last decade have demonstrated that bone loss is common in patients with overt hyperthyroidism and, to a lesser extent, in those with subclinical hyperthyroidism. Data from India is sparse regarding the effects of thyrotoxicosis on bone and mineral metabolism.

BMD is an indicator of areal bone mineral content, expressed in g/cm2 when measured using dual-energy X-ray absorptiometry (DXA), a standard tool for diagnosing osteoporosis. However, BMD only accounts for 60 – 70% of variations in bony strengths and increased fracture risks (10). Other variables like clinical risk factors (CRF) such as old age, history of previous fragility fractures, smoking, alcohol intake (>3 units per day), chronic glucocorticoid use, etc., also contribute to increased fracture risk. Additionally, bone strength is also affected by bone quality, an umbrella term used to describe structural and material properties of the bone, which in turn affects bone turn-over rates.

The structural properties of bone include geometry and micro-architecture (trabecular thickness, connectivity, separation and number, and cortical thickness and porosity). In contrast, the material properties include bone mineral content (crystal size and orientation), collagen composition, and damage accumulation (11). Evaluation of the bone micro-architecture and remodeling includes invasive procedures like histomorphometric or micro-computed tomography (micro-CT) analysis of the transiliac crest bone biopsy or complex noninvasive technologies including high-resolution peripheral quantitative computed tomography (HR-pQCT) and micro-magnetic resonance imaging (micro-MRI), which have limited availability due to high costs, and are mainly used in research settings(12).

To this end, trabecular bone score (TBS) was introduced in 2008 as a new tool for assessing bone micro-architecture (13). It is a textural index based on pixel-grey level variations identifiable on lumbar spine DXA (14), an indirect indicator of the trabecular micro-architecture.

An increased TBS assessment is associated with better bone micro-architecture, while a reduced TBS estimation correlates with fragile skeletal micro-architecture. It was shown that TBS is related to the structure of the bone tissue, and it may detect differences between DXA scans that show identical BMD values. It is an easy tool, and clinical studies suggest that it improves (in addition to clinical risk factors and BMD) the prediction of fracture risk in osteoporosis and some metabolic bone diseases. TBS also seems more sensitive than BMD in identifying secondary osteoporosis, namely hyperparathyroidism, adrenal adenomas, and iatrogenic Cushing. (14,15)

As far as we know, there is no data on TBS in patients of hyperthyroidism from India. This cross-sectional case-control study aimed to evaluate the effects of hyperthyroidism on morphometric markers of bone health (BMD and TBS) and to look for a correlation with disease activity.

REVIEW OF LITERATURE

Thyroid hormones regulate metabolism and cell differentiation throughout the body due to their omnipresent receptors. Therefore, both increases and decreases in levels of thyroid hormones impact all body systems.

Hyperthyroidism and thyrotoxicosis, though used interchangeably, are not synonymous. Thyrotoxicosis is a state of thyroid hormone excess, whereas hyperthyroidism results from increased thyroid gland function. Graves' disease is the most common cause of hyperthyroidism (70% of cases in iodine-sufficient areas). Other causes of hyperthyroidism include toxic multinodular goiter (MNG) and toxic adenoma. (16)

Graves' disease gets its name from Irish physician Robert James Graves who described a case series of 3 female patients with violent and long palpitations with peculiar enlargement of the thyroid gland. Graves' disease is a consequence of uncontrolled autoimmunity (TSHR-S autoantibody) principally at TSH receptors, mimicking action of TSH on the thyroid gland leading to hyperthyroidism. As the effect of TSHR-S autoantibodies, diffusely enlarged thyroid is the hallmark of the disease, but thyroid nodules can sometimes develop. Graves' ophthalmopathy (30 – 45%) and, rarely, pretibial myxoedema are other typical physical findings.

Toxic adenoma and multinodular toxic goiter are other frequent causes of hyperthyroidism, especially in iodine-deficient areas. Toxic adenomas are monoclonal benign encapsulated tumors synthesizing thyroid hormones independent of TSH stimulation. They are characterized by heterozygous gain-of-function mutations involving the TSHR (20-80%) or the Gsα protein genes (8-75%), which induce a permanent and TSH-independent activation of the adenylate-cyclase pathway (17). Toxic adenoma grows slowly over many years in an otherwise normal thyroid gland. The coexistence of two or more toxic adenomas (multiple adenomatoses) is uncommon. In the early phases, the amount of secreted thyroid hormones is insufficient to completely suppress TSH secretion (partial autonomy) and the function of the extranodular tissue. Eventually, overt thyrotoxicosis ensues,

with frankly elevated thyroid hormone levels (hyperthyroidism) when adenomas attain a size greater than 3 cm in most cases (18).

Multinodular toxic goiter is also often detected in iodine-deficient countries and has been noted to be decreasing with iodine prophylaxis (19). Similar somatic activating mutations of the TSHR demonstrated in toxic adenoma have also been observed in toxic multinodular goiter. However, in many nodules, neither TSHR nor Gsα protein mutations have been observed (17). The natural history of multinodular toxic goiter is similar to toxic adenoma, with the slow formation of multiple autonomously functioning nodular areas in an overall nodular goiter. Because of the slow progression through several degrees of thyrotoxicosis and their advanced age, patients with multinodular toxic goiter may report fewer symptoms.

Role of radioactive iodine (RAI) uptake in thyrotoxicosis

The causality of thyrotoxicosis is highly varied, ranging from as simple as thyrotoxicosis factitia to infection-induced or drug-related or autoimmune thyroiditis to autonomous functioning nodules. The differentiation of these aetiologies is vital for managing the disease and ascertaining long-term prognosis, summarised in table 1.

Amongst the battery of tests for evaluating thyrotoxicosis, a radioactive iodine uptake scan occupies an important place. The advantages of performing RAI uptake have been listed below:

1. RAI uptake helps in differentiating hyperthyroidism from among the causes of thyrotoxicosis. RAIU and scintigraphy allow differentiation from various stages of silent thyroiditis or factitious hyperthyroidism.
2. When considering RAI as a treatment option for hyperthyroidism, pretherapeutic measurement of RAI uptake helps in radioactive iodine dose estimation and subsequent response to therapy.
3. Thyroid scintigraphy can identify the presence of a “cold” nodule within the diffuse toxic goiter, which usually requires further work-up to exclude malignancy. It is advantageous over technetium pertechnetate scan in evaluating certain solitary cold thyroid nodules. Up to 10% of solitary cold thyroid nodules are malignant, requiring further workup. Occasionally, a

nodule will appear “warm” on a Tc-99m scan but cold on an RAI scan (ie, the lesion traps Tc-99m but does not organify iodine); this is termed a discordant nodule. Since a discordant nodule requires further workup (such as fine-needle aspiration biopsy), depending on the patient’s risk factors for malignancy (20).

4. Thyroid enlargement is not necessarily present in all cases of Graves disease; the gland could be normal in size, particularly in the early stages of the disease. RAIU and scintigraphy allow differentiation from various stages of silent thyroiditis.

The radioactive iodine uptake in Graves’ hyperthyroidism is elevated. Early uptakes are sometimes as high as, or higher than, the 24-hour uptake because of rapid thyroidal iodine turnover. In elderly patients with Graves’ disease, the radioactive iodine uptake tends to be lower and may sometimes even be in the normal range.

In toxic multinodular goiter and toxic autonomously functioning adenoma, uptakes may be in the normal range. Nevertheless, the radioactive iodine uptake clearly distinguishes these states of thyroid overactivity from thyrotoxic thyroiditis states (subacute, silent, and postpartum thyroiditis), in which the values are low, usually less than 1% and no more than 3%.

Table 1: Causes and distinctive features of hyperthyroidism

Disease	**Distinctive features**	**RAI uptake**
Thyrotoxicosis of thyroidal origin associated with hyperthyroidism		
Graves’ disease	Diffuse goitre, Ophthalmopathy Positive TSHR autoantibodies	High
Toxic adenoma	Single ‘hot’ nodule at thyroid scan	High
Multinodular toxic goitre	Multiple ‘hot’ nodules at thyroid scan	High
TSH-secreting adenomas	Inappropriately high TSH level	High
Non-autoimmune congenital and familial hyperthyroidism	TSH receptor gene mutations by DNA analysis	High

Type I amiodarone-induced thyrotoxicosis	High urinary iodine	Normal, high, low
Drugs-induced hyperthyroidism (Interferon α)	Positive TSHR autoantibodies	High
Thyrotoxicosis of thyroidal origin associated with thyroid destruction		
Subacute thyroiditis	Neck pain, High ESR	Low
Silent thyroiditis	Positive thyroid autoantibodies	Low
Type II amiodarone-induced thyrotoxicosis	High urinary iodine	Low
Other drugs-induced thyrotoxicosis (thalidomide, alemtuzumab, ipilimumab, lithium, HAART)	Negative TSHR autoantibodies	Low
Thyrotoxicosis of non-thyroidal origin		
Factitious thyrotoxicosis History	Low serum thyroglobulin	Low
Dermoid tumors (struma ovarii)	Abdominal RAIU	Low
Metastatic differentiated thyroid cancer	Bone RAIU	Low

The systemic manifestations of thyroid hormone excess are similar despite the underlying pathology, only the rapidity of occurrence (rapid onset in infective and inflammatory pathologies affecting the thyroid gland), age of presentation (older age in toxic MNG), and specific symptomatology (Graves' – ophthalmopathy, dermopathy, acropachy) differs across various causes. Systemic effects are summarized in table 1.

Table 2: Systemic Effects of thyrotoxicosis

System	**Effects**
General	Heat intolerance, weight loss, fatigue, insomnia, nervousness, tremulousness
Skin	Fine, warm, and moist, hyperpigmentation, hyperhidrosis, onycholysis, fine and often straight hair, urticaria, pruritus

Eye	Exophthalmos, lid edema, lid lag, globe lag, chemosis, ophthalmoplegia, optic nerve involvement
Mental	Irritability, restlessness, anxiety, inability to concentrate, lability, depression, psychiatric reactions
Neurological	Syncope, delirium, stupor, coma, choreoathetosis
Neuromuscular	Tremulousness, hypermetric reflexes, weakness of proximal muscles, muscle atrophy, myopathy, periodic paralysis
Cardiovascular	Tachycardia, widened pulse pressure, and bounding pulse. Occasionally cardiomegaly, congestive heart failure, angina pectoris, and paroxysmal tachycardia or atrial fibrillation
Respiratory	Dyspnoea
Gastrointestinal	Hyperphagia, increased thirst, diarrhoea, or increased frequency of stools, hepatomegaly
Metabolic	Hypercalcemia, decreased serum magnesium, increased bone alkaline phosphatase, hypercalciuria
Osseous	Osteopenia or osteoporosis
Reproductive	Irregular menses or amenorrhoea, gynaecomastia, decreased fertility
Hematopoietic	Anemia (usually normochromic, normocytic), lymphocytosis, splenomegaly, lymphadenopathy, enlarged thymus

Hyperthyroidism and Bone

The effects of thyroid hormone excess on the skeletal system are often overlooked because other signs and symptoms dominate the clinical picture. The earliest report of hyperthyroid bone disease was published in 1891 by Von Recklinghausen, who described the “worm-eaten appearance of long bones” in a young woman who died of hyperthyroidism. Plummer in 1920 published similar reports. With the advent of Anti-thyroid drugs (ATDs) in the 1940s, the clinically apparent hyperthyroid bone disease became less common. However, the effect of hyperthyroidism on bone persisted in patients, though reduced significantly compared to patients without any treatment, posing an increased fracture risk compared to controls, as demonstrated in their study by Wejda et al. in 1995. (21)

Physiopathology of Thyroid Effect on Bone Metabolism

The bone remodeling cycle is crucial for bone changes in thyroid pathology. The bone undergoes a continuous bone formation and resorption process throughout its lifetime called the bone remodeling cycle. The bone remodeling process is started by osteoclasts, which are osteoblast-derived cells. They are connected by a dendritic network and initiate the bone resorption performed by osteoclasts. After the osteoclasts finish the osteolytic process, osteoblasts function in bone formation at the site. Apart from local factors, the bone remodeling process is regulated by systemic factors such as calcitonin, parathyroid hormone, vitamin D3, estrogen, thyroid hormone, glucocorticoids, and growth hormones (22).

Thyroid hormones and TSH

The T3 hormone acts on TRα receptors present in both osteoblasts and osteoclasts. On osteoblasts, it is considered to increase osteoblast formation and bone formation, while on osteoclasts, it could increase osteoclast formation and the bone resorption process. It is unclear whether T3 acts on osteoclasts directly or indirectly using the osteoprotegerin/receptor activator of the nuclear factor kappa-B ligand (OPG/RANKL) pathway (Figure 1). The pituitary gland can also act directly on the bone cells by thyroid-stimulating hormone (TSH) action on the TSH receptor (TSHR) found in both osteoblasts and osteoclasts, similar to T3 (23,24).

This may be the consequence of the stimulatory effect of thyroid hormones on bone turn-over (4). The increased level of thyroid hormones in hyperthyroidism causes a reversible bone loss due to an expansion of the remodelling space, an irreversible loss due to a negative net bone balance, and eventually an increased risk of trabecular perforations (25).

TSH receptor antibody (TRAb)

The direct effects of TSH on osteoblastic bone formation and osteoclastic bone resorption are mediated by the TSH receptor on osteoblast and osteoclast precursors. In this way, the receptor antibodies could play a direct role in BMD by a similar mechanism to TSH. There have been various studies on the effect of TRAb on bone turn-over, as summarized below:

a. In the study by Majima et al., they found that higher serum TSH receptor stimulating (TSHR-S) antibodies significantly correlated with a reduction in BMD at the distal radius in male patients with untreated Graves' disease. In addition, higher TSHR-S antibody significantly correlated with higher urinary N-terminal telopeptide of type I collagen (26).
b. The study by Ercolano A. et al. demonstrated the negative correlation of the Z-score of lumbar spine BMD with TRAb (27).
c. A study by Greenspan et al. suggests that past history of previously treated Graves' disease is responsible for bone loss in women receiving long-term levothyroxine therapy. This also indicates some autoimmune effects of TRAb on bone metabolism (28).

There are different sites of action for TSH and antibodies on the TSH receptor; thus, the TSHR-blocking antibodies do not allow the stimulatory action of TSH on osteoblast (29).

It is known that the disappearance of TRAb in serum comes gradually over a considerable period of time and much later than attaining euthyroid status. A differential higher risk of fracture in such patients treated with radioiodine versus those treated with surgery also supports this hypothesis of TRAb being involved in bone metabolism, considering that surgical treatment of Graves' disease is associated with less pronounced or shorter duration of elevated TRAb levels in contrast to radioiodine therapy, where higher TRAb levels persist longer (30).

Interleukin-6

Increased serum interleukin-6 (IL-6) concentrations in hyperthyroid patients may also play a role in thyroid hormone-stimulated bone loss. High intrathyroidal IL-6 production has been postulated to be caused by TRAb-mediated stimulation of follicular thyroid cells. This increase in intrathyroidal IL-6 causes increased HLA class 1 and aberrant expression of HLA class 2. This expression appears to be essential for antigen presentation to autoreactive T-cells, a phenomenon relevant for the initiation and/or perpetuation of intrathyroidal autoimmune reactions. The cytokine might stimulate intrathyroidal autoantibody production by the induction of terminal differentiation of lymphocytes (31).

IL-6 is produced by bone cells, primarily by osteoblasts. IL-6 stimulates bone resorption by enhancing osteoclast proliferation and differentiation. In vitro, thyroid hormones do not stimulate IL-6 production directly in fetal rat limb bones. However, in the presence of physiological concentrations of thyroid hormones, the IL-1-stimulates IL-6 response and bone resorption is significantly increased in these cultures (32).

IL-6 has been suggested to serve as a paracrine mediator of estrogen action on bone cells. Estrogen withdrawal induces elevated IL-6 production in murine bone cells. Estrogen also inhibits IL-6 production in human osteoblastic cells (33). This effect seems to be mediated by an inhibitory effect of estrogen on the IL-6 gene promoter. Thus, the decline in levels of estrogen in post-menopausal females might explain IL-6-mediated bone loss. Interleukin-6 also stimulates osteoclast production and may affect the action of parathyroid hormone (PTH) on bone.

In the above context, Lakatos P. et al., in 1996, published the possible involvement of interleukin-6 (IL-6) in the bone loss of hyperthyroidism. The relationships between thyroid status, biochemical and densitometric parameters of bone metabolism and IL-6 were studied in female subjects. Patients with hyperthyroidism caused by either toxic nodular goiter or Graves' disease had significantly higher serum IL-6 concentrations than normal controls (6). A subgroup of hyperthyroid patients with the lowest BMC (values more than 1 SD below normal age-matched controls) also had serum IL-6 concentrations significantly greater than those of hyperthyroid patients showing less reduction of BMC. The correlations observed in that study supported the possibility that IL-6 plays a role in mediating the bone loss that results from excess thyroid hormone (34).

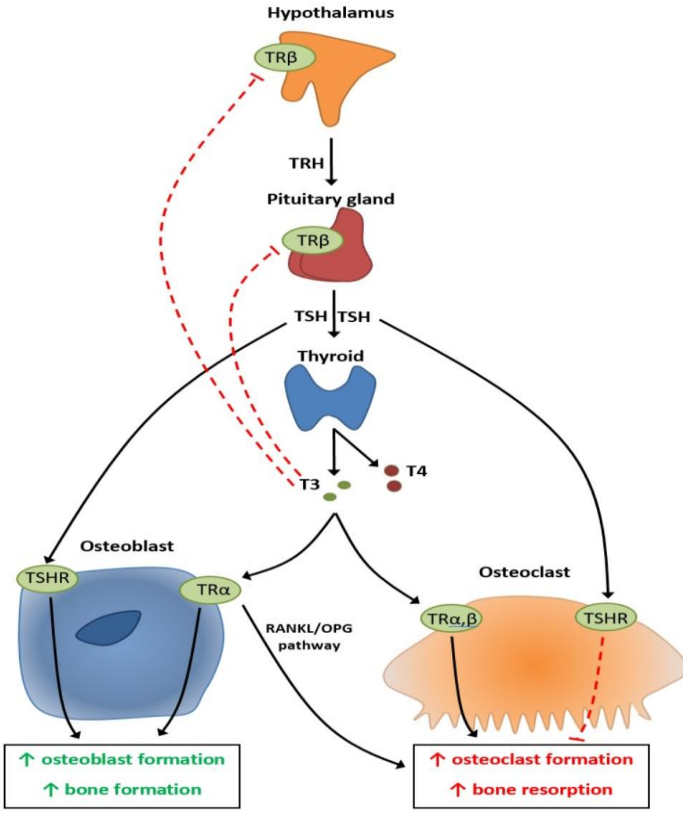


Figure 1: Physiopathology of the effect of hypothalamic–pituitary–thyroid axis on bone metabolism (35).

OPG = osteoprotegerin; RANKL = receptor activator of nuclear factor kappa-B ligand; TRH = thyrotropin-releasing hormone; TSH = thyroid-stimulating hormone; TSHR = thyroid-stimulating hormone receptor.

Bone Mineral Density in Hyperthyroidism

In the typical bone remodeling sequence, osteoclastic resorption and osteoblastic bone formation are synchronized. In overt hyperthyroidism, osteoclastic resorption is stimulated out of proportion to osteoblastic remineralization. As a result, the typical cycle duration of approximately 200 days is reduced to 100 days. Thus, each cycle is associated with a 9.6 percent loss of mineralized bone. Therefore, lower bone mass is found in hyperthyroidism, leading to an increased risk of fractures (24,36).

With the introduction of measures of bone mineral density, like photon absorptiometry in the 1970s – 1980s and DXA in 1991, BMD measurement in hyperthyroid patients has gained interest (37). Vertebral fractures are among the most common fractures in osteoporosis. Their diagnosis is essential because they

usually remain unnoticed until they lead to significant morbidity and predict future fragility fractures all over the skeleton (38).

The extent of reduction in bone density in hyperthyroid patients ranges from 10 to 20 percent. In contrast, the studies examining bone density changes after hyperthyroidism treatment have yielded variable results, ascertaining incomplete recovery in bone mineral density of 3.7 to 6.6 percent after one year of treatment (39,40).

Table 3 : Studies comparing BMD in hyperthyroid individuals with matched healthy controls.

Author, site of study	**Methodology**	**Inference**
Krolner B et al., 1983, Denmark (40)	25 hyperthyroid patients vs. 27 euthyroid controls, observed bone mineral content with photon absorptiometry at baseline and 12 months after treatment completion	Median lumbar BMC in patients with thyrotoxicosis was 12.6% ($p < 0.05$) lower than that of normal individuals before the beginning of treatment. The median gain in bone mineral density after one year was 3.7% ($p < 0.01$).
Tsai KS et al., 1991, China (41)	They compared the effects of prolonged thyrotoxicosis on BMD on 24 untreated patients with symptoms of thyrotoxicosis for at least one year with 116 healthy women as normal controls.	BMDs of thyrotoxic patients for the whole-body skeleton, lumbar spine, femoral neck, greater trochanter, and Ward's triangle were all significantly lower than those of normal controls.
Diamond T et al., 1994, Australia (39)	15 Graves patients (6 premenopausal + 9 postmenopausal) Vs. 15 healthy matched (age, sex, and menopausal status)	Significant reduction in BMD at spine and femoral greater trochanter, with significant improvement in BMD at spine at 12 months follow-up (6.6 % in the first year)

Grant DJ et al., 1995, UK (42)	Compared BMD of treated 106 post-menopausal women (with a previous history of hyperthyroidism) with 102 post-menopausal controls	Post-menopausal women with previous hyperthyroidism treated with radioiodine have reduced BMD.
Udayakumar N et al., 2006, India (43)	Investigated 50 patients with thyrotoxicosis for three-year to compare BMD before and one year after completion of treatment and compared BMD with age-matched controls.	29.8% decline in BMD was observed in thyrotoxic patients, with a 4% BMD increase found in 1-year post-treatment.
Tsevis K et al., 2018, Greece (44)	Compared BMD at spine in 32 hyperthyroid patients with 123 age, BMI matched controls	16.5% reduction in spine was noted.

Trabecular Bone Score in Hyperthyroidism

The trabecular bone score (TBS) is a relatively recent and indirect index of bone micro-architecture that may improve the prediction of fracture risk in osteoporosis and some metabolic bone diseases (table 5).

TBS is determined by constructing a variogram of the projected image (containing the region of interest) and computing the sum of the square of gray-level differences between pixels at a specific distance (figure 2), followed by calculating the slope of the "log-log transform" of this variogram (45). The TBS software (TBS iNsight; Medimaps Group, Geneva, Switzerland) generates results (unitless) for the whole LS (L1 to L4) and each vertebra using the same region of interest as for BMD. Vertebrae excluded from BMD calculation (fractures or osteoarthritis) may also be excluded from the TBS analysis.

As TBS is only a software-derived result of the BMD spine DXA image, TBS can be obtained regardless of the timing of obtaining the image. Thus, TBS can be readily applied to any available DXA image obtained from a GE Lunar (Prodigy and iDXA; Madison, WI, USA) or Hologic (Delphi, QDR 4500, and Discovery; Waltham, MA,

USA) densitometers (46), though inter-machine results may vary depending upon the variation in BMD results.

An important point worth considering is that TBS does not directly assess bone micro-architecture as DXA lacks the resolution to detect the trabeculae of the bones. TBS was validated using two-dimensional projections of three-dimensional micro-CT images of human cadaveric bone specimens (47). Significant correlations were found between TBS and bone volume fraction, trabecular spacing, and the number of trabeculae obtained using cadaveric vertebra, femoral neck, and distal radius samples. Eventually, the technique was extended to DXA images acquired ex vivo in cadaveric vertebrae, and significant correlations were found between trabecular indices obtained by micro CT and TBS; notably, these results were independent of the patient's BMD (48).

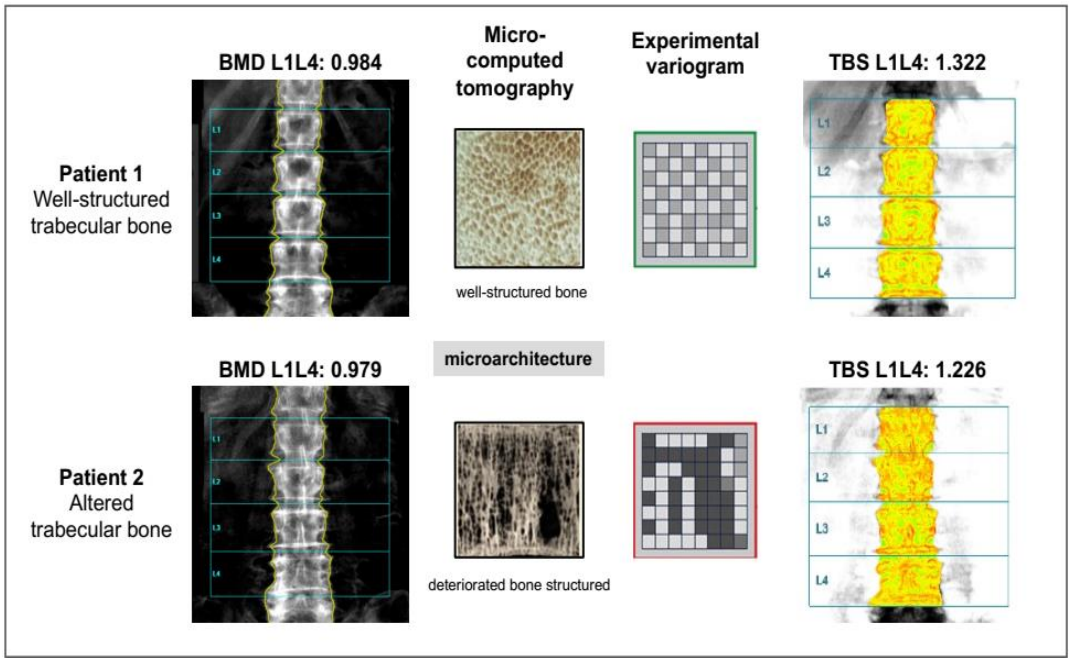


Figure 2: Example of two different patients with equivalent BMD but different TBS (46)

Higher scores in TBS reflect stronger and more fracture-resistant micro-architecture, whereas lower scores indicate bone that is weaker and more susceptible to fracture. It was shown that TBS is a qualitative marker of bone health, and it may detect differences between DXA scans that show identical BMD quantifications. It has been proposed: TBS ≥1.350 is considered normal; while TBS between 1.200 and 1.350 is considered to be partially degraded micro-architecture; and TBS ≤1.200 as degraded micro-architecture. A working group of TBS users established these cut-off points

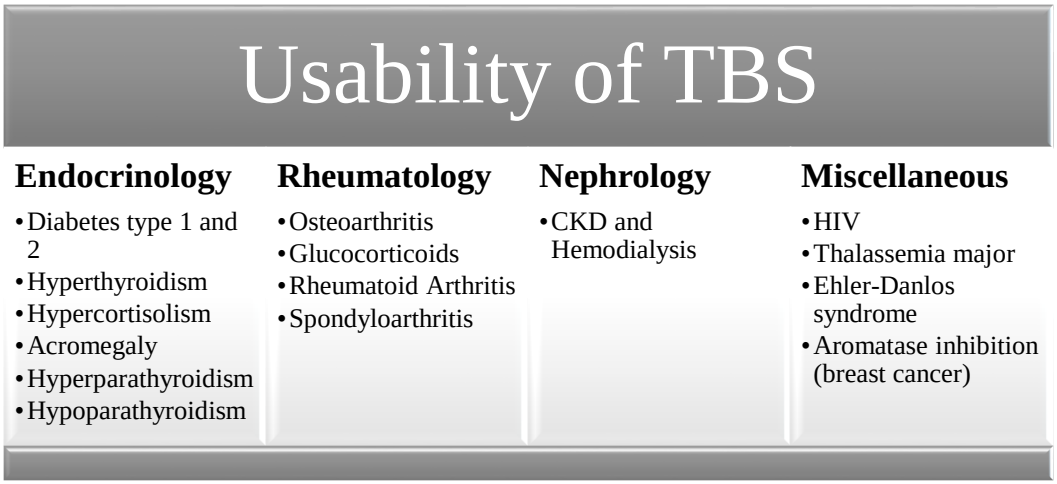
from different countries by analogy with the three BMD categories, i.e., normal bone mass, osteopenia, and osteoporosis (49).

Table 4: Interpretation of TBS score compared to BMD values and diagnosis (50)

Bone Mineral Density	Trabecular Bone Score
Normal, T score ≥ -1.0	Normal, TBS ≥ 1.350
Osteopenia, -1.0 < T score < -2.50	Partially degraded, 1.200 < TBS < 1.350
Osteoporosis, T score ≤ -2.50	Degraded, TBS ≤ 1.200

So, in addition to clinical risk factors and BMD, TBS can be even more sensitive than BMD or add value to BMD in identifying secondary osteoporosis, like in cases of hyperparathyroidism, adrenal adenomas, and other iatrogenic causes(15). Clinical studies with TBS data are very scarce worldwide in hyperthyroidism and even rarer in India (26–28).

Table 5: List of special conditions (causing secondary osteoporosis) known to increase fracture risk with TBS adding to clinical value.



Limitations of TBS: Technical limitations

1. Both bone and soft tissue absorb X-rays. Thus, an increase in the amount of soft tissue can interfere with the TBS analysis as it does with accurate acquisition of DXA images, referred to as “noise”.

2. The adjustment in TBS for BMI is only optimized when BMI ranges from 15 to 35 kg/m2. Thus, the results of TBS are not validated in individuals with a BMI beyond these limits.
3. TBS results are not comparable across different make and model of DXA machines.
4. TBS value is a software-generated interpretation of a DXA image. Thus, TBS depends on the quality of the DXA acquisition. Its clinical utility is valid only when DXA is performed with quality-control safeguards. As for now, TBS done individually (without BMD spine) is not validated, as no phantom for TBS standardization is available yet.

Limitations of TBS: Clinical Limitations

1. The clinical practice lacks validated TBS cut-off points that defines normal and abnormal TBS values across a wide population. The TBS reference range proposed so far applies to postmenopausal women only, and a large population study would be required to determine the optimal ranges across age and sex.
2. The use of TBS in individuals with BMI below 15 kg/m2 & over 35 kg/m2 has not yet been validated.
3. Finally, although TBS highly correlates with mCT in ex vivo studies, studies in vivo have shown only moderate correlations.
4. There is a lack of association between TBS and trabecular thickness, indicating that TBS does not fully depict trabecular micro-architecture as assessed by higher-resolution imaging modalities (50).

Table 6: Studies assessing TBS in patients with hyperthyroidism and their inferences.

Author, site of study	**Methodology**	**Inference**
Ock SY et al., 2016, Korea (51)	Compared BMD and TBS in 30 Graves' disease patients (13 were premenopausal females, and 17 were male), post-treatment over a mean follow-up period was 20.7 ± 8.5 months	Both bone quality and density improved after anti-thyroid treatment in premenopausal female and male Graves' disease patients

Barbosa et al., 2017, Portugal (53)	Compared TBS and BMD in hyperthyroid patients with age and stature matched control in men > 50ys. Sample size: 41 + 41 controls	Significant reduction in BMD at distal 1/3 radius. No significant change in TBS was noted.
Kuzma et al., 2018, Slovakia (52)	Compared TBS and fractalkine amongst active, cured graves with control (age, gender, and BMI matched) in premenopausal women. Sample size: 37 (21+16), 23 (controls)	Significant reduction in BMD at spine, TBS, and fractalkine levels.
Barbosa et al., 2020, Portugal (54)	Compared TBS and BMD in hyperthyroid patients with age and stature-matched control in premenopausal women. Sample size: 40 and 40 controls.	Significant reduction in BMD at the total hip, femoral neck, whole body, and TBS.

Fracture risk in Hyperthyroidism

A history of overt hyperthyroidism is a risk factor for hip fracture later in life, which is one of the causes of excess late mortality in previously hyperthyroid patients, which was demonstrated in a retrospective case-control study by Wejda B et al., 1995. They compared the incidence of hyperthyroidism (treated or untreated) in post-menopausal women with matched controls. Hyperthyroidism was seen 2.5-fold more often in hip fracture patients than in controls (55). In a retrospective study of 621 patients treated for hyperthyroidism with radioiodine, the risk of the spine (RR = 8.9, 95% CI: 1.6-48.4) and forearm fractures (RR = 3.1, 95% CI: 1.6-6.2) was found to be increased as compared with age-matched healthy controls (56). It is, therefore, reasonable to assume that in some hyperthyroid patients, bone density does not return to normal after antithyroid treatment.

The impact of low serum TSH concentrations on fracture risk was investigated in a prospective cohort study of 686 white women over age 65 years, followed by a mean of 3.7 yr. Women with serum TSH concentrations of 0.1 mU/l or less at baseline were at increased risk for hip and vertebral fractures (relative risk 3.6 and 4.5,

respectively). History of hyperthyroidism was a risk factor for hip fracture, even after adjustment for serum TSH concentration and bone mineral density (57).

Bone turn-over markers in Hyperthyroidism

The increased bone turn-over seen in hyperthyroid patients, is reflected in high levels of bone turn-over markers (BTMs). BTMs studied in hyperthyroid patients include ALP, osteocalcin, urinary deoxypyridinoline, PINP, CTx, fractalkine, etc. All BTMs tend to rise in the hyperthyroid phase and subsequently decline once euthyroidism is achieved, apart from ALP, which remains elevated for about a month.

Procollagen type 1 N-terminal Propeptide (PINP)

Type 1 collagen in the organic bone matrix (> 90%) is formed in the bone from procollagen type 1. Procollagen type 1 is synthesized by fibroblasts and osteoblasts. Procollagen type 1 has N-terminal and C-terminal extensions, which are removed by specific proteases during the conversion of procollagen to collagen. The procollagen type 1, including P1CP and PINP, subsequently conjugates onto the bone matrix (produced in equimolar concentrations). The bone formation biomarker of PINP is a specific indicator of type 1 collagen deposition. PINP has been demonstrated to be a more sensitive bone biomarker to measure the bone formation rate in osteoporosis (58). Both sub-clinical and overt hyperthyroidism has been associated with increased PINP levels (52,59).

C-terminal telopeptide of type I collagen

The CTX-I is a product of the breakdown of type I collagen-containing pyridinium cross-links. Serum levels are correlated significantly with histomorphometric measures of bone resorption. CTX-1 and NTX-1 are both released during collagen degradation. Studies have shown CTX-1 to be a specific and sensitive biomarker of bone resorption that can rapidly indicate the response to bisphosphonate therapy for postmenopausal osteoporosis (58). Increased levels of CTX have also been noted in patients with an active hyperthyroid state (52).

In summary, hyperthyroidism affects bone health, more so in females than in males, which is multifactorial, but the data available on risk factors (markers of disease activity), leading to altered bone density and microstructure, still needs to be elaborated.

AIMS AND OBJECTIVES

Aim:

To assess Bone Mineral Density (BMD) and bone quality (TBS) in patients with hyperthyroidism and to determine risk factors for alterations in BMD and TBS in this group of patients.

Objectives :

Primary Objective:

- To compare BMD and TBS in patients with hyperthyroidism (Graves, toxic multinodular goiter, toxic adenoma) with a matched control group.

Secondary Objectives:

To correlate BMD and TBS in hyperthyroid patients with :

- Thyroid hormone levels, TRAb levels and IL-6 levels.
- Thyroid uptake and size - Nuclear uptake scan (Tc99m uptake, RAI Uptake), USG Thyroid
- Bone turn-over markers (PINP and CTX)
- Clinical parameters (duration of disease, duration in hyperthyroid phase, cumulative dose of ATDs)

MATERIALS AND METHODS

Study Setting:

This was an observational case-control study among patients with hyperthyroidism in the Department of Endocrinology & Metabolism at AIIMS, Jodhpur.

Study Type:

A case-control study.

Study Participants:

Patients with hyperthyroidism presenting to Endocrinology and Metabolism and referred from Internal Medicine, ENT, and General Surgery OPD at AIIMS Jodhpur.

Inclusion Criteria:

1. Age 20-70 yrs.
2. Patients presenting with hyperthyroidism (TSH below reference range, T4/FT4, T3/FT3 above reference range).
3. Nuclear scan s/o Graves/ Toxic adenoma/ Toxic MNG and/or TRAB positive (for Graves).

Exclusion Criteria:

1. Chronic Smokers[$], chronic alcoholic[#]
2. Steroid intake >5mg for >3 months.
3. Pregnancy.
4. CKD stages 3-5(60), CLD Child Pugh class C (61)
5. History of presence of Rheumatoid arthritis and other endocrinopathies (hyperparathyroidism, male hypogonadism, Cushing's disease).
6. Post-menopausal females.

> 24gm/ day for more than 10 years. (62).

$ > 20 pack years (63).

Sampling:

Hyperthyroid patients presenting to the departments of Endocrinology and Metabolism, Internal Medicine, ENT, and General Surgery at AIIMS Jodhpur fulfilling the inclusion criteria were duly explained about the study and enrolled after obtaining informed consent.

Healthy age and sex-matched controls were enrolled after considering the qualifying criteria to enter the study.

Study Duration:

18 months (From January 2021 after getting Ethical approval to June 2022)

Sample size:

- Trabecular bone score and vertebral fracture assessment in premenopausal Portuguese women with hyperthyroidism by Barbosa et al, 2020 was chosen to be the index study for sample size calculations (64).

$$\text{Number of samples per group (n)} = \frac{2\,x\,(Z_{(1-\alpha/2)}+Z_{\beta})^2 x\,\sigma^2}{\Delta^2}$$

Here:

- $Z_{(1-\alpha/2)}$ denotes Standard normal deviate at a 5 % level of significance
- Z_{β} indicates Standard normal deviate at 20% Type 2 error
- σ denotes pooled standard deviation
- $\triangle$ denotes mean difference
- The sample was calculated to be 31 subjects in both case and control arms.

Data Collection:

Subjects with hyperthyroidism were enrolled in the study after applying the inclusion and exclusion criteria. The hyperthyroid patient group, referred to as cases, included patients with suppressed TSH (<0.35), fT3/T3, fT4/T4 (above reference range), and increased uptake on the nuclear scan(Tc99m, RAI Uptake)(65). All the clinical parameters pertaining to hyperthyroidism were noted, including clinical history (patient details, geographical location of primary stay, use of iodine-fortified salt, symptoms leading to diagnosis, duration of symptoms, clinical course of disease,

family history, drug history, history of substance abuse, treatment history), anthropometric measurements and clinical examination (BP, PR, presence of dysrhythmia, presence of goiter, tremors, lid retraction, gynecomastia, warm or moist skin). Clinical indices of disease activity were quantified as follows:

- **Time to diagnosis (TD) –** duration(months) of hyperthyroid symptoms, after which biochemical evidence is confirmed.
- **Time in hyperthyroid state (THS) –** duration(months) TFTs remain suggestive of hyperthyroidism till BMD evaluation.
- **Time to normalization of hyperthyroid activity (TNHA)** – duration(months) from the start of ATD to biochemical euthyroidism (normal T4/fT4 and T3/fT3 levels).
- **Time to rebound of TSH (TRT)** – duration (months) from the start of ATD to TSH normalization (TSH >0.35).
- **Cumulative ATD to TRT (CAT)** - the sum of products of carbimazole equivalent doses (mg) and duration of the dose given (months) till euthyroidism is achieved.
- **Cumulative ATD Index (CAI)** – the sum of products of carbimazole equivalent doses (mg) and duration of the dose given (months) till BMD evaluation.

Carbimazole equivalence - 40 CBZ = 30 MMI = 400 PTU

The biochemical battery of tests were done to confirm the diagnosis of active hyperthyroidism and related complications and to rule out other known causes of osteoporosis.

Biochemical and radiological tests done in the cases included:

- Thyroid profile
- Complete Hemogram
- LFT, KFT
- Bone-Mineral Panel (Ca/PO_4/ALP/25 (OH) D, iPTH)
- TRAB (if feasible)
- IL -6
- Bone Markers
- Tc99m pertechnetate uptake

- RAI uptake study
- USG thyroid

Biochemical and radiological tests in controls included bone turn-over markers and BMD by DXA scan.

Then the cases and controls were evaluated for Bone Mineral Density in both Axial (AP Lumbar spine), hip joint, and distal 33% radius (Non-dominant arm), Trabecular Bone Score (TBS) by Hologic Horizon-A DXA scanner (using APEX^{TM} QDR^{TM} and TBS iNsight software v3.1.1) and interpreted with similar age and sex-matched controls fulfilling inclusion criteria.

Figure 3: Hologic Horizon-A DXA scanner

PINP levels were done using Human PINP(Procollagen I N-Terminal Propeptide) ELISA Kit by Elabscience® (E-EL-H0185). The kit had a sensitivity of 9.38 pg/ml with a detection range of 15.53 – 1000 pg/ml. The kit had a coefficient of variation

of < 10% with no significant cross-reactivity or interference between Human PINP and analogs.

CTX levels were done using Human CTXI(Cross Linked C-telopeptide of Type I Collagen) ELISA Kit by Elabscience® (E-EL-H0835). The kit had a sensitivity of 0.10 ng/ml with a detection range of 0.16 – 10 ng/ml. The kit had a coefficient of variation of < 10% with no significant cross-reactivity or interference between Human PINP and analogs.

Study Flow:

Figure 4: Study flow of Cases

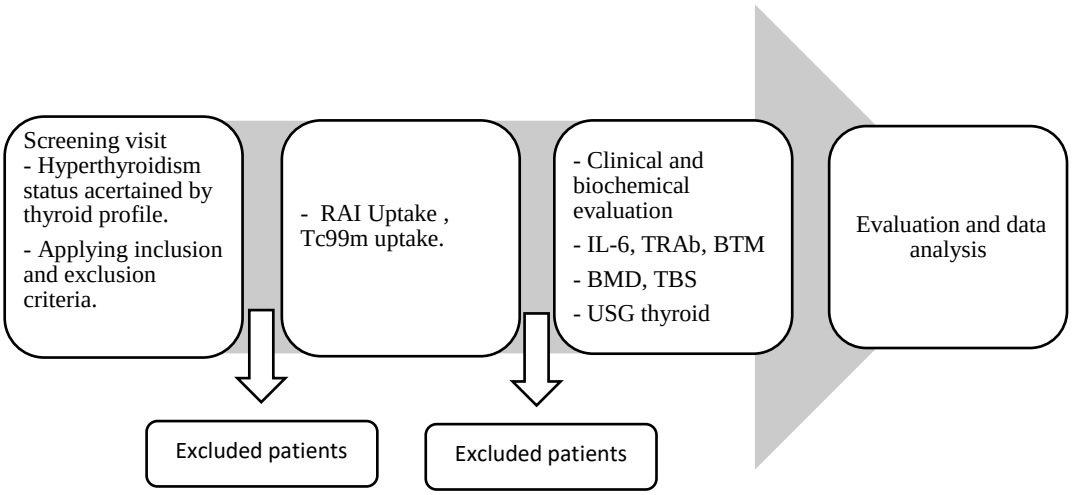


Figure 5: Nuclear scans procedure in Cases

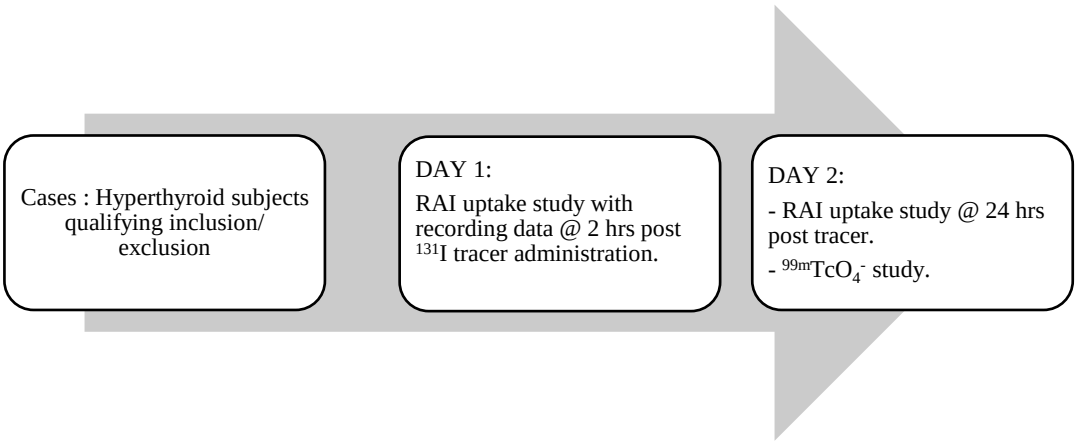
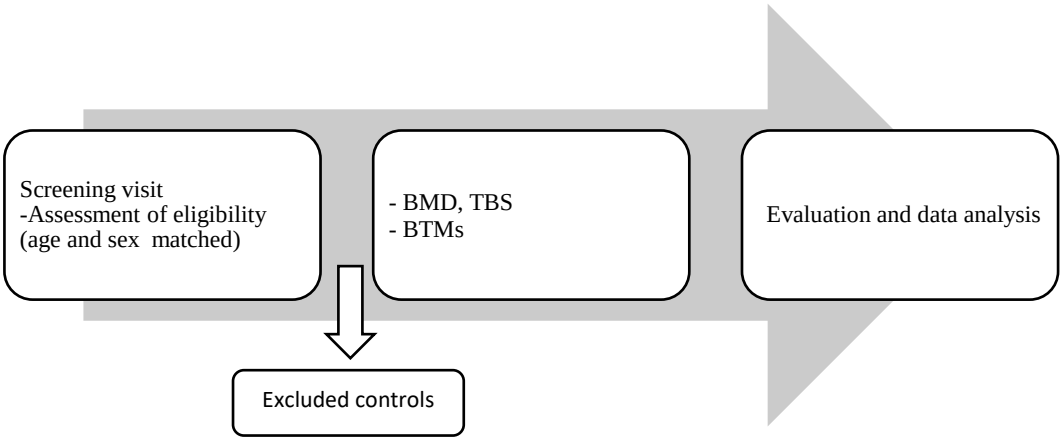


Figure 6: Study flow of Controls



Statistical analysis

Data are expressed as mean ± standard deviation or median with IQR(Q1 – Q3). Independent Student t-test was used to compare continuous variables between two groups, ANOVA for comparison of >2 groups. The chi-square test was used to compare categorical variables. Analysis was done using SPSS version 23. Multivariate analysis for the correlation of variables was used.

RESULTS

Out of 155 screened patients with thyrotoxicosis, 54 were excluded: 34 were lost to follow-up without undergoing BMD evaluation,16 patients were post-menopausal females, 2 were pregnant females, and four were diagnosed as sub-acute thyroiditis during evaluation.

Overall, 103 patients (Cases) and age, gender, and BMI-matched healthy controls (n=40) were included in the study after obtaining informed consent. All the enrolled hyperthyroid patients (Cases) underwent a detailed history taking, clinical examination, relevant biochemical investigations, BMD, and TBS evaluation.

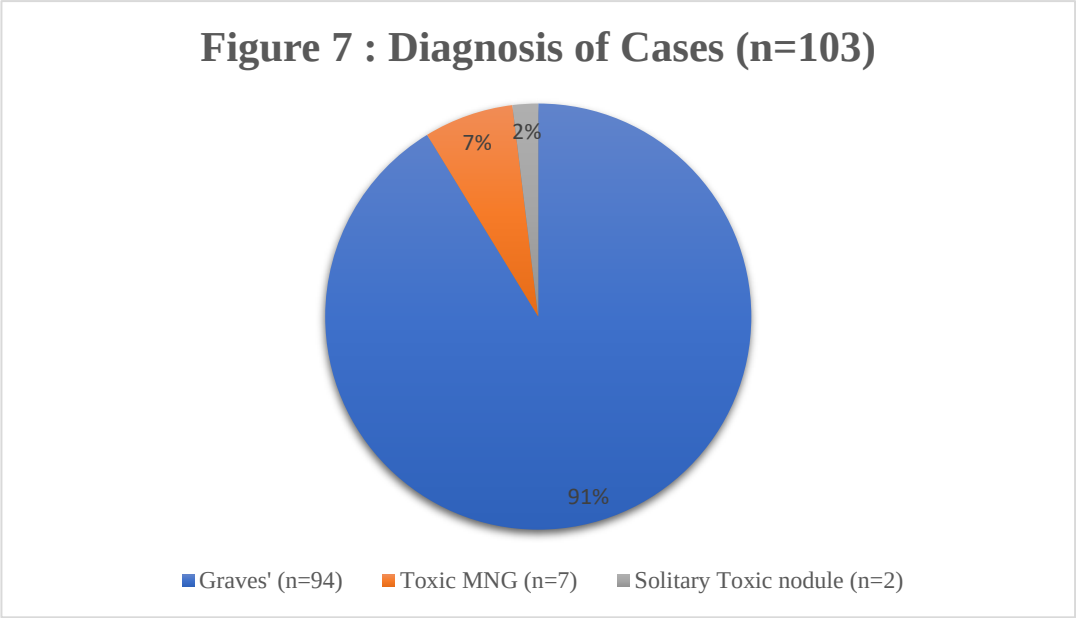
Baseline characteristics

Cases included 31.5 % (n=32) males and 68.9% (n=71) females, while the control group included 35%(n=14) males and 65% (n=26) females (p=0.69). The mean age of cases was 35.63 ± 9.7 years, while that of the control group was 32.97 ± 9.08 (p=0.135). The BMI of cases was 21.76 ± 4.62 $kg/m^{2,}$ while that of the control group was 23.11 ± 3.75 kg/m^2 (p=0.10). Therefore, cases and controls were deemed as age, sex, and BMI matched, as detailed in table 7.

Table 7 : Demographic details of Cases and Controls

Variables	**Cases (n = 103) Mean ± SD**	**Controls (n = 40) Mean ± SD**	**p value**
Age (years)	35.63 ± 9.6	32.97 ± 9.1	0.135
Females	72	25	0.69
BMI (kg/m2)	21.76 ± 4.62	23.11 ± 3.75	0.10

In the hyperthyroid cohort (cases) 12.6% (n =13) had a positive family history of hypothyroidism, while 2.9%(n = 3) had a positive family history of graves disease. 9.7%(n = 10) of the cases were active smokers, and no other addictions were found in this cohort. At the point of contact with cases in the study, 27.8%(n=29) were treatment naïve, whereas 57.7%(n=60) were on anti-thyroid drugs (ATDs).



Evaluation of cases revealed the diagnosis of 91.2%(n=94) patients as Graves' disease, 6.9%(n=7) as toxic multinodular goiter (MNG), and 1.9%(n=2) as solitary toxic adenoma, shown in figure 6. Ophthalmopathy was seen in 22.1%(n=23) of cases, while dermopathy was present in 3.9%(n=4) of cases. A detailed description of the baseline characteristics of cases has been summarised in table 8.

Table 8: Baseline characteristics of Cases

Variables	**N(%)**
Cause of hyperthyroidism - Graves - Toxic multinodular goitre - Toxic adenoma	 94(91.3) 7(6.8) 2(1.9)
Family history - Hypothyroidism - Graves	 13(12.6) 3(2.9)
Smoking	10(9.7)
Ophthalmopathy	23 (24.4)
Dermopathy	4(4.2)
Acropachy	0

The median time to diagnosis (TD) of hyperthyroidism in cases was 2 months (IQR 1-5). The median time in the hyperthyroid state (THS) was 9 months (IQR 3 – 28.5) at the point of contact in the study.

The median cumulative dose received by the cases was 170 (IQR 55 - 400). 32 patients achieved at least one event of normalization of thyroid hormone levels (both fT3/T3 and fT4/T4 within normal limits), and the median time to normalization of hyperthyroid activity (TNHA) was 14 months (IQR 6 - 59). Twenty patients achieved a rebound increase in TSH during their treatment phase; the median time to rebound TSH (TRT) was 18 months (IQR 5 – 43.5), as demonstrated in figure 7. The median cumulative ATD dose to TRT (CAT) was 210 (IQR 132.5 - 400). The clinical indices of cases are detailed in table 9.

Figure 8 : Clinical indices of cases

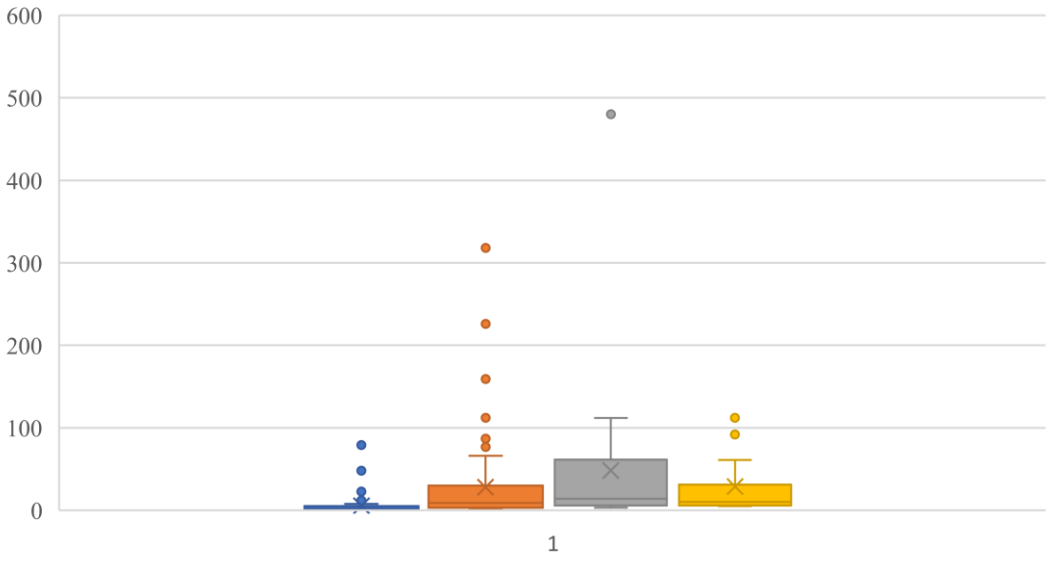


Table 9: Clinical indices of cases

Clinical Indices	**N**	**Median (IQR)**
Time to diagnosis (months) **(TD)**	103	2 (1 - 5)
Time in HTH state (months) **(THS)**	103	9 (3 – 28.5)
Time to normalization of hyperthyroid activity (months) **(TNHA)**	31	14 (6 - 59)
Time to rebound TSH **(TRT)**	27	18 (5-43.5)
Cumulative ATD index **(CAI)**	103	170 (55 - 400)
Cumulative ATD dose to **TRT (CAT)**	27	210 (132.5 - 400)

Biochemical parameters of Cases

In our study the median free T4 levels (2.36 pg/dl (IQR 1.87 – 4.1)) were 1.31 times ULN, while the median free T3 levels (6.46 ng/dl (IQR 4.79 – 12.59)) were 1.54 times ULN, with supressed median TSH levels (0.011 MIU/L (IQR 0.006 – 0.04)). The median TRAb levels were more than 5 times ULN. Other pertinent biochemical parameters in the study population have been summarised in Table 10.

Table 10: Biochemical and radiological investigations of Cases

Variables	**Median (IQR)/ N**
FT4 (pg/ml)	2.36 (1.87 – 4.1), n= 103
FT3 (ng/dl)	6.46(4.79 – 12.59), n= 103
TSH (MIU/L)	0.011 (0.006 – 0.04), n= 103
TRAB (IU/L)	9.0 (3.65 – 19.71), n= 73
IL-6 (pg/ml)	1.79 (0.8 – 3.8), n= 65
RAIU 2 HR (%)	36.2 (22.47 – 40.5), n= 55
RAIU 24 HRS (%)	64.2 (51.29 – 65.28), n= 55
THYROID VOLUME USG (cc)	14.41 (9.57 – 24.18), n= 100

Reference values: fT3: 2.2 – 4.2 ng/dL; Free T4: 0.80 - 1.80 pg/dL; TSH: 0.3 – 3.6 mIU/L; TRAb: <1.8 IU/L; IL-6: < 4.4 pg/ml

In our study we found statistically significant decrease in bone mineral density (BMD) at spine, femoral neck, total hip, distilled 1/3 radius, and whole body in cases as compared with controls. We also found significant decrease in trabecular bone scores(TBS) in cases as compared with controls. The details have been summarized in table 1.

Table 11 : Comparison BMD and TBS in Cases with Controls

Variable	**Cases (n=103)**	**Controls (n=40)**	**p value**
Spine (L1-L4)	0.91± 0.17	1.01± 0.12	0.003
Femoral neck	0.74± 0.13	0.81± 0.11	<0.001
Total hip	0.85± 0.15	0.96± 0.11	<0.001
Distal 1/3 left radius	0.65± 0.13	0.72± 0.07	<0.001
Whole body BMD	1.02± 0.13	1.10± 0.08	<0.001
TBS	1.32± 0.11	1.38± 0.06	0.002

Comparison of BMD and TBS between cases and controls has been represented in figure 8 and 9 respectively.

Figure 9 : BMD comparison Cases Vs Controls

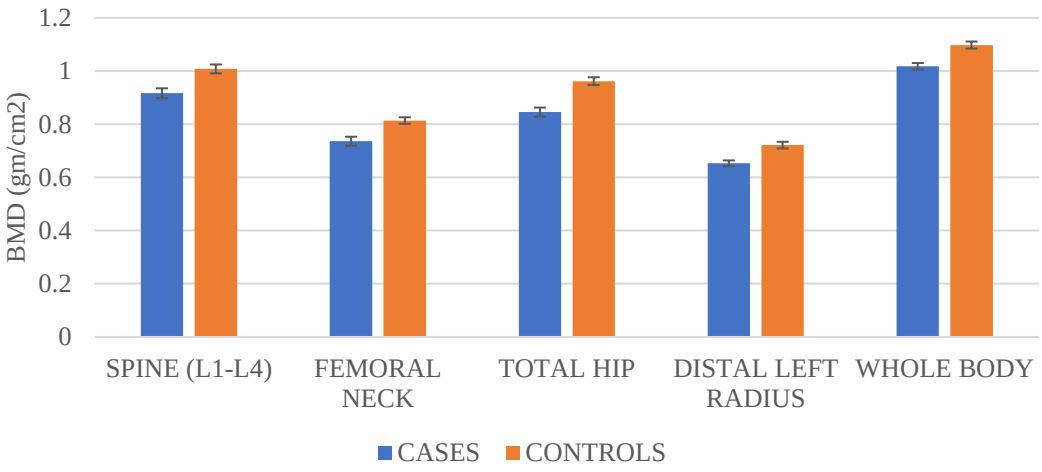
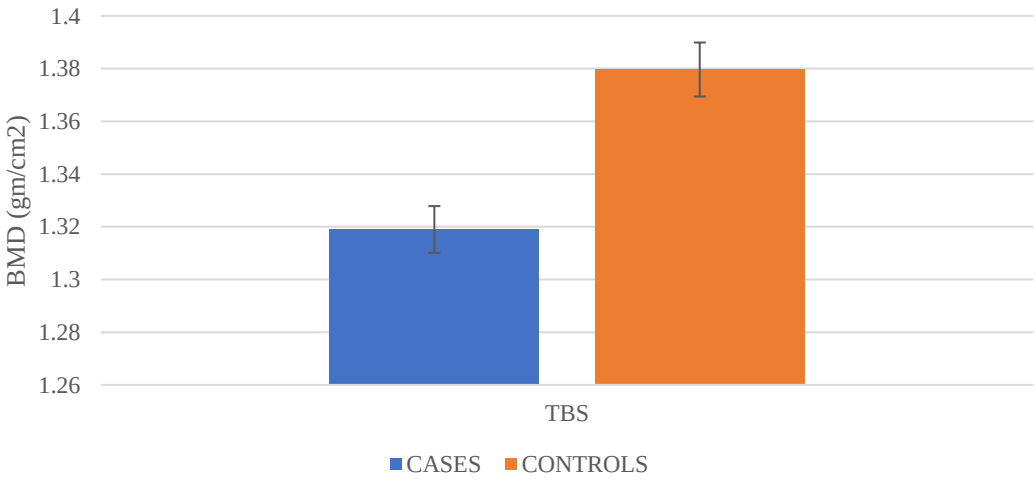


Figure 10 : TBS comparison Cases Vs Controls

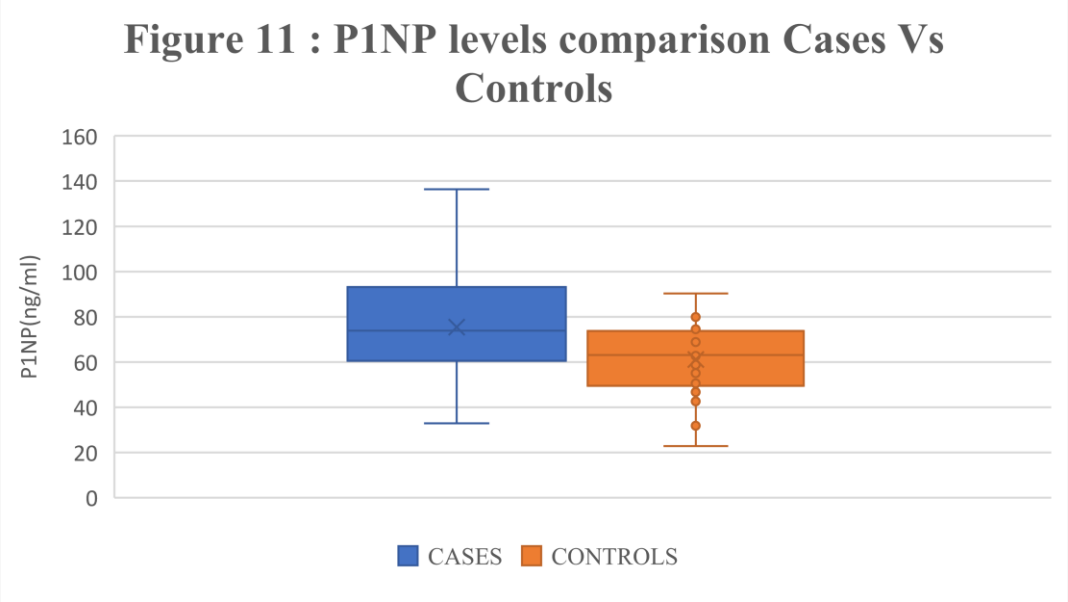


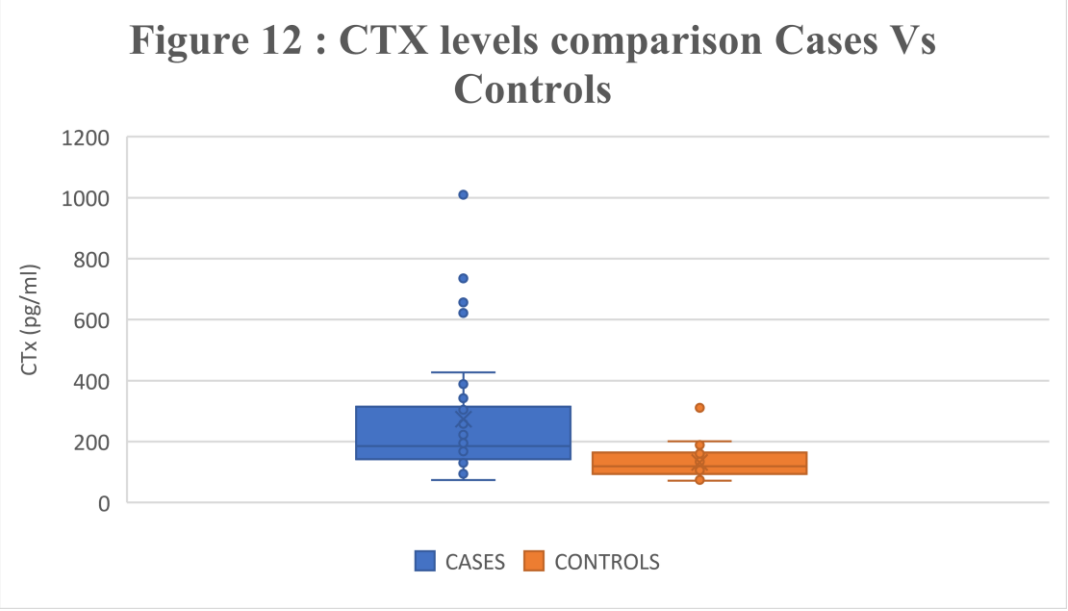
Bone turnover markers (BTMs), PINP (bone formation marker) and CTX (bone resorption marker) were found to be significantly higher in cases as compared with controls, summarized in table 12.

Table 12 : Comparison of BTMs in Cases and Controls

Variable	Cases (n=103)	Controls (n=40)	p value
PINP (ng/ml)	73.9 (IQR 61.3 – 92.3)	63.1 (IQR 49.8 – 73.1)	0.003
CTx (pg/ml)	173.5(IQR 118.3 – 271.3)	119.5(IQR 94 – 162.3)	0.001

Comparison of PINP and CTX levels in case and controls has been represented in figure 10 and 11 respectively.





Correlation of BMD parameters with biochemical and radiological parameters

We found no significant correlation between the free T3, free T4, and TSH levels in cases and the bone mineral densities at all skeletal sites, detailed in table 14. No significant correlation was found between the free T3, free T4, and TSH levels in cases and theie TBS, detailed in table 13.

Table 13: Correlation of thyroid function tests with BMD parameters in cases

Variable		Spine (L1-L4)	Femoral neck	Total hip	Distal left radius (1/3)	Whole body BMD	TBS
FT4	Pearson coefficient (ρ)	-0.004	-0.052	-0.073	-0.177	-0.034	-0.075
	p value	0.97	0.60	0.46	0.07	0.73	0.45
FT3	Pearson coefficient (ρ)	0.148	0.078	0.004	-0.028	0.094	0.066
	p value	0.136	0.43	0.96	0.77	0.34	0.51
TSH	Pearson coefficient (ρ)	0.080	0.161	0.120	0.047	-0.0023	0.022
	p value	0.423	0.104	0.226	0.631	0.981	0.828

In our study, a significant linear negative correlation was found in cases between TRAb levels and bone mineral desity at total hip, distal radius and whole body, which has been represented in figure 12, 13 and 14 respectively. No significant correlation was found between TRAb levels in cases and BMD at the lumbar spine and the femoral neck, or the TBS, detailed in table 14.

Table 14: Correlation of TRAb levels with BMD parameters in cases

Variable		Spine (L1-L4)	Femoral neck	Total hip	Distal left radius (1/3)	Whole body BMD	TBS
TRAB levels	Pearson coefficient (ρ)	-0.114	-0.183	-0.338	-0.298	-0.253	-0.148
	p value	0.334	0.121	**0.003**	**0.01**	**0.03**	0.21

Figure 13 : Correlation of Total Hip BMD with TRAb levels in cases

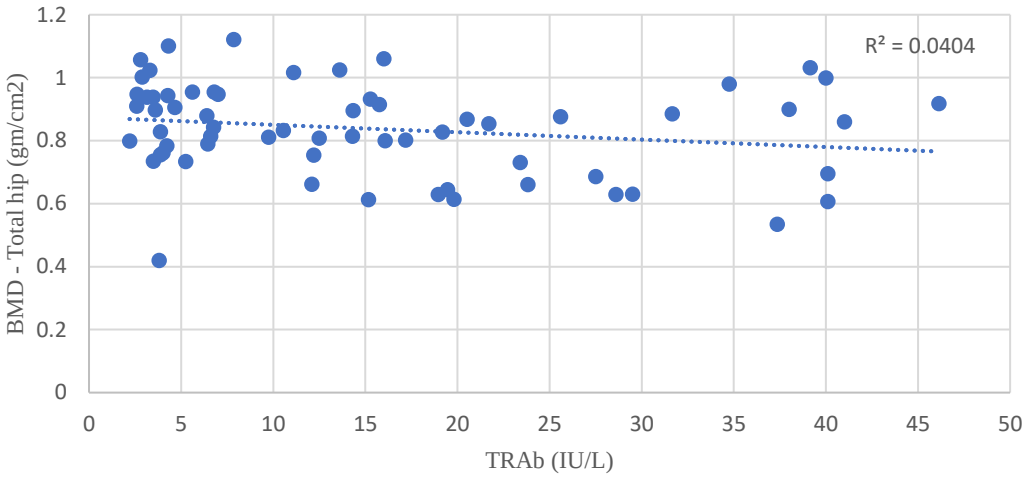


Figure 14 : Correlation of Distal 1/3 radius BMD with TRAb in cases

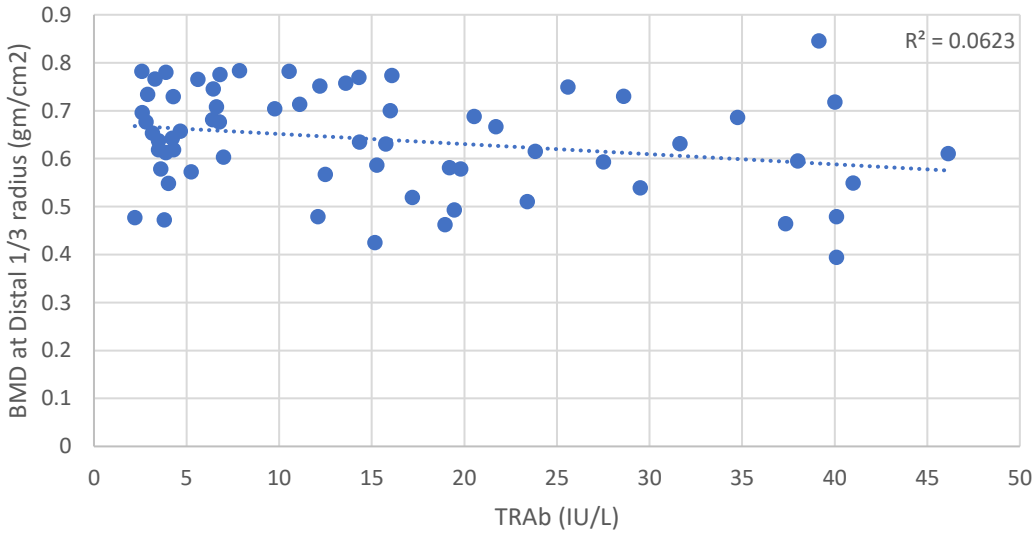
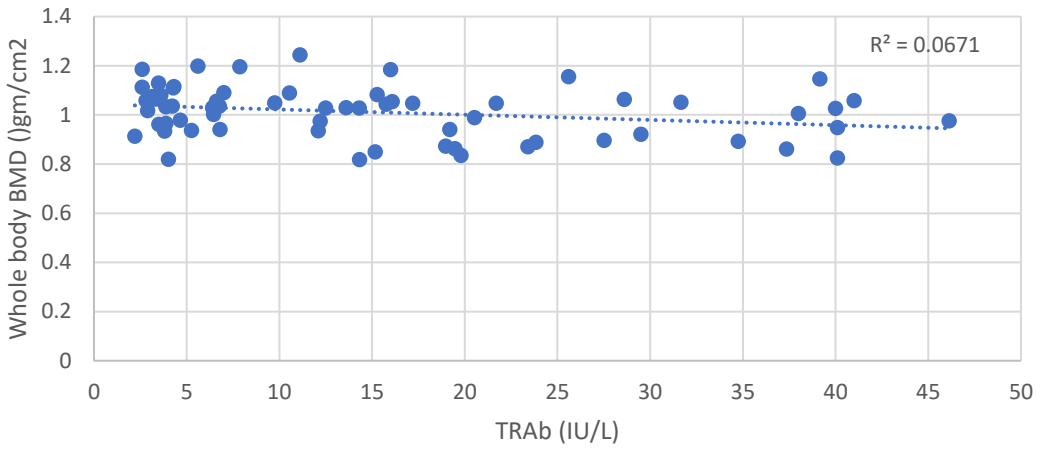


Figure 15 : Correlation of Whole body BMD with TRAb in cases



We did not find any significant correlation between the IL-6 levels in cases and the bone mineral density at all skeletal sites, as summarised in table 15.

Table 15: Correlation of IL-6 levels with BMD parameters in cases

Variables		**Spine (L1-L4)**	**Femoral neck**	**Total hip**	**Distal left radius (1/3)**	**Whole body BMD**	**TBS**
IL-6	Pearson coefficient (ρ)	0.135	0.182	0.144	0.131	0.154	0.075
	p value	0.285	0.146	0.252	0.304	0.235	0.553

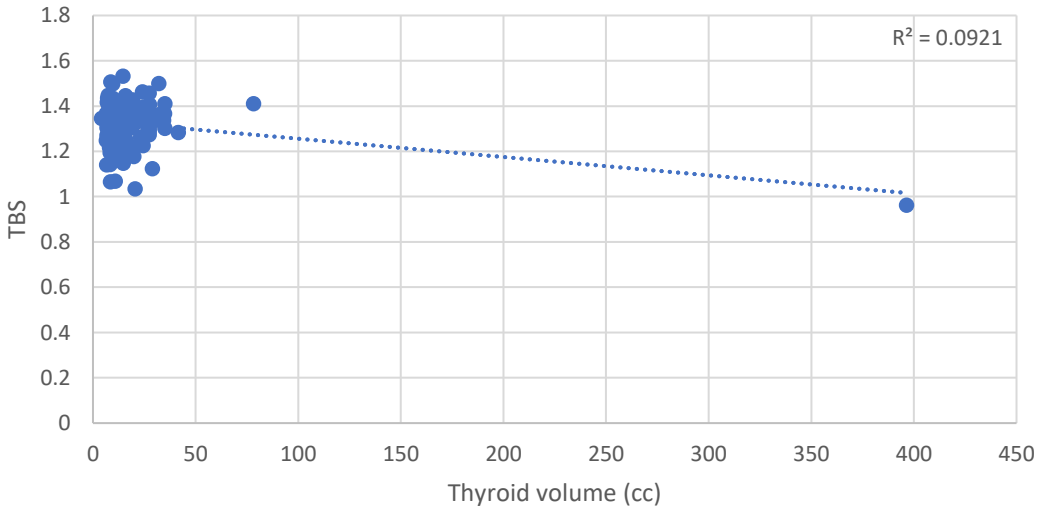
No significant correlation was found in patients with Graves' disease between RAI uptake at 2 or 24 hrs with BMD at all skeletal sites, as detailed in table 16.

We found a significant negative correlation in cases between the thyroid volume (measured by USG) and the trabecular bone score (pearson's $\rho = -0.296$, p value < 0.01), demonstrated in figure 16.

Table 16: Correlation of RAI uptake and thyroid volume with BMD parameters in cases

Variables		Spine (L1-L4)	Femoral neck	Total hip	Distal left radius (1/3)	Whole body BMD	TBS
RAIU 2hr (%)	Pearson coefficient (ρ)	0.064	0.001	0.095	0.164	-0.123	-0.118
	p value	0.641	0.999	0.495	0.237	0.382	0.390
RAIU 24hr (%)	Pearson coefficient (ρ)	0.210	0.157	0.084	-0.028	0.020	0.143
	p value	0.123	0.251	0.542	0.843	0.888	0.296
Thyroid volume	Pearson coefficient (ρ)	-0.05	0.084	0.067	-0.071	-0.079	-0.296
	p value	0.62	0.40	0.50	0.47	0.43	**<0.01**

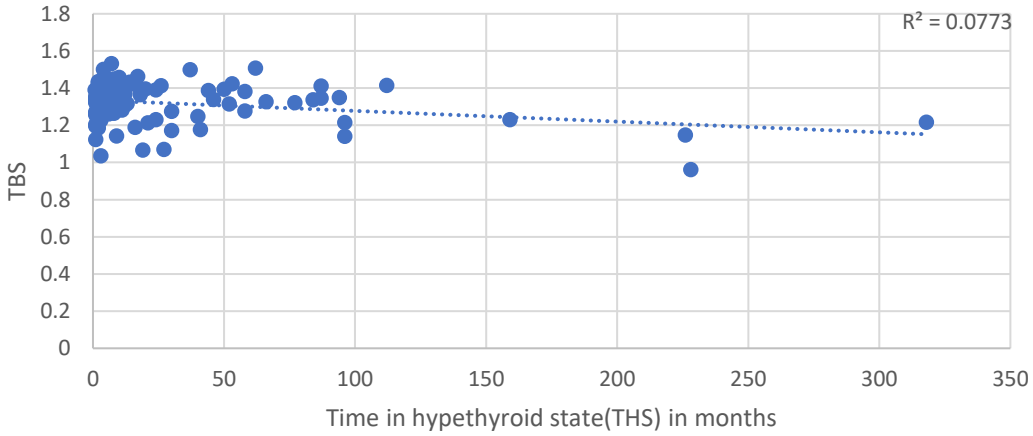
Figure 16 : Correlation of TBS with thyroid volume in cases



Correlation of BMD parameters with clinical indices

Our study found a statistically significant negative correlation in cases between the patient's time in hyperthyroid state(THS) and their trabecular bone score(TBS) (pearson's ρ = -0.278, p-value = 0.004), demonstrated in figure 11. We found no correlation between THS and BMD at the lumbar spine (Pearson's ρ = -0.02, p value = 0.82), total hip (pearson's ρ = -0.07, p value = 0.47), distal radius (pearson's ρ = -0.016, p value = 0.87) and whole body (pearson's ρ = -0.024, p value = 0.81).

Figure 17: Correlation of TBS with THS in cases



Time to diagnosis (TD) in cases did not correlate with BMD at the lumbar spine, femoral neck, total hip, whole body, distal left 1/3 radius, and the trabecular bone score(TBS), detailed in table 17.

In our study, cumulative ATD index (CAI) in cases did not correlate with BMD at all skeletal sites. CAI in cases also did not correlate significantly with the trabecular bone score(TBS) , as detailed in table 17.

We did not find any significant correlation between time to normalization of hyperthyroid activity (TNHA) in cases with BMD at all skeletal sites. TNHA in cases also did not correlate significantly with the trabecular bone score(TBS) , as detailed in table 17.

Time to rebound TSH (TRT) in cases did not correlate with BMD at all skeletal sites. TRT in cases also did not correlate significantly with the trabecular bone score(TBS), as detailed in table 17.

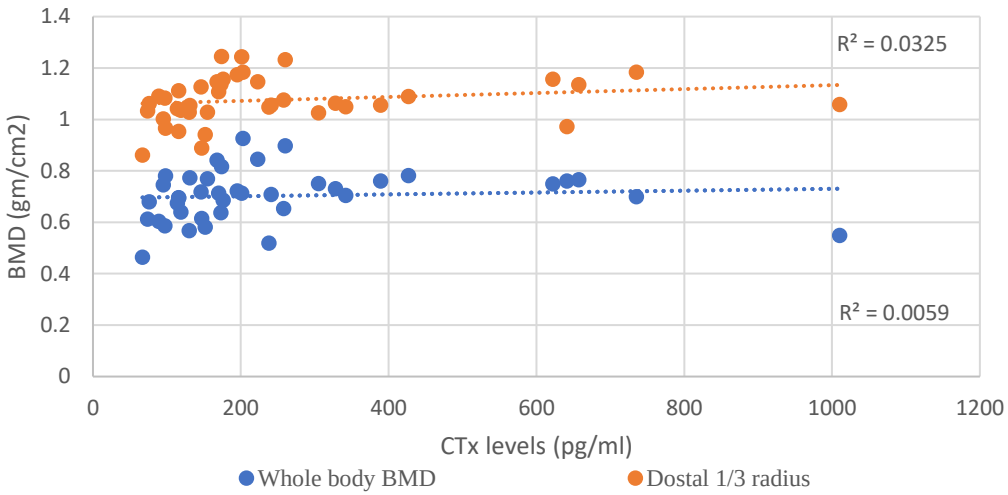
Table 17: Correlation of clinical indices with BMD parameters in cases

Variables		Spine (L1-L4)	Femoral neck	Total hip	Distal left radius (1/3)	Whole body BMD	TBS
Time to diagnosis (TD)	Pearson coefficient (ρ)	0.103	0.034	0.019	-0.003	0.014	0.142
	p value	0.29	0.73	0.84	0.97	0.88	0.15
Time in hyperthyroid state (THS)	Pearson coefficient (ρ)	-0.022	0.044	-0.07	-0.016	-0.024	-0.278
	p value	0.82	0.65	0.47	0.87	0.81	**0.004**
Cumulative ATD index (CAI)	Pearson coefficient (ρ)	0.034	-0.01	0.027	- 0.011	-0.07	-0.142
	p value	0.74	0.98	0.79	0.91	0.49	0.15
Time to normalization of hyperthyroid activity (TNHA)	Pearson coefficient (ρ), n= 31	-0.010	-0.24	-0.14	-0.162	0.057	-0.081
	p value	0.95	0.19	0.45	0.37	0.75	0.65
Time to rebound TSH (TRT)	Pearson coefficient (ρ), n= 27	0.047	0.060	-0.001	-0.065	0.074	-0.018
	p value	0.81	0.76	0.99	0.74	0.71	0.92

Correlation of BMD parameters with bone turnover markers

Amongst the bone turnover markers, the CTX levels (bone resorption marker) in cases were found to significantly correlate positively with distal 1/3 radius (Pearson's ρ = -0.341, p-value = 0.03) and whole-body BMD (Pearson's ρ = -0.345, p-value = 0.03) demonstrated in figure 17. While, no statistically significant correlation between CTX levels in cases and bone mineral density at lumbar spine, femoral neck and total hip could be established in cases. CTX levels in cases also did not correlate significantly with the trabecular bone scores.

Figure 18 :Correlation of BMD at whole body and Distal 1/3 radius with CTX in cases



We found no correlation between PINP levels in cases and BMD at various skeletal sites. No correlation was found between PINP levels in cases and trabecular bone score, as detailed in table 18.

Table 18: Correlation of bone turnover markers with BMD parameters in cases

Variables		**Spine (L1-L4)**	**Femoral neck**	**Total hip**	**Distal left radius (1/3)**	**Whole body BMD**	**TBS**
PINP	Pearson coefficient (ρ)	0.16	0.086	0.159	-0.066	0.115	-0.182
	p value	0.92	0.60	0.328	0.691	0.498	0.26
CTx	Pearson coefficient (ρ)	0.174	0.237	0.269	0.341	0.345	0.048
	p value	0.28	0.14	0.09	**0.03**	**0.03**	0.77

Correlation of bone turnover markers (BTMs) with biochemical and radiological parameters

In our study, we found no correlation between free T3, free T4 levels and TSH levels in cases with PINP levels (bone formation marker), as detailed in table 20. CTX levels in cases also did not correlate with significantly with the free T3, free T4 levels and TSH levels, as detailed in table 19.

Table 19: Correlation of thyroid function tests with bone turnover markers in cases

Variables		**PINP**	**CTX**
FT4	Pearson coefficient (ρ)	0.168	0.033
	p value	0.30	0.84
FT3	Pearson coefficient (ρ)	0.179	-0.090
	p value	0.27	0.58
TSH	Pearson coefficient (ρ)	-0.187	0.098
	p value	0.28	0.549

TRAb levels in cases were not found to correlate significantly with the PINP levels or the CTX levels in cases, as detailed in table 20.

Table 20: Correlation of TRAb levels with bone turnover markers in cases

Variables		**PINP**	**CTx**
TRAB levels	Pearson coefficient (ρ)	0.305	-0.017
	p value	0.07	0.92

In our study, IL-6 levels in cases did not correlate significantly with theie PINP levels or the CTX levels, as detailed in table 21.

Table 21: Correlation of IL-6 levels with bone turnover markers in cases

Variables		**PINP**	**CTx**
IL-6	Pearson coefficient (ρ)	0.065	0.082
	p value	0.689	0.61

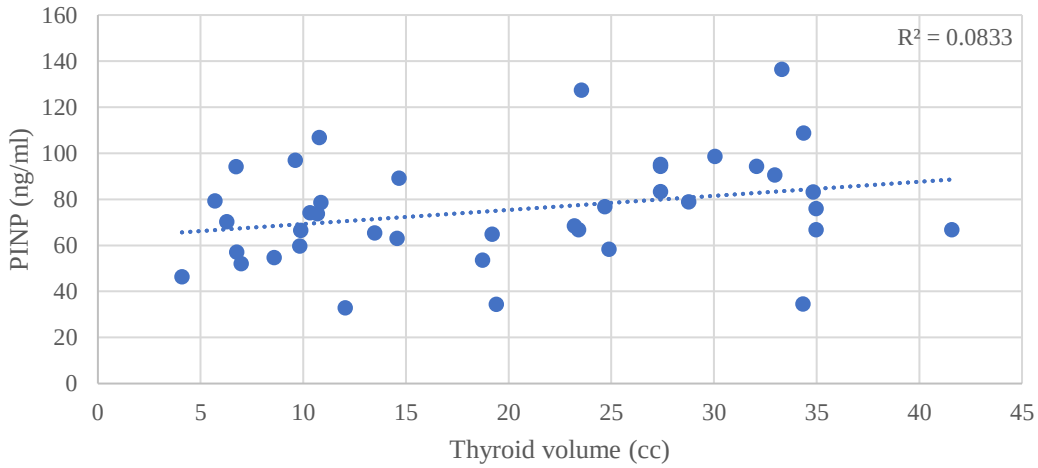
We did find any correlation in patients of Graves' disease with RAI uptake at 2 and 24 hours with PINP levels or the CTX levels, as detailed in table 23.

We found a statistically significant positive correlation (Pearson's ρ = 0.308, p-value = 0.05) between the PINP levels in cases and their thyroid volume measured by USG, detailed in table 23 and illustrated in figure 18. But no statistically significant correlation could be established between CTX levels in cases and their thyroid volumes, detailed in table 22.

Table 22: Correlation of RAI uptake and thyroid volume with bone turnover markers in cases

Variables		**PINP**	**CTX**
RAIU 2hr (%)	Pearson coefficient (ρ)	0.276	-0.034
	p value	0.08	0.83
RAIU 24hr (%)	Pearson coefficient (ρ)	0.100	0.033
	p value	0.539	0.840
Thyroid volume	Pearson coefficient (ρ)	0.308	-0.163
	p value	**0.05**	0.31

Figure 19: Correlation of Thyroid volume with PINP levels in cases



Correlation of bone turnover markers (BTMs) with clinical indices

In our study, amongst bone turnover markers, PINP levels in cases were found to correlate negatively with time in the hyperthyroid state (THS) (Pearson's ρ = -0.368, p-value = 0.02) and time to normalization of hyperthyroid activity (TNHA)

(Pearson's ρ = -0.688, p-value = 0.04), summarised in table 24, demonstrated in figure 19 and 20.

In our study, amongst bone turnover markers, PINP levels in cases were found to correlate negatively with time in the hyperthyroid state (THS) (Pearson's ρ = -0.368, p-value = 0.02) and time to normalization of hyperthyroid activity (TNHA) (Pearson's ρ = -0.688, p-value = 0.04), detailed in table 24, demonstrated in figure 19 and 20. We found no significant correlation of PINP levels in cases and Cumulative ATD index (CAI), Time to rebound TSH (TRT), Cumulative dose to TRT (CAT).

Figure 20 : Correlation of PINP levels with THS in cases

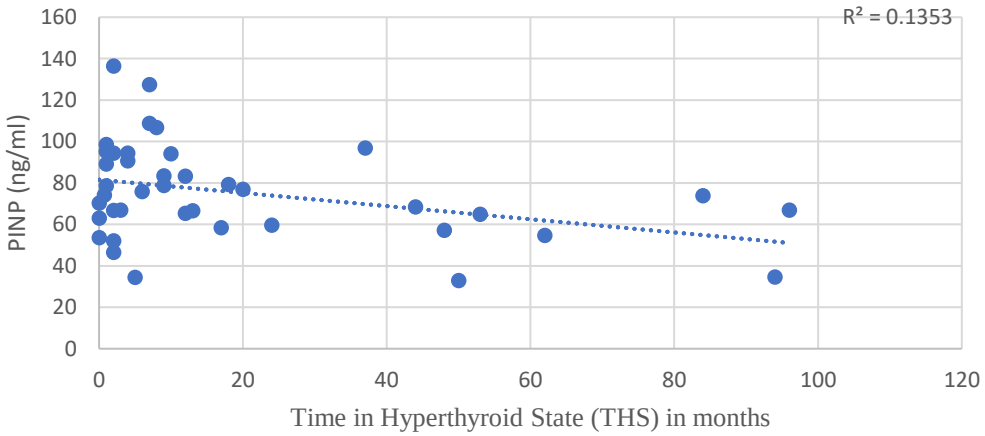


Figure 21 : Correlation of PINP levels with TNHA in cases

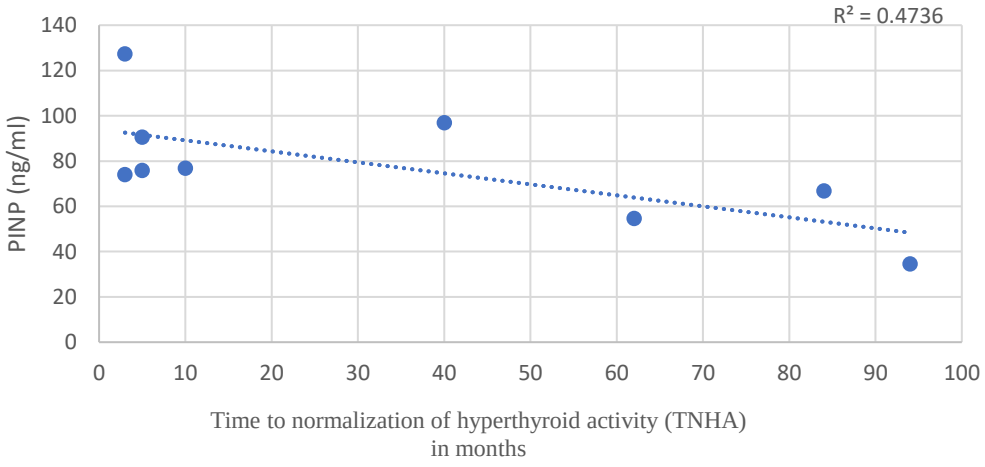


Table 23: Correlation of clinical indices with bone turnover markers in cases

Variables		**PINP**	**CTX**
Time in hyperthyroid state (THS)	Pearson coefficient (ρ)	-0.368	-0.144
	p value	**0.02**	0.37
Cumulative ATD index (CAI)	Pearson coefficient (ρ)	-0.163	-0.050
	p value	0.316	0.74
Time to normalization of hyperthyroid activity (TNHA)	Pearson coefficient (ρ), (N = 9)	-0.688	-0.401
	p value	**0.04**	0.28
Time to rebound TSH (TRT)	Pearson coefficient (ρ), (N = 5)	-0.788	-0.491
	p value	0.11	0.40

We found no significant correlation between CTX levels in cases and time in the hyperthyroid state (THS), time to normalization of hyperthyroid activity (TNHA), Cumulative ATD index (CAI), and Time to rebound TSH (TRT), as detailed in table 24.

DISCUSSION

Cases included 31.5 % (n=32) males and 68.9% (n=71) females, while the control group included 35%(n=14) males and 65% (n=26) females (p=0.69). Female preponderance has been associated with hyperthyroidism, more so with Graves' disease, where female to male ratio is 5-10 to 1 in different series(66), whereas 5-6 to 1 for autonomous functioning toxic adenomas and toxic multinodular goiter (67).

The mean age of cases was 35.63 ± 9.7 years, while that of the control group was 32.97 ± 9.08 (p=0.135). The mean age of presentation in patients with Graves' disease was 35.1 years, 27 years for toxic adenoma, and 44.4 years for toxic multinodular goiter. The mean age of patients with Graves' disease is congruent with the available data, i.e., 30-60 years at diagnosis with a mean age of 47 years (68).

In our study, the patients with toxic adenoma had a mean age of 27 years Vs. 30-40 years in the available literature. Various studies mention toxic adenoma manifests with thyrotoxic symptoms at a size > 3 cm, which is generally reached at an age more than 60 yrs, but, in our study, patients with toxic adenoma were symptomatic at a much earlier age, contrary to the data available. The above observation can be due to low sample size (n = 2) or non-iodinated rock salt, which is quite prevalent in this region. Similar arguments also hold for earlier manifestations (mean age 44.4 in the study vs 60 -70 yrs in literature) of toxic MNG in this region (69).

Ophthalmopathy was seen in 24.4%(n=23), while dermopathy was present in 4.2%(n=4) of patients with Graves' disease. The incidence of orbitopathy matches the literature from an extensive series of patients from Denmark and Italy (20 – 25%) (30,70).

At the point of contact with cases in the study, 27.8%(n=29) were treatment naïve, whereas 71.8% (n=74) had prior received intermittent anti-thyroid drugs (ATDs) before coming to us, but were still in thyrotoxicosis. Around 3% of cases had received either of the multiple treatment options (RAI ablation or surgery with ATDs) but were still thyrotoxic. ATDs have been the most common treatment modality used in India for the management of hyperthyroidism (71).

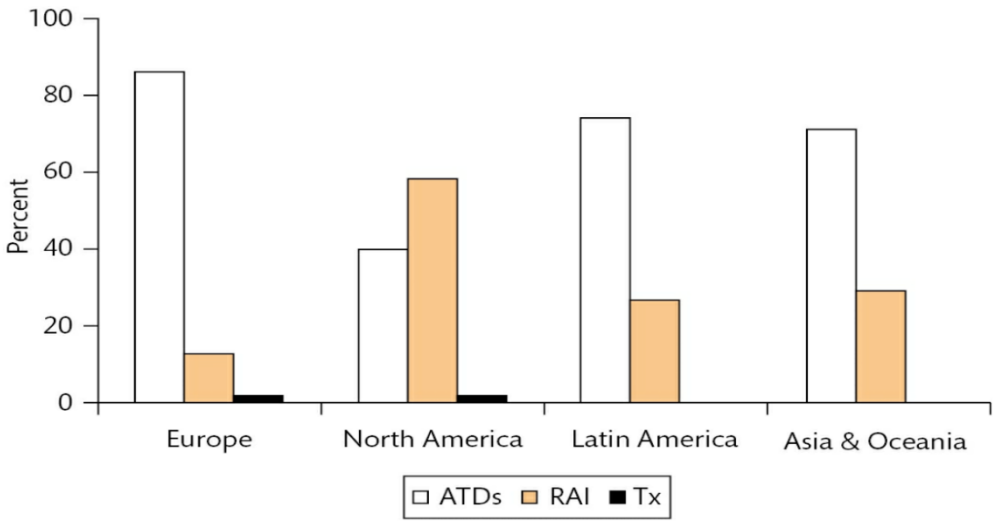


Figure 22. First-line treatment for newly diagnosed hyperthyroidism due to Graves' disease in different continents.(72,73)

The median time to diagnosis (TD) of hyperthyroidism in cases was two months (IQR 1.0 – 5.0). Patients maximally presented to the clinician within five months of the onset of symptoms, which is congruent with the available literature (74). The low median time to diagnosis reflects prompt identification of the symptoms of thyrotoxicosis and timely intervention by the physicians before the patient attends a tertiary care center.

At presentation, around 72% of cases were on treatment for hyperthyroidism, which was uncontrolled, with the median time in the hyperthyroid state (THS) being nine months (IQR 3 – 28.5). The persistent thyrotoxicosis in patients was due to poor compliance, loss of follow-up during the COVID pandemic, inadequate ATD dosing, etc. The median cumulative dose received by the cases was 170 (IQR 55 - 400).

Thirty-one patients achieved at least one event of normalization of thyroid hormone levels (both fT3/T3 and fT4/T4 within normal limits), and the median time to normalization of hyperthyroid activity (TNHA) was 14 months (IQR 6 - 59). Twenty-seven patients achieved a rebound increase in TSH during their treatment phase; the median time to rebound TSH (TRT) was 18 months (IQR 5 – 43.5), as demonstrated in figure 3. The mean cumulative ATD dose to TRT (CAT) was 210 (IQR 132.5 - 400).

Bone mineral density (BMD)

Lower bone mineral density has been associated with thyrotoxicosis. Bone loss has varied from 10 – 20% in various studies. In our study, we found a significant decrease in bone mineral density (BMD) in cases as compared with controls at the spine (0.91 ± 0.17 gm/cm^2 Vs. 1.01± 0.12 gm/cm^2, p = 0.003), femoral neck (0.74± 0.13 gm/cm^2 Vs. 0.81± 0.11 gm/cm^2, p <0.001), total hip (0.85± 0.15 gm/cm^2 Vs. 0.96± 0.11 gm/cm^2, p<0.001), distal 1/3 radius (0.65± 0.13 gm/cm^2 Vs. 0.72± 0.07 gm/cm^2, p<0.001), and whole body (1.02± 0.13 gm/cm^2 Vs. 1.10± 0.08 gm/cm^2, p<0.001).

Similar observations were made in the study conducted by Barbosa et al., 2020 in Portugal amongst 40 premenopausal women with hyperthyroidism, compared with age and BMI-matched controls. They found a significant reduction in BMD at the femoral neck (CT 0.883 ± 0.150 Vs. HT 0.800 ± 0.090, p= 0.037), total hip (CT 0.989 ± 0.110 Vs. HT 0.908 ± 0.100 0.0009), whole body (CT 1.174 ± 0.080 Vs. HT 1.108 ± 0.090, p= 0.0009) (54). While in another study by Barbosa et al. 2017, in 41 hyperthyroid men, the researchers for decreased BMD at distal radius (CT 0.769 ± 0.05 Vs. HT 0.722 ± 0.08, p = 0.005) (53). While in the study by Kuzma et al., 2018 only significant reductions in the lumbar spine (CT 1.051 ± 0.03 Vs. HT 1.016 ± 0.03, p < 0.05) were noted in patients with Graves' disease as compared with healthy matched healthy controls (52).

The low bone mass in thyrotoxicosis is due to the desynchronized bone remodeling sequence in thyrotoxicosis. Thyrotoxicosis leads to stimulation of osteoclastic resorption out of proportion to osteoblastic remineralization. As a result, the average cycle duration of around 200 days is reduced to almost 100 days. Each cycle is associated with a 9.6 percent loss of mineralized bone (36). The deleterious effect of thyrotoxicosis on bone mineral content is postulated to be multifactorial.

- Increased free T3 hormones directly/indirectly stimulate RANKL/OPG-mediated osteoclastogenesis (23).

In our study we found no significant correlation in cases between free T3 levels and BMD at various sites (lumbar spine (ρ = 0.15, p = 0.14), femoral neck (ρ = 0.08, p = 0.43), total hip(ρ = 0.004, p = 0.96), distal 1/3 radius(ρ = - 0.03, p = 0.77) and whole body(ρ = 0.0.1, p = 0.34)) or free T4 levels and BMD at various

sites (lumbar spine (ρ = -0.004, p = 0.97), femoral neck (ρ = -0.05, p = 0.60), total hip(ρ =-0.07, p = 0..46), distal 1/3 radius(ρ =-0.18, p=0.07) and whole body(ρ = -0.03, p = 0.73)).

The lack of significant correlation can be due to the variability of thyroxine levels with prior or ongoing treatment. Thus, the current free thyroxine levels (free T3 and free T4) at the time of enrolment in the study might reflect only the photographic image of the disease state rather than the natural history of the disease. It seems that bone loss in hyperthyroid patients is more of a cumulative effect of the disease rather than an instantaneous effect of the raised thyroxine levels. Therefore, prolonged exposure to hyperthyroxinemia would correlate better with bone loss rather than a singular event.

A similar absence of correlations between serum thyroxine levels and BMD parameters has been reported in the study by Barbosa et al., 2020 (54). But, in the Rotterdam study, correlations were observed between thyroid functions (free T4 and TSH) and bone mineral density in apparently normal older adults with relatively stable thyroid functions over a long period (75). This further ascertains that though increased thyroxine levels negatively impact bone health, significant correlations can only be established in the face of stable thyroid functions.

- Increased TRAb levels stimulate TSH receptor-dependent activation of osteoclasts and osteoblasts (24). TRAb-mediated increase in interleukin-6 levels leads to increased osteoclastic proliferation and differentiation (6).

Thus, we have ascertained that hyperthyroidism causes a decrease in BMD as compared with matched healthy controls. This observation supports the notion of a high bone turnover state in hyperthyroidism.

Trabecular bone score (TBS)

The trabecular bone score (TBS), a textural index, is an indirect index of bone microarchitecture that improves the prediction of fracture risk in osteoporosis and metabolic bone diseases (48). TBS changes reflect the deterioration of the bone

micro-architecture that can be traced earlier than the more evident changes in the bone mineral density as documented by the DXA scan (46).

In our study, we found a significant decrease in trabecular bone scores in cases as compared with controls (1.32± 0.11 Vs. 1.38± 0.06, p=0.002). The trabecular bone score in our hyperthyroid subjects (cases) falls in the partially degraded category compared to normal trabecular bone scores in the control group.

Thyrotoxicosis, a hypermetabolic state, if prolonged, as in the case of the multiple causalities of hyperthyroidism, can lead to rapid bone loss, which might not be evident clinically or with the use of routine radiological procedures. Therefore, we strongly recommend using TBS measurement to screen hyperthyroid patients for fracture risk assessment so that earlier diagnosis and treatment are incorporated into standard clinical practice.

Similar results were observed in the study of Barbosa et al., 2020 in Portugal amongst 40 premenopausal women with hyperthyroidism, compared with age and BMI-matched controls. They found a significant reduction in the trabecular bone score in the hyperthyroid women compared to the matched control group (CT 1.463 ± 0.080 Vs. HT 1.406 ± 0.080, p= 0.005) (54).

Kuzma et al., 2018 also demonstrated significant reductions in the trabecular bone score (CT 1.469± 0.02 Vs. HT 1.395 ± 0.02) in patients with Graves' disease compared to healthy matched healthy controls (52).

While in the previous study by Barbosa et al. 2017, in 41 hyperthyroid men, the researchers did not find a significant change in the trabecular bone score. The researchers explained that the time with nontreated hyperthyroidism was not long enough to develop bone microarchitecture changes measured by TBS (53).

Thyroid receptor antibody (TRAb)

TRAb levels represent the autoimmune milieu in patients with Graves' disease. TRAb levels have also been found to correlate with free T3 levels and thyroid gland size at diagnosis (76).

In our study, the median TRAb levels (9.0 IU/L (IQR 3.65 – 19.71)) were more than five times ULN in patients with Graves' disease as compared to controls. The levels of TRAb grossly parallels the degree of hyperthyroidism in Graves' disease, as assessed by the serum levels of thyroid hormones. TRAb levels more likely reflect the intensity or duration of the intrathyroidal inflammatory autoimmune reactions.

In our study, we found a statistically significant negative correlation in cases between TRAb levels and bone mineral density at total hip (ρ = -0.338, p = 0.003), distal left 1/3 radius (ρ = -0.298, p=0.01), and whole body (ρ = -0.253, p = 0.03). While, TRAb levels in cases did not correlate with bone mineral density at the lumbar spine (ρ = -0.114, p=0.33), and femoral neck (ρ = -0.183, p=0.12).

A similar negative correlation of TRAb levels with bone mineral density at distal radius has been documented in a study of male graves' patients done by Majima et al. (26). While, in the study by Ecolano A. et al. TRAb levels have been found to correlate negatively with bone mineral density at the lumbar spine (27).

TRAb levels tend to be stable in individuals with Graves' disease despite lowering thyroxine levels till there is underlying continuing autoimmunity: also serving as a marker to demarcate the attainment of remission in Graves' patients (77,78). Therefore, TRAb remains stable over long periods; thus, more profoundly is found to correlate with bone health.

In our study, in cases, we found no correlation between PINP and TRAb levels (ρ = 0.305, p = 0.07) or between CTX and TRAb levels (ρ = -0.017, p = 0.92). In the literature, we did not find any data correlating TRAb levels with PINP or CTX in patients with hyperthyroidism.

TRAb seems to affect bone metabolism by modulating TSH receptors at osteoblast and osteoclast, thereby inhibiting the positive impact of TSH. Thus increased TRAb levels cause an increase in bone turnover, thereby increasing levels of BTMs. The correlation not reaching significance levels might be due to the small sample size of cases with TRAb levels availability (N=35).

Interleukin-6 levels (IL-6)

Increased levels of IL-6 have been reported in patients with hyperthyroidism, both in Graves' disease and autonomous functioning thyroid nodules, but studies have revealed mixed results.

- Increased serum IL-6 levels in hyperthyroid patients (Graves' disease and toxic nodular goitre) as compared with controls have been demonstrated by Lakatos et al. (34)
- Weetmann et al. found increased intrathyroidal but not serum IL-6 levels in a small group of patients with GD. (79)
- The increase in IL-6 was limited to the bone microenvironment, but not serum IL-6, as a paracrine cytokine to thyroxine secretion was reported by Tarjan et al. (32)

The median serum interleukin-6 (IL-6) levels in the hyperthyroid patients were 1.79 pg/ml (IQR 0.8 – 3.8 pg/ml). The normal levels of IL-6 as reported by our laboratory, is less than 4.4 pg/ml.

In our study, we found no significant correlation in cases between IL-6 levels and BMD at various sites (lumbar spine ($\rho = 0.14$, $p = 0.28$), femoral neck ($\rho = 0.18$, $p = 0.14$), total hip($\rho = 0.144$, $p = 0.25$), distal 1/3 radius($\rho = 0.13$, $p = 0.30$) and whole body($\rho = 0.154$, $p = 0.23$)). Also, no correlation was found between IL-6 levels and TBS ($\rho = 0.07$, $p = 0.55$).

There were also no significant correlations in cases between IL-6 and PINP ($\rho = 0.06$, $p = 0.68$) or IL-6 and CTX ($\rho = 0.08$, $p = 0.61$) levels.

The reason for these observations can be multifactorial:

- Increased IL-6 levels in hyperthyroidism are possibly intrathyroidal (TRAb mediated, as in the case of Graves') or in the bone microenvironment (due to increased local free T3 levels per se); thus, increased serum levels could not be found in our study.
- IL-6 production is also transiently suppressed by the action of thionamides on follicular cells, marking their immunomodulatory effect (80).

As in our study, around 72% of hyperthyroid patients had received some ATD in the past before coming to us.

Thyroid volume

A good agreement has also been observed between TRAb and thyroid volume in untreated patients with Graves' disease. An increase in thyroid gland size has also been correlated with increased disease severity and duration (81).

In our study, the median volume of the thyroid gland in cases measured by ultrasonography was 14.41 cc (IQR 9.57 – 24.18 cc). The normative data for thyroid volume in India, for males, is 7.82 ± 4.76 cc and for females is 5.36 ± 2.31 ml) (82).

Increased thyroid gland size has been a known marker of thyroid gland overactivity in patients with hyperthyroidism. In Graves' thyroid gland size has been correlated with exuberant autoimmunity, represented as TRAb levels and thereby reflecting as proportional hyperthyroxinemia. Whereas, in toxic adenoma and toxic multinodular goiter, the size of the nodule(s), thereby increased total thyroid volume, correlates with thyroid hormone levels (83).

Our study did not find a correlation of thyroid volume (by USG) in cases with bone mineral density at the lumbar spine (ρ = -0.05, p = 0.62), femoral neck (ρ = 0.08, p = 0.40), total hip (ρ = 0.67, p = 0.50), distal 1/3 radius(ρ = -0.07, p = 0.47) or whole body(ρ = -0.08, p = 0.43). Our study found a statistically significant negative correlation between thyroid volume (by USG) and trabecular bone score (ρ = -0.296, p-value < 0.01).

Thyroid volume in hyperthyroid individuals correlates with the severity and duration of the disease, affecting the bone as well. In our study, the median time in the hyperthyroid state (THS) in cases was nine months (IQR 3 – 28.5).

In our study, TBS was found to correlate with thyroid volume but not with BMD. This might be due to the fact that the trabecular bone score is a representation of bone micro-architecture. It is impacted earlier than the entire bone morphology such that it is reflective of the deterioration of bone strength earlier than detected by bone

mineral density. This further underlines the importance of obtaining a trabecular bone score along with the assessment of bone mineral density.

We found no studies correlating thyroid volume with BMD or TBS in the literature.

In our study, a significant correlation was found in cases between PINP levels (a marker of bone formation) and thyroid gland volume (measured by ultrasonography) ($\rho = 0.308$, p-value = 0.05). No correlation was found between levels of CTX in cases and thyroid volume ($\rho = -0.163$, p-value = 0.31).

Thus, an increased thyroid gland size correlates with increased bone turnover.

Radioactive iodine uptake (RAIU)

Radioactive iodine uptake reflects the hyperthyroid activity, i.e., activity of the thyroid follicles, which too translates to the thyroxine levels in the blood. RAI uptake in Graves' disease is a maker of iodine turnover. Normal radioactive iodine uptake at 24 hrs is 8 – 35% (20).

In our study, the median radioactive iodine uptake levels in patients with Graves' disease (n = 55) at 2 and 24 hours were 36.2% (IQR 22.47 – 40.5) and 64.2% (IQR 51.29 – 65.28), respectively.

Our study found no significant correlation between RAI uptake at 2 hours in cases with bone mineral density at all sites or TBS.

In the literature, we did not find any study correlating the RAIU in patients with hyperthyroidism with the bone mineral density at any site.

Radioactive iodine uptake is easily influenced by many factors such as iodinated contrasts, high iodine-containing foods (iodized salt, kelp), drugs like amiodarone, salicylates, glucocorticoids, sulphonamides and lithium, thyroxine supplementation, and thionamides (84). Therefore, as with thyroxine levels, RAI uptake levels too reflect the current disease status alone; both are readily modifiable with ATDs. Hence the increased radioactive uptake seems to reflect on transient parameters rather than bone loss which is a marker of the prolonged disease state.

In our study, we found no correlation between the RAI uptake, in patients with Graves' disease, at 2 hours ($\rho = 0.276$, p-value = 0.08) and 24 hours (pearson's ρ = 0.10, p-value = 0.54) with PINP levels (bone formation marker). Also, CTX (bone resorption marker) did not correlate with RAI uptake at 2 hours ($\rho = -0.034$, p-value = 0.83) or 24 hours ($\rho = 0.033$, p-value = 0.84) in patients with Graves' disease.

In the literature, we did not find any study correlating the RAIU in patients with hyperthyroidism with the levels of bone turnover markers.

Radioactive iodine uptake, a marker of increased thyroid gland activity, reflects the spontaneous activity of the thyroid gland rather than a prolonged increase in the thyrotoxic milieu. RAI uptake also undergoes marked reduction during the use of anti-thyroid medication.

Bone turnover markers

Bone turnover markers increase in hyperthyroid patients irrespective of the etiology. The rise in BTMs occurs as early as three days post rise in blood thyroxine levels (sub-acute thyroiditis) to 15 days post thyrotoxicosis. Likewise, reversal of BTMs to baseline occurs in studies up to one-month post achievement of euthyroid levels (85). Both PINP and CTX levels have been found found to be elevated in hyperthyroid individuals compared to controls.

In our study, PINP, the marker of bone formation, was found to be significantly higher (73.9 ng/ml (IQR 61.3 – 92.3) Vs. 63.1 ng/ml (IQR 49.8 – 73.1), p=0.003) in cases as compared with controls. Also, CTX, the marker of bone resorption, was found to be significantly higher (173.5 pg/ml (IQR 118.3 – 271.3) Vs. 119.5 pg/ml (IQR 94 – 162.3), p = 0.001)in cases as compared with controls. Bone turnover seems to be increased substantially in patients with hyperthyroidism as both the marker of bone formation (PINP) and bone resorption (CTX) were found to be elevated in our study.

Similar observations were made by Kuzma et al., 2018 in Slovakia while comparing 37 patients of active Graves' disease with 23 matched controls. They found significantly raised PINP (162.43 µg/L ± 16.6 Vs. 43.95 µg/L ± 11.76, p<0.001) and CTx (840.8 pg/ml ± 106.04 Vs. 322.3 pg/ml ± 67.1, p<0.001) (52).

Our study found no significant correlation between markers of bone turnover (PINP and CTX) in cases and free thyroid hormone (free T3, free T4) or TSH levels.

The study by El Hadidy et al. also reported a significant correlation of BTMs CTX levels with thyroid hormone levels in patients with hyperthyroidism (86). The population enrolled in this study were known cases of uncontrolled hyperthyroidism for 1 – 7 years (mean 2.9 ± 1.2 years), while the median time in the hyperthyroid state in our study population was six months (IQR 3 – 28.5 months). It seems that a longer disease duration also has a significant role in the prolonged increase in bone turnover markers apart from instantaneous thyroid hormone levels.

Fluctuations in levels of thyroid hormones levels in our cases could have translated to an inconsistent rise in levels of BTMs, hence such an observation. Around 70% of our cases took ATDs intermittently before coming to us.

In our study, only CTX was found to correlate significantly but positively in cases with distal 1/3 radius ($\rho = 0.341$, p-value = 0.03) and whole-body BMD ($\rho = 0.345$, p-value = 0.03), while no correlation was found between CTX levels in cases and TBS. PINP levels in the cases did not show any correlations with BMD at all sites or TBS.

In the literature, we did not find any study correlating the bone mineral density in patients with hyperthyroidism with the levels of bone turnover markers.

Therefore, BTMs seem to represent the ongoing or the instantaneous effect of the disease state, hyperthyroidism in our case, while bone mineral density reflects the impact of the pathology on bone health due to prolonged hyperactivity. This explains why the correlation between BTMs and bone mineral density could not be met.

But still, CTx levels were found to correlate positively with higher BMD at distal left 1/3 radius and whole-body BMD while not at other sites.

The clinical indices

In our study, we defined time in hyperthyroid state (THS) as the duration in months the thyroid function tests remained suggestive of hyperthyroidism until BMD evaluation was done. We found a significant negative correlation in cases between

the patients' time in the hyperthyroid state (THS) and their trabecular bone score (ρ = -0.278, p-value = 0.004). No significant correlations were present between THS and BMD at various sites.

We defined time to diagnosis (TD) as the duration (months) of hyperthyroid symptoms, after which biochemical evidence is confirmed. We did not find any correlations between TD in cases and their BMD at various sites or the TBS.

We defined time to normalization of hyperthyroid activity (TNHA) as the duration(months) from the start of ATD to biochemical euthyroidism (normal T4/fT4 and T3/fT3 levels). We did not find any correlations between TNHA in cases and their BMD at various sites or the TBS.

We defined the time to rebound of TSH (TRT) as the duration (months) from the start of ATD to TSH normalization (TSH >0.35). We did not find any correlations between TRT in cases and their BMD at various sites or the TBS.

We defined cumulative ATD to TRT (CAT) as the sum of products of carbimazole equivalent doses (mg) and the duration of the dose given (months) till euthyroidism is achieved. We did not find any correlations between CAT in cases and their BMD at various sites or the TBS.

We defined cumulative ATD to TRT (CAT) as the sum of products of carbimazole equivalent doses (mg) and the duration of the dose given (months) till euthyroidism is achieved. We did not find any correlations between CAT in cases and their BMD at various sites or the TBS.

THS quantifies the periods for which the hyperthyroid individual had the active disease; thus, aptly, the effects are reflected in bone health as lower TBS values. TBS, a marker of bone micro-architecture, seems to be affected earlier in diseases hastening bone metabolism (87). Thus, prolonged THS has been shown to correlate with TBS rather than other BMD parameters. A longer follow-up study is probably needed to ascertain the effect of THS on other BMD parameters.

Though the cumulative ATD index (CAI) also reflects the longevity of hyperthyroidism, it may not be a true reflection of the biochemical or clinical thyrotoxic phase. Around 70% of the cases in our study included were already on ATDs at some point in their hyperthyroid phase. Thus, CAI may not be an accurate

marker of increased thyroid hormone exposure to the bone. Therefore no correlation could be found with BMD parameters.

In our study, it was observed that the median TNHA (n = 31/72 treated patients)was 14 months (IQR 6- 59) while TRT(n = 27/72 treated patients) was 18 months (IQR 5 – 43.5). TNHA and TRT are also unlikely to truly represent the thyrotoxic milieu during the treatment phase as to for what time the individual was in a thyrotoxic state during these periods. Thus, true BMD correlations seem less likely using the above parameters.

In our study, the median time in the hyperthyroid state (THS) in the population for which BTMs were available (n=40) was 7.5 months. Amongst bone turnover markers, PINP levels in cases were found to correlate negatively with time in the hyperthyroid state (THS) ($\rho = -0.368$, $p = 0.02$). No significant correlation was found between TSH in cases and CTX levels.

The study by El Hadidy et al. demonstrated that the duration of hyperthyroidism positively correlated with levels of BTMs (osteocalcin, CTx) (86).

The median time to normalization of hyperthyroid activity (TNHA) was ten months in the population for which BTMs were available (n=9). We found a negative correlation (Pearson's $\rho = -0.688$, $p = 0.04$) of TNHA in cases with PINP levels. At the same time, CTX levels in cases did not correlate with TNHA.

TNHA or any similar clinical indices have not been studied in the past.

In our study, BTMs (PINP and CTX) did not correlate with TD, TRT, CAI, or CAT in cases.

CONCLUSION

Hyperthyroidism, a state of excess thyroid hormone secretion, has myriad manifestations, including deleterious effects on bone health. While its impact on bone mineral density (BMD) has been well-known, evidence of its effect on bone micro-architecture can be assessed non-invasively using the trabecular bone score (TBS). There is scarce data on TBS in hyperthyroidism, especially in the Indian context. Our study aimed to compare BMD, TBS, and bone turnover markers (BTMs) in hyperthyroidism patients with matched healthy controls and to ascertain the determinants affecting them.

We conducted a case-control study including 103 hyperthyroid patients(cases) with Graves' disease (n=94), toxic multinodular goitre (n=7), and toxic adenoma (n=2) and age, BMI, and sex-matched healthy controls. Both cases and controls underwent clinical examination, BMD, and TBS evaluation using Hologic-horizon-DXA, the amino-terminal peptide of procollagen type-1 (PINP), and c-terminal telopeptide-X (CTX). Additionally, thyroid function test, thyroid receptor antibody (TRAb), Tc99m pertechnetate scan and /or radioactive iodine uptake study (RAIU), and ultrasonography of thyroid for thyroid volume were done in cases.

Cases included 31.5 % (n=32) males and 68.9% (n=71) females, while the control group included 35%(n=14) males and 65% (n=26) females (p=0.69). The mean age of cases was 35.63 ± 9.7 years, while that of the control group was 32.97 ± 9.08 (p=0.135). Ophthalmopathy was present in 24.4%(n=23), while dermopathy was present in 4.2%(n=4) of patients with Graves' disease.

In our study, 26.2%(n=27) of cases were treatment naïve. While 67.9%(n=71) had received some form of prior intermittent anti-thyroid drugs (ATDs). However, all included patients were thyrotoxic at the time of enrolment into the study. The median duration in the hyperthyroid phase and the cumulative ATD index for cases were nine months (IQR 3 – 28.5) and 170 (IQR 55 - 400), respectively.

The cases had median free T4 and free3 levels of 2.36 pg/ml (1.3 times ULN) and 6.46 ng/dl (1.5 times ULN), respectively, with suppressed TSH. Cases with Graves' disease had median TRAb levels of 9.0 IU/L (> five times ULN). In cases the median

volume of the thyroid gland was 14.41 ml which was more than two times the ULN of the gender-specific Indian normative data.

The salient conclusions from our study were as follows:

- Compared with controls, cases had statistically significantly lower BMD (kg/m^2) at all measured sites, including the lumbar spine, femoral neck, total hip, distal left radius, and whole-body BMD. This puts patients with hyperthyroidism, irrespective of the cause, at a higher risk of fracture than the euthyroid population.
- Trabecular Bone Score was significantly lower in cases than in controls, signifying a deterioration in bone micro-architecture in hyperthyroid individuals. This observation highlights that bone quality is affected in these patients and there is a need for addition of TBS to the clinical fracture risk assessment tool for appropriate risk estimation in patients of hyperthyroidism.
- A significant negative correlation was also seen between thyroid volume and trabecular bone score. Thyroid volume in hyperthyroid individuals has been documented to correlate with the severity and duration of the disease and with higher levels of free T3 and TRAb. The thyroid gland volume thus serves as a surrogate for the severity of hyperthyroidism. The greater bone loss as detected by BMD thus correlates with the severity of hyperthyroidism as deduced from the thyroid gland volume.
- A significant negative correlation was observed between TRAb levels and bone mineral density at the total hip, distal left 1/3 radius, and whole body. High TRAb levels could serve as an independent prognostic marker, identifying a subset of Graves' disease patients at a greater risk for bone loss. The numbers in Non-Graves hyperthyroid patients was limited to draw any meaningful difference between their bone loss and Graves' patients.
- No significant correlation was observed between FT4, FT3, IL-6, and RAI uptake with either BMD or TBS.
- A significant negative correlation was seen between the Time in Hyperthyroid State (THS) and trabecular bone scores (TBS). This duration of a patient being in thyrotoxic state is reflected in the greater effect on bone quality as evidenced by deterioration in TBS. TBS, a marker of bone micro-

architecture, seems to be affected earlier than other BMD parameters. This may build a case for doing TBS to determine the early effects of hyperthyroidism on bone.

- P1NP (bone formation) and CTx (bone resorption) were significantly higher in cases than in controls showing increased bone turnover in patients with hyperthyroidism. However, the correlation with loss of bone seen with BMD was observed with only CTx and at distal 1/3 radius and whole-body BMD. The effect on distal radius may signify an earlier initiation of bone resorption at areas of higher cortical bone.
- A significant correlation was observed between PINP and thyroid gland volume denoting the severity of the disease have a higher bone turnover. Thyroid gland volume correlates with FT3 and TRAb levels in patients with Graves' disease implying greater severity.

In summary, Hyperthyroidism is associated with significant implications for bone health. Our study confirmed lower BMD, greater deterioration in TBS, and higher BTMs in patients of hyperthyroidism. Higher levels of autoimmunity (TRAb) have an independent effect on bone loss. Increased thyroid gland size is also associated with lower BMD and TBS although other surrogates of severity like FT3 and IL-6 did not show correlation with bone loss in our study.

From "wormian bones" to the prevention of fractures, we have come a long way in managing bone health in hyperthyroidism patients. To the best of our knowledge, this is the first detailed study in India of TBS, BMD and BTMs in hyperthyroidism patients. Based on the results of our study, we conclude that besides doing BMD in these patients for detecting and documenting bone loss, TBS may be an additional tool to identify effects on bone micro-architecture and pick up early bone disease.

BIBLIOGRAPHY

1. Reddy PA, Harinarayan CV, Sachan A, Suresh V, Rajagopal G. Bone disease in thyrotoxicosis. Indian J Med Res. 2012 Mar;135(3):277–86.

2. Fibrous or deforming ostitis, osteomalacia, and osteoplastic carcinosis in their mutual relationships (89 pp., 5 whole plates, 4 of them in color). von Recklinghausen, Friedrich Daniel v .: (1891) | Antiq. F.-D. Söhn - Medicusbooks.Com [Internet]. [cited 2020 Nov 19]. Available from: https://www.zvab.com/erstausgabe/fibr%C3%B6se-deformierende-Ostitis-Osteomalacie-osteoplastische-Carcinose/1239516015/bd

3. Baxter JD, Bondy PK. Hypercalcemia of thyrotoxicosis. Ann Intern Med. 1966 Sep;65(3):429–42.

4. Bassett JHD, O'Shea PJ, Sriskantharajah S, Rabier B, Boyde A, Howell PGT, et al. Thyroid hormone excess rather than thyrotropin deficiency induces osteoporosis in hyperthyroidism. Mol Endocrinol Baltim Md. 2007 May;21(5):1095–107.

5. Abe E, Marians RC, Yu W, Wu XB, Ando T, Li Y, et al. TSH is a negative regulator of skeletal remodeling. Cell. 2003 Oct 17;115(2):151–62.

6. Lakatos P, Foldes J, Horvath C, Kiss L, Tatrai A, Takacs I, et al. Serum interleukin-6 and bone metabolism in patients with thyroid function disorders. J Clin Endocrinol Metab. 1997 Jan;82(1):78–81.

7. Krølner B, Jørgensen JV, Nielsen SP. Spinal bone mineral content in myxoedema and thyrotoxicosis. Effects of thyroid hormone(s) and antithyroid treatment. Clin Endocrinol (Oxf). 1983 May;18(5):439–46.

8. Langdahl BL, Loft AG, Eriksen EF, Mosekilde L, Charles P. Bone mass, bone turnover, body composition, and calcium homeostasis in former hyperthyroid patients treated by combined medical therapy. Thyroid Off J Am Thyroid Assoc. 1996 Jun;6(3):161–8.

9. Vestergaard P, Mosekilde L. Hyperthyroidism, Bone Mineral, and Fracture Risk—A Meta-Analysis. Thyroid. 2003 Jun;13(6):585–93.

10. Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, et al. Predictive value of BMD for hip and other fractures. J Bone Miner Res Off J Am Soc Bone Miner Res. 2005 Jul;20(7):1185–94.

11. Felsenberg D, Boonen S. The bone quality framework: determinants of bone strength and their interrelationships, and implications for osteoporosis management. Clin Ther. 2005 Jan;27(1):1–11.

12. Whittier DE, Boyd SK, Burghardt AJ, Paccou J, Ghasem-Zadeh A, Chapurlat R, et al. Guidelines for the assessment of bone density and microarchitecture in vivo using high-resolution peripheral quantitative computed tomography. Osteoporos Int J Establ Result Coop Eur Found Osteoporos Natl Osteoporos Found USA. 2020 Sep;31(9):1607–27.

13. Pothuaud L, Carceller P, Hans D. Correlations between grey-level variations in 2D projection images (TBS) and 3D microarchitecture: applications in the study of human trabecular bone microarchitecture. Bone. 2008 Apr;42(4):775–87.

14. Silva BC, Leslie WD, Resch H, Lamy O, Lesnyak O, Binkley N, et al. Trabecular bone score: a noninvasive analytical method based upon the DXA image. J Bone Miner Res Off J Am Soc Bone Miner Res. 2014 Mar;29(3):518–30.

15. Bousson V, Bergot C, Sutter B, Levitz P, Cortet B, Scientific Committee of the Groupe de Recherche et d'Information sur les Ostéoporoses. Trabecular bone score (TBS): available knowledge, clinical relevance, and future prospects. Osteoporos Int J Establ Result Coop Eur Found Osteoporos Natl Osteoporos Found USA. 2012 May;23(5):1489–501.

16. Rapoport B, Chazenbalk GD, Jaume JC, McLachlan SM. The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies. Endocr Rev. 1998 Dec;19(6):673–716.

17. Krohn K, Führer D, Bayer Y, Eszlinger M, Brauer V, Neumann S, et al. Molecular pathogenesis of euthyroid and toxic multinodular goiter. Endocr Rev. 2005 Jun;26(4):504–24.

18. Oxford Textbook of Endocrinology and Diabetes. OUP Oxford. 3e ed.

19. Aghini Lombardi F, Fiore E, Tonacchera M, Antonangeli L, Rago T, Frigeri M, et al. The effect of voluntary iodine prophylaxis in a small rural community: the Pescopagano survey 15 years later. J Clin Endocrinol Metab. 2013 Mar;98(3):1031–9.

20. Intenzo CM, dePapp AE, Jabbour S, Miller JL, Kim SM, Capuzzi DM. Scintigraphic Manifestations of Thyrotoxicosis. RadioGraphics. 2003 Jul;23(4):857–69.

21. Wejda B, Hintze G, Katschinski B, Olbricht T, Benker G. Hip fractures and the thyroid: a case-control study. J Intern Med. 1995;237(3):241–7.

22. Siddiqui JA, Partridge NC. Physiological Bone Remodeling: Systemic Regulation and Growth Factor Involvement. Physiol Bethesda Md. 2016 May;31(3):233–45.

23. Bassett JHD, Williams GR. Role of Thyroid Hormones in Skeletal Development and Bone Maintenance. Endocr Rev. 2016 Apr;37(2):135–87.

24. Baliram R, Latif R, Zaidi M, Davies TF. Expanding the Role of Thyroid-Stimulating Hormone in Skeletal Physiology. Front Endocrinol. 2017 Oct 3;8:252.

25. Mosekilde L, Eriksen EF, Charles P. Effects of thyroid hormones on bone and mineral metabolism. Endocrinol Metab Clin North Am. 1990 Mar;19(1):35–63.

26. Majima T, Komatsu Y, Doi K, Takagi C, Shigemoto M, Fukao A, et al. Negative correlation between bone mineral density and TSH receptor antibodies in male patients with untreated Graves' disease. Osteoporos Int J Establ Result Coop Eur Found Osteoporos Natl Osteoporos Found USA. 2006;17(7):1103–10.

27. Ercolano MA, Drnovsek ML, Silva Croome MC, Moos M, Fuentes AM, Viale F, et al. Negative correlation between bone mineral density and TSH receptor antibodies in long-term euthyroid postmenopausal women with treated Graves' disease. Thyroid Res. 2013 Sep 11;6(1):11.

28. Greenspan SL, Greenspan FS, Resnick NM, Block JE, Friedlander AL, Genant HK. Skeletal integrity in premenopausal and postmenopausal women receiving long-term L-thyroxine therapy. Am J Med. 1991 Jul;91(1):5–14.

29. Weetman AP, McGregor AM. Autoimmune thyroid disease: further developments in our understanding. Endocr Rev. 1994 Dec;15(6):788–830.

30. Laurberg P, Berman DC, Bülow Pedersen I, Andersen S, Carlé A. Incidence and clinical presentation of moderate to severe graves' orbitopathy in a Danish population before and after iodine fortification of salt. J Clin Endocrinol Metab. 2012 Jul;97(7):2325–32.

31. Bartalena L, Brogioni S, Grasso L, Martino E. Interleukin-6 and the thyroid. Eur J Endocrinol. 1995 Apr;132(4):386–93.

32. Tarjan G, Stern PH. Triiodothyronine potentiates the stimulatory effects of interleukin-1 beta on bone resorption and medium interleukin-6 content in fetal rat limb bone cultures. J Bone Miner Res Off J Am Soc Bone Miner Res. 1995 Sep;10(9):1321–6.

33. Passeri G, Girasole G, Jilka RL, Manolagas SC. Increased interleukin-6 production by murine bone marrow and bone cells after estrogen withdrawal. Endocrinology. 1993 Aug;133(2):822–8.

34. Lakatos P, Foldes J, Horvath C, Kiss L, Tatrai A, Takacs I, et al. Serum interleukin-6 and bone metabolism in patients with thyroid function disorders. J Clin Endocrinol Metab. 1997 Jan;82(1):78–81.

35. Apostu D, Lucaciu O, Oltean-Dan D, Mureșan AD, Moisescu-Pop C, Maxim A, et al. The Influence of Thyroid Pathology on Osteoporosis and Fracture Risk: A Review. Diagnostics. 2020 Mar 7;10(3):149.

36. Reddy PA, Harinarayan CV, Sachan A, Suresh V, Rajagopal G. Bone disease in thyrotoxicosis. Indian J Med Res. 2012 Mar;135(3):277–86.

37. Tsai KS, Lai SM, Huang KM, Chieng PU, Su CT, Chen FW. Decreased bone mineral density in patients with prolonged thyrotoxicosis before and after treatment. J Formos Med Assoc Taiwan Yi Zhi. 1991 Mar;90(3):250–5.

38. Black DM, Arden NK, Palermo L, Pearson J, Cummings SR. Prevalent vertebral deformities predict hip fractures and new vertebral deformities but not wrist fractures. Study of Osteoporotic Fractures Research Group. J Bone Miner Res Off J Am Soc Bone Miner Res. 1999 May;14(5):821–8.

39. Diamond T, Vine J, Smart R, Butler P. Thyrotoxic bone disease in women: a potentially reversible disorder. Ann Intern Med. 1994 Jan 1;120(1):8–11.

40. Krølner B, Jørgensen JV, Nielsen SP. Spinal bone mineral content in myxoedema and thyrotoxicosis. Effects of thyroid hormone(s) and antithyroid treatment. Clin Endocrinol (Oxf). 1983 May;18(5):439–46.

41. Tsai KS, Lai SM, Huang KM, Chieng PU, Su CT, Chen FW. Decreased bone mineral density in patients with prolonged thyrotoxicosis before and after treatment. J Formos Med Assoc Taiwan Yi Zhi. 1991 Mar;90(3):250–5.

42. Grant DJ, McMurdo ME, Mole PA, Paterson CR. Is previous hyperthyroidism still a risk factor for osteoporosis in post-menopausal women? Clin Endocrinol (Oxf). 1995 Sep;43(3):339–45.

43. Udayakumar N, Chandrasekaran M, Rasheed MH, Suresh RV, Sivaprakash S. Evaluation of bone mineral density in thyrotoxicosis. Singapore Med J. 2006 Nov;47(11):947–50.

44. Tsevis K, Trakakis E, Pergialiotis V, Alhazidou E, Peppa M, Chrelias C, et al. The influence of thyroid disorders on bone density and biochemical markers of bone metabolism. Horm Mol Biol Clin Investig. 2018 Sep 15;35(1):/j/hmbci.2018.35.issue-1/hmbci-2018-0039/hmbci-2018-0039.xml.

45. Silva BC, Leslie WD, Resch H, Lamy O, Lesnyak O, Binkley N, et al. Trabecular Bone Score: A Noninvasive Analytical Method Based Upon the DXA Image. J Bone Miner Res. 2014;29(3):518–30.

46. Palomo T, Muszkat P, Weiler FG, Dreyer P, Brandão CMA, Silva BC. Update on trabecular bone score. Arch Endocrinol Metab. 2022 Nov 11;66(5):694–706.

47. Pothuaud L, Carceller P, Hans D. Correlations between grey-level variations in 2D projection images (TBS) and 3D microarchitecture: applications in the study of human trabecular bone microarchitecture. Bone. 2008 Apr;42(4):775–87.

48. Hans D, Barthe N, Boutroy S, Pothuaud L, Winzenrieth R, Krieg MA. Correlations between trabecular bone score, measured using anteroposterior dual-energy X-ray absorptiometry acquisition, and 3-dimensional parameters of bone microarchitecture: an experimental study on human cadaver vertebrae. J Clin Densitom Off J Int Soc Clin Densitom. 2011;14(3):302–12.

49. Cormier DC, Lamy O, Poriau S. TBS IN ROUTINE CLINICAL PRACTICE: PROPOSALS OF USE.

50. Silva BC, Leslie WD, Resch H, Lamy O, Lesnyak O, Binkley N, et al. Trabecular Bone Score: A Noninvasive Analytical Method Based Upon the DXA Image: TRABECULAR BONE SCORE. J Bone Miner Res. 2014 Mar;29(3):518–30.

51. Ock SY, Chung YS, Choi YJ. Changes in bone mineral density and trabecular bone score in Graves' disease patients after anti-thyroid therapy. Osteoporos Sarcopenia. 2016 Sep;2(3):175–9.

52. Kužma M, Vaňuga P, Binkley N, Ságová I, Pávai D, Blažíček P, et al. High Serum Fractalkine is Associated with Lower Trabecular Bone Score in Premenopausal Women with Graves' Disease. Horm Metab Res. 2018 Aug;50(08):609–14.

53. Barbosa AP, Mascarenhas MR, Bicho M, Janeiro J, Oliveira AG. The main autoimmune and nonautoimmune etiologies of endogenous hyperthyroidism do not seem to influence the increased prevalence of morphometric vertebral

fractures and osteoporosis in Portuguese men. Osteoporos Sarcopenia. 2017 Sep;3(3):149–54.

54. Barbosa APG dos S, Mascarenhas MRG, Bicho MDP, Oliveira AMG de. Показатель качества трабекулярной костной ткани и оценка вертебральных переломов у португальских пременопаузальных женщин с гипертиреозом. PAIN Jt SPINE. 2020;10(2):65–71.

55. Wejda B, Hintze G, Katschinski B, Olbricht T, Benker G. Hip fractures and the thyroid: a case-control study. J Intern Med. 1995 Mar;237(3):241–7.

56. Vestergaard P, Rejnmark L, Weeke J, Mosekilde L. Fracture risk in patients treated for hyperthyroidism. Thyroid Off J Am Thyroid Assoc. 2000 Apr;10(4):341–8.

57. Bauer DC, Ettinger B, Nevitt MC, Stone KL, Study of Osteoporotic Fractures Research Group. Risk for fracture in women with low serum levels of thyroid-stimulating hormone. Ann Intern Med. 2001 Apr 3;134(7):561–8.

58. Kuo TR, Chen CH. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. Biomark Res. 2017 May 18;5(1):18.

59. Murphy E, Glüer CC, Reid DM, Felsenberg D, Roux C, Eastell R, et al. Thyroid Function within the Upper Normal Range Is Associated with Reduced Bone Mineral Density and an Increased Risk of Nonvertebral Fractures in Healthy Euthyroid Postmenopausal Women. J Clin Endocrinol Metab. 2010 Jul 1;95(7):3173–81.

60. Nickolas TL, McMahon DJ, Shane E. Relationship between moderate to severe kidney disease and hip fracture in the United States. J Am Soc Nephrol JASN. 2006 Nov;17(11):3223–32.

61. K P kumar, Narayanasamy K, J JJ, A C, R SK. Bone Mineral Density Assessment in Chronic Liver Disease. Arch Cancer Res [Internet]. 2017 Mar 27 [cited 2020 Nov 16];5(1). Available from:

https://www.acanceresearch.com/abstract/bone-mineral-density-assessment-in-chronic-liver-disease-18817.html

62. Kanis JA, Johansson H, Johnell O, Oden A, De Laet C, Eisman JA, et al. Alcohol intake as a risk factor for fracture. Osteoporos Int. 2005 Jul 1;16(7):737–42.

63. Vogel JM, Davis JW, Nomura A, Wasnich RD, Ross PD. The Effects of Smoking on Bone Mass and the Rates of Bone Loss Among Elderly Japanese-American Men. J Bone Miner Res. 1997;12(9):1495–501.

64. Paula Gouveia dos Santos Barbosa A, Rui Guerreiro Mascarenhas M, Diamantino Pires Bicho M, Manuel Gouveia de Oliveira A. Trabecular bone score and vertebral fracture assessment in portuguese premenopausal women with hyperthyroidism. PAIN Jt SPINE. 2020 Mar 1;10(2):65–71.

65. Bahn R, Burch H, Cooper D, Garber J, Greenlee M, Klein I, et al. Hyperthyroidism and other Causes of Thyrotoxicosis: Management Guidelines of the American Thyroid Association and American Association of Clinical Endocrinoloigists. Endocr Pract. 2011 May;17(3):456–520.

66. Aghini-Lombardi F, Antonangeli L, Martino E, Vitti P, Maccherini D, Leoli F, et al. The spectrum of thyroid disorders in an iodine-deficient community: the Pescopagano survey. J Clin Endocrinol Metab. 1999 Feb;84(2):561–6.

67. Hamburger JI. Evolution of toxicity in solitary nontoxic autonomously functioning thyroid nodules. J Clin Endocrinol Metab. 1980 Jun;50(6):1089–93.

68. DeGroot LJ. Graves' Disease and the Manifestations of Thyrotoxicosis [Internet]. Endotext [Internet]. MDText.com, Inc.; 2015 [cited 2022 Dec 15]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK285567/

69. Carlé A, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Rasmussen LB, et al. Epidemiology of subtypes of hyperthyroidism in Denmark: a population-based study. Eur J Endocrinol. 2011 May;164(5):801–9.

70. Tanda ML, Piantanida E, Liparulo L, Veronesi G, Lai A, Sassi L, et al. Prevalence and natural history of Graves' orbitopathy in a large series of patients

with newly diagnosed graves' hyperthyroidism seen at a single center. J Clin Endocrinol Metab. 2013 Apr;98(4):1443–9.

71. Mithal A, Shah A, Kumar S. The management of Graves' disease by Indian thyroidologists. Natl Med J India. 1993;6(4):163–6.

72. Burch HB, Burman KD, Cooper DS. A 2011 Survey of Clinical Practice Patterns in the Management of Graves' Disease. J Clin Endocrinol Metab. 2012 Dec 1;97(12):4549–58.

73. Bartalena L, Kahaly GJ, Baldeschi L, Dayan CM, Eckstein A, Marcocci C, et al. The 2021 European Group on Graves' orbitopathy (EUGOGO) clinical practice guidelines for the medical management of Graves' orbitopathy. Eur J Endocrinol. 2021 Oct 1;185(4):G43–67.

74. Vos XG, Smit N, Endert E, Tijssen JGP, Wiersinga WM. Variation in phenotypic appearance of Graves' disease: effect of genetic anticipation and duration of complaints. Eur J Endocrinol. 2009 Jul;161(1):113–8.

75. Effects of serum TSH and FT4 levels and the TSHR-Asp727Glu polymorphism on bone: the Rotterdam Study - Van Der Deure - 2008 - Clinical Endocrinology - Wiley Online Library [Internet]. [cited 2022 Dec 16]. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1365-2265.2007.03016.x

76. Michalek K, Morshed SA, Latif R, Davies TF. TSH receptor autoantibodies. Autoimmun Rev. 2009 Dec;9(2):113–6.

77. Ross DS, Burch HB, Cooper DS, Greenlee MC, Laurberg P, Maia AL, et al. 2016 American Thyroid Association Guidelines for Diagnosis and Management of Hyperthyroidism and Other Causes of Thyrotoxicosis. Thyroid. 2016 Oct;26(10):1343–421.

78. Laurberg P, Wallin G, Tallstedt L, Abraham-Nordling M, Lundell G, Tørring O. TSH-receptor autoimmunity in Graves' disease after therapy with anti-thyroid drugs, surgery, or radioiodine: a 5-year prospective randomized study. Eur J Endocrinol. 2008 Jan;158(1):69–75.

79. Weetman AP, Bright-Thomas R, Freeman M. Regulation of interleukin-6 release by human thyrocytes. J Endocrinol. 1990 Nov;127(2):357–61.

80. Pedro ABB, Romaldini JH, Takei K. Changes of Serum Cytokines in Hyperthyroid Graves' Disease Patients at Diagnosis and during Methimazole Treatment. Neuroimmunomodulation. 2011;18(1):45–51.

81. Orgiazzi J. ANTI–TSH RECEPTOR ANTIBODIES IN CLINICAL PRACTICE. Endocrinol Metab Clin North Am. 2000 Jun;29(2):339–55.

82. Prasad MA, Agrawal SK, Srivastava V, Chauhan M. Normal reference ranges of thyroid volume in North Indian subjects using ultrasound. Int J Med Health Res. 2020 Jan 1;6(7):19–21.

83. Bliddal H, Hegedüs L, Hansen JM, Bech K, van der Gaag R, Drexhage HA. The relationships between serum T3 index, thyroid volume, and thyroid stimulating, TSH receptor binding and thyroid growth stimulating antibodies in untreated Graves' disease. Clin Endocrinol (Oxf). 1987 Jul;27(1):75–84.

84. Meier DA, Kaplan MM. RADIOIODINE UPTAKE AND THYROID SCINTISCANNING. Endocrinol Metab Clin North Am. 2001 Jun;30(2):291–313.

85. Engler H, Oettli RE, Riesen WF. Biochemical markers of bone turnover in patients with thyroid dysfunctions and in euthyroid controls: a cross-sectional study. Clin Chim Acta. 1999 Nov;289(1–2):159–72.

86. El Hadidy EHM, Ghonaim M, El Gawad SSA, El Atta MA. Impact of severity, duration, and etiology of hyperthyroidism on bone turnover markers and bone mineral density in men. BMC Endocr Disord. 2011 Aug 6;11(1):15.

87. Silva BC, Leslie WD, Resch H, Lamy O, Lesnyak O, Binkley N, et al. Trabecular Bone Score: A Noninvasive Analytical Method Based Upon the DXA Image. J Bone Miner Res. 2014;29(3):518–30.

ANNEXURES

Annexure 1

Institutional Ethical Committee Clearance Certificate

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

No. AIIMS/IEC/2021/3458 Date: 12/03/2021

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2021/3293**

Project title: **"Assessment of Bone Mineral Density (BMD) and Trabecular Bone score (TBS) in patients of Hyperthyroidism"**

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

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

INFORMED CONSENT FORM

Title of Thesis/Dissertation: **Assessment of BMD and TBS in patients of hyperthyroidism.**

Name of DM Senior Resident : Dr. PRASOON RASTOGI

Mob No. 8920542552 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 BMD and TBS in patients of hyperthyroidism**.", 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

Witness 1 Witness 2
_____ _____

सूचित सहमति प्रपत्र

प्रधानअन्वेषक: डॉ प्रसून रस्तोगी **टेलीफोन नंबर:** 8920542552

रोगी / स्वयंसेवीपहचानसंख्या: _____

मैं,_____पुत्र/पुत्री_____ __निवासी_____फ़ोन_स्वयं को अध्ययन का हिस्सा होने के लिए अपनी पूर्ण स्वैच्छिक सहमति देता हूँ। इस अध्ययन का शीर्षक है :

: **Assessment of BMD and TBS in patients of hyperthyroidism**.

मेरी पूर्ण संतुष्टि के लिए मेरी खुद की भाषा में मुझे समझाया गया है।मैं इस बात की पुष्टि करता हूं कि मुझे सवाल पूछने का पूर्ण अवसर मिला है।मुझे पता है कि मेरी पहचान उस व्हाट्सएप ग्रुप के सभी सदस्यों के लिए खुली होगी और मैं इसके लिए अपनी स्वैच्छिक सहमति देता हूं।मैं यह समझताहूँ कि मेरी भागीदारी स्वैच्छिक है और बिना कोई कारण बताए किसी भी समय इस अध्ययन सेस्वयं को वापस लेने के लिए मेरे अधिकार के बारे में मुझे पता है।मैं यह समझता हूँ कि मेरे मेडिकल रिकॉर्ड की एकत्रित की गई जानकारी" अखिल भारतीय आयुर्विज्ञान संस्थान जोधपुर" या नियामक अधिकारियों द्वा रादेखी जा सकती है।मैं इन व्यक्तियों को मेरे रिकॉर्ड के उपयोग के लिए अनुमति देता हूँ।

दिनांक: हस्ताक्षर/
वामअंगूठेकानिशान
स्थान:
यह प्रमाणित किया जाता कि इस संस्करण की सहमति मेरी उपस्थिति में प्राप्त की गयी है:

दिनांक: प्रमुख अन्वेषक के हस्ताक्षर
1. साक्षी1 2. साक्षी 2

हस्ताक्षर: _____
हस्ताक्षर:_____

ANNEXURE 3

Patient Information Sheet

You are being invited to willing fully participate in the study entitled **"Assessment of BMD and TBS in patients of hyperthyroidism."**

Hyperthyroidism is a disease of increased production of thyroid hormone (thyroxine). Thyroxine has influence on all body systems, needed at its normal

levels to maintain basal metabolic rate. But when thyroxine in excess as in your disease, it adversely impacts all body systems including cardiovascular system, eye problems, skeletal system amongst others. But the deterioration of bone health is often overlooked due to its lack of symptoms, potentially leading to fractures and impaired quality of life. Our study would enable assessment of bone quantity and quality by bone mineral density (BMD) and trabecular bone score (TBS) using latest technique by a single DXA scan, helping early detection and appropriate management.

Study Design

You will be undergoing nuclear scans (RAI uptake and Tc99m uptake) to assess the activity of your thyroid gland and determining the cause of excess thyroid hormone. If found eligible to participate in the study, you would evaluated to ascertain the effect of disease on various body systems especially bones with state of the art Hologic horizon A DXA scanner.

General instructions:
Whole body DXA scan use X RAYs 1/150 of a normal spine XRAY, posing no risks to general wellbeing.

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.

रोगी सूचना पत्र

आपको " **हाइपरथायरायडिज्म के रोगियों में बीएमडी और टीबीएस के आकलन** " नामक अध्ययन में स्वेच्छा से भाग लेने के लिए आमंत्रित किया जा रहा है।

हाइपरथायरायडिज्म थायराइड हार्मोन (थायरोक्सिन) के उत्पादन में वृद्धि का एक रोग है। थायरोक्सिन का शरीर की सभी प्रणालियों पर प्रभाव पड़ता है, इसकी सामान्य स्तर पर जरूरत होती है ताकि बेसल चयापचय दर को बनाए रखा जा सके।लेकिन जब थायरोक्सिन आपकी बीमारी के रूप में अधिक होता है, तो यह हृदय प्रणाली, आंख की समस्याओं, कंकाल प्रणाली सहित अन्य सभी प्रणालियों पर प्रतिकूल प्रभाव डालता है।लेकिन हड्डी के स्वास्थ्य की गिरावट को अक्सर इसके लक्षणों की कमी, संभावित रूप से फ्रैक्चर और जीवन की खराब गुणवत्ता के कारण अनदेखा किया

जाता है। हमारा अध्ययन अस्थि खनिज घनत्व (बीएमडी) और त्रिकोणीय हड्डी स्कोर (टीबीएस) द्वारा हड्डी की मात्रा और गुणवत्ता का आकलन करने में सक्षम होगा, जो कि एक एकल डीएक्सए स्कैन द्वारा नवीनतम तकनीक का उपयोग करके, प्रारंभिक पहचान और उचित प्रबंधन में मदद करेगा।

अध्ययन योजना

आपके थायरॉयड ग्रंथि की गतिविधि का आकलन करने और अतिरिक्त थायराइड हार्मोन के कारण का निर्धारण करने के लिए आपको परमाणु स्कैन (आरएआई अपटेक और टीसीपीएम अपटेक) से गुजरना होगा। यदि अध्ययन में भाग लेने के योग्य पाया जाता है, तो आप विभिन्न शरीर प्रणालियों पर बीमारी के प्रभाव का पता लगाने के लिए मूल्यांकन करेंगे, विशेष रूप से हड्डियों के राज्य के साथ होलोग्राम क्षितिज एक डीएक्सए स्कैनर।

सामान्य निर्देश:

संपूर्ण शरीर DXA स्कैन X RAYs 1/150 एक सामान्य रीढ़ XRAY का उपयोग करता है, जिससे सामान्य रूप से कोई जोखिम नहीं होता है।

गोपनीयता

आपके मेडिकल रिकॉर्ड और पहचान को गोपनीय दस्तावेजों के रूप में माना जाएगा। यदि आवश्यक हो तो वे केवल अध्ययन के अन्य डॉक्टरों / वैज्ञानिकों / मॉनिटर / ऑडिटर्स के सामने आएंगे। अध्ययन के परिणाम एक वैज्ञानिक पत्रिका में प्रकाशित हो सकते हैं लेकिन आपको नाम से नहीं पहचाना जाएगा। अध्ययन के लिए आचार समिति की मंजूरी मिल गई है।

आपकी भागीदारी और अधिकार

अध्ययन में आपकी भागीदारी पूरी तरह से स्वैच्छिक है और आप बिना कारण बताए कभी भी अध्ययन से हट सकते हैं। किसी भी मामले में, आप अपनी स्थिति के लिए उपयुक्त उपचार प्राप्त करेंगे। अध्ययन में भाग लेने के लिए आपको कोई राशि नहीं दी जाएगी। आपको नियमित जांच के लिए भुगतान करना होगा।

Annexure 4

Case Record Proforma (Cases)

S. No.

Study ID:

Patient Particulars

Name	
Age	
Sex	
Address (Major Location of stay)	
Phone No.	
AIIMS Registration Number	

Occupation	
Menopausal status (in females)	

Anthropometry

Weight (kg)	
Height (cm)	
BMI ($kg/m^{2)}$	

Clinical Parameters-

Onset of symptoms (mm/yyyy)	
Confirmation of Diagnosis(mm/yyyy)	
Duration of Disease (in months)	
Palpitations	
Fatigue	
Heat intolerance/ Excessive sweating	
Weight loss	
Anxiety/Nervousness	
Appetite increased	
Sweaty/moist palms	
Tremors	
Gynecomastia (in males)	

Menstrual History

Cycle length		
Cycle duration		
Flow		
Remarks		

Comorbidities

DM		
HTN/CAD		

Others		

Addictions:

	Amount/Frequency	Duration
Smoking		
Alcohol intake		
Others		

Family history :

Hypothyroidism	
Graves'	
Other autoimmune disorders	

Treatment History-

	Name	Dosing	Duration	Remarks
Anti-thyroid drug				
Beta Blocker				
Others - RAI - Surgery				
Drugs for other comorbidities				

Clinical indices

Time to diagnosis (TD) (Duration in months of hyperthyroid symptoms after which hyperthyroidism with TFTs is confirmed)	

Time in hyperthyroid state (THS) Duration in months TFTs remain suggestive of hyperthyroidism	
Time to normalization of hyperthyroid activity (TNHA) Duration in months from start of ATD to TFT with normal T4/FT4 and T3/FT3 levels	
Time to rebound of TSH (TRT) Number of months from start of ATD to TSH >0.35	
Cumulative ATD dose to TRT (CAT) (Σ CBX * Duration till euthyroid)[#]	
Cumulative ATD Index (CAI) (Σ CBX * Duration till BMD evaluation)[#]	

[#] **CBX (Carbimazole equivalent)** **40 CBZ = 30 MMI = 400 PTU**

Examination Findings

Vitals:

BP (mm hg)				
Pulse (beats /min)				

Examination

Orbitopathy				
Dermopathy				
Acropachy				
Thyroid Gland Examination	Size	Grade	Nodularity	Bruit
Systemic Examination	Respiratory System - Cardiovascular System - Gastrointestinal System - Central Nervous System -			

Thyroid ophthalmopathy

Disease severity score : NOSPECS

No symptoms or signs	
Only signs (upper lid retraction, without lid lag or proptosis)	
Soft tissue involvement with symptoms	
Proptosis	
Extraocular muscle involvement	
Corneal involvement	
Sight loss	

Severity of Graves' Ophthalmopathy : EUGOGO classification

Mild	**Moderate**	**Severe**

Mild GO	• minor lid retraction (<2 mm) • mild soft-tissue involvement - absence of festoons - upper lid skin fold appears angled in >45^0 - absent eyelid erythema - equivocal or minimal redness - absence of conjunctival edema - absent inflammation of caruncle • exophthalmos <3 mm above normal • no or intermittent diplopia • corneal exposure responsive to lubricants.
Moderate GO	• lid retraction >2 mm • moderate to severe soft tissue involvement - absence of festoons - upper lid skin fold appears angled at 45^0 - <50% definite conjunctival redness - Presence of conjunctival edema - inflammation of caruncle • exophthalmos >3 mm above normal • inconstant or constant diplopia
Severe GO	• DON - Decreased visual acuity - Afferent pupillary defect - Affected colour vision - Optic disc atrophy/ edema • corneal breakdown • severe soft tissue involvement - Lower lid festoon +nt - upper lid skin fold appears angled at 45^0 - >50% definite conjunctival redness

Disease Activity Score (CAS)

Pain	• Spontaneous retrobulbar pain • Pain on up gaze, side gaze, or down gaze	
Redness	• Redness of the eyelids • Redness of the conjunctiva	
Swelling	• Swelling of the eyelids • Swelling of the caruncle and/or plica • Chemosis	
Total Score (Max 7)		

Great Score (Risk of Recurrent Graves Hyperthyroidism After 1-Year Treatment With ATD)

Age (yr) - ≥40 0 - <40 +1	
FT4 (pmol/L) – conv. Factor 12.872 to ng/dl - ≥40 0 - <40 +1	
TBII (U/L) - <6 0 - 6 – 19.9 +1 - >20 +2	
Goiter size - Grade 0-I 0 - Grade II -III +2 Grade 0 – only visible Grade I – palpable (normal position) & visible with head in raised position Grade II – palpable & visible with head in normal position Grade III – visible from a distance	
Total score	**Risk of Recurrence at 1 yr** **Class I (score 0–1) 16%** **Class II (score 2–3) 44%** **Class III (score 4–6) 68%**

PREDIGO Score

Baseline variable Cut-off Score	
CAS - 0 0 - ≥1 5	
TBII - <2 0 - 2-10 2 - >10 (U/L) 5	

Duration of hyperthyroid symptoms - <1 months 0 - 1-4 months 1 - >4 months 3	
Current smoking - No 0 - Yes 2	
Total Score	

Investigations

Bio-chemical Parameters-

Date					Remarks (Conversion)
FT3(pg/ml) / **T3** (ng/ml)					
FT4 (ng/ml) / **T4** (mcg/ml)					
TSH (mIU/L)					
TRAB (if feasible) (IU/L)					
IL- 6 (pg/ml)					
Fasting lipid profile - **TG** (mg/dl) - **LDL** (mg/dl) - **HDL** (mg/dl) - **TC** (mg/dl)					
Kidney Function Test - **Urea** (mg/dl) - **Creatinine** (mg/dl)					
Haemogram - **Hb** (gm %) - **TLC** (/mm^3) - **DLC** (%) - **MCV** (fL) - **PLT** (lac/ mm^3)					

Liver function test			
- **S. Bil** (T/D) (mg/dl)			
- **TP/ ALB/ G** (g/dl)			
- **SGOT** (IU/ml)			
- **SGPT** (IU/ml)			
Bone and Mineral Panel			
- **S. Ca** (mg/dl)			
- **S. PO_4** (mg/dl)			
- **ALP** (IU/L)			
- **25 OH Vit D** (ng/ml)			
- **iPTH** (pg/ml)			
Bone Markers			
ECG			
USG thyroid ± doppler			
Tc 99m thyroid scan			
RAI Uptake 2 hrs			
24 hrs			

BMD parameters-

Site	Bone mineral content (g)	Bone mineral density (g/cm^2)	T-Score	Z-Score	Remarks
Spine (L1 – L4)					
Femoral neck					
Total Hip					
Distal left Radius 33 %					
Whole body BMD					

Other BMD parameters-

Trabecular Bone Score (Spine)	
Total lean mass	
Total Body Fat content	

ANNEXURE 5

Case Record Proforma (Controls)

S. No.

Study ID:

Patient Particulars

Name	
Age	
Sex	
Address (Major Location of stay)	
Phone No.	
AIIMS Registration Number	
Occupation	
Menopausal status (in females)	

Anthropometry

Weight (kg)	
Height (cm)	
BMI ($kg/m^{2)}$	

Menstrual History

Cycle length		
Cycle duration		
Flow		
Remarks		

Comorbidities

DM		
HTN/CAD		
Others		

Addictions:

	Amount/Frequency	Duration
Smoking		
Alcohol intake		
Others		

Family history :

Hypothyroidism	
Graves'	
Other autoimmune disorders	

Examination Findings

Vitals:

BP (mm hg)				
Pulse (beats /min)				

Investigations

Bio-chemical Parameters-

Date					Remarks (Conversion)
FT3(pg/ml) / **T3** (ng/ml)					
FT4 (ng/ml) / **T4** (mcg/ml)					
TSH (mIU/L)					

BMD parameters-

Site	**Bone mineral content (g)**	**Bone mineral density (g/cm^2)**	**T-Score**	**Z-Score**	**Remarks**
Spine (L1 – L4)					
Femoral neck					
Total Hip					
Distal left Radius 33 %					
Whole body BMD					

Other BMD parameters-

Trabecular Bone Score (Spine)	
Total lean mass	
Total Body Fat content	