Study the Role of Signaling Pathway Ligands (WNT5A and TGF-β3) in Osteoporosis: A Cross Sectional Study in Iraqi Cohort

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Keywords: Comorbidity-Driven Bone Loss, Osteopenia, Osteoporosis, TGF-β3, WNT5A Signalling.

Abstract: Osteoporosis is a prevalent systemic metabolic disorder characterized by a reduction in bone mass and bone density, damage in microstructure of bone tissue, and raised bone fragility causing of fracture susceptibility. This study included 189 Iraqi adults aged 45–75 years, evaluating serum levels TGF-β3 and WNT5A across three groups: healthy individuals (n = 31) as control, those with primary osteoporosis/osteopenia (n = 61), and with comorbidity-related osteoporosis (linked to β-thalassemia, parathyroid disorders or diabetes; n = 97). Among those, β-thalassemia-associated osteoporosis patients showed the most distinct decrease in bone mineral density (0.57 ± 0.078 g/cm², $p < 0.0001$) along with marked increases in WNT5A (0.89 ± 0.68). Based on disease context, the level of TGF-β3 varied, peaking in thalassemia cases (0.28 ± 0.18 ng/mL). Significant disease-specific correlations were noticed, comprising moderate positive relationships between TGF-β3 and WNT5A ($r = 0.433$, $p = 0.017$) in diabetes patients, as well as a negative correlation between HbA1c and TGF-β3 in diabetes ($r = -0.503$, $p = 0.047$). Receiver Operating Characteristic (ROC) analysis highlighted WNT5A (AUC ≤ 0.882, sensitivity ≤ 81%) and TGF-β3 (AUC ≤ 0.891) as effective biomarkers for distinguishing thalassemia-related osteoporosis. These results underscore the role of comorbidities in altering stress response and signaling pathways, and support the use of WNT5A and TGF-β3 as promising diagnostic markers for targeted osteoporosis management in high-risk populations.

1 INTRODUCTION

Osteoporosis is a prevalent systemic metabolic disorder marked by reduced bone mineral density and mass, deterioration of the bone's microarchitectural structure, and heightened fragility, which together increase susceptibility to fractures [1], [2]. The likelihood of osteoporotic fractures rises as bone mass declines and microstructural integrity worsens after peak bone mass is achieved, with bone loss accelerating significantly after menopause [2]. Over a lifetime, the probability of experiencing such a fracture is about 50% for women and 20% for men aged 50 years and older [3].

The disease becomes more common with advancing age. According to the United Nations' World Population Prospects 2019, individuals aged 65 years and above are projected to comprise 16% of the global population by 2050, with the total number of osteoporosis cases expected to reach 221 million. Given global population aging, osteoporosis has emerged as a major public health challenge [1], [2].

Its development results from a complex interplay between local and systemic regulators of bone cell activity, influenced by factors such as genetic makeup, transcriptional control, signaling pathways, hormonal balance, and cytokine activity [2].

Recent advances in multi-omics technologies have identified key molecular players, including signaling pathways such as Wingless-related integration site (Wnt) and transforming growth factor beta (TGFβ), which regulate bone cell differentiation, stress responses, and matrix remodeling [4], [5]. Wnts are lipid-modified secreted proteins that interact with various cell surface receptors to trigger either canonical or non-canonical Wnt signaling pathways, thereby regulating a wide range of biological processes during embryonic development and in adult tissues [6].

WNT5A, a ligand in the non-canonical Wnt pathway, regulates osteoblastogenesis and osteoclast differentiation. While canonical Wnt/β-catenin signaling promotes bone formation, WNT5A enhances osteoclastogenesis via RANKL-independent mechanisms, creating a delicate balance

critical for skeletal integrity [7]. Dysregulated WNT5A expression is observed in osteoporosis, where it may accelerate bone loss by disrupting osteoblast-osteoclast coupling [8].

TGFβ3 is a member of the multifunctional polypeptide cytokine TGFβ superfamily, plays a complex role in bone metabolism and osteoporosis. It has been identified as a potential marker for osteoporosis severity which may have a protective effect on bone density. A study by (Haghighizadeh E., 2019) found a significant direct correlation between TGF-β3 serum levels and BMD T-score, indicating that lower TGF-β3 levels may be associated with lower bone density and osteoporosis [9]. Other studies suggest that TGF-β3 can inhibit osteogenic differentiation of human mesenchymal stem cells (MSCs) and the high concentrations of TGF-β3 can suppress bone formation [10].

Current diagnostic reliance on BMD and fracture history has limitations in sensitivity and predictive power. Biomarkers such as WNT5A and TGFβ offer potential for early detection, risk stratification, and personalized therapy. For example, metabolomic studies highlight TGFβ-associated metabolites as predictors of low BMD [11]. Similarly, proteomic analyses identify WNT5A as mediators of oxidative stress and inflammation in bone [12], [13]. However, the interplay between these biomarkers in osteoporosis remains poorly characterized, particularly in understudied populations like Iraq.

This study aimed to examine the relationship between circulating levels of TGFβ and WNT5A and the severity of osteoporosis in a group of Iraqi adults, using biochemical analysis. Serum samples from both osteoporotic patients and healthy individuals have been assessed to measure these biomarkers and evaluate their diagnostic potential in comparison with standard bone mineral density (BMD) tests. The research also investigated how these biomarkers interact with known bone turnover markers to better understand their role in bone remodeling. Based on these insights, the study aimed to develop a multi-biomarker panel tailored for early diagnosis and treatment monitoring, particularly in settings with limited access to advanced tools like DEXA. Ultimately, by clarifying the roles of TGFβ and WNT5A in osteoporosis, this research intends to support precision medicine approaches for at-risk populations and help reduce the incidence of osteoporotic fractures in underserved regions.

2 MATERIALS AND METHODS

2.1 Study Population

This cross-sectional study included 212 participants aged 45 to 75, recruited from Alyarmouk Teaching Hospital between December 4th and March 29th. After applying strict exclusion criteria such as the presence of malignancies, Paget's disease, chronic liver/kidney conditions, cardiovascular disease, or use of steroids or alcohol 23 individuals were excluded. The final sample consisted of 189 adults, categorized into three groups: (1) 97 individuals with osteoporosis or osteopenia and coexisting metabolic or endocrine disorders (e.g., diabetes mellitus (DM), parathyroid issues, or β-thalassemia), (2) 61 individuals with primary osteoporosis or osteopenia and no significant medical history, and (3) 31 healthy, age-matched controls. Venous blood samples were collected after obtaining written informed consent. Serum was separated *via* centrifugation (3,000 × rpm for 15 minutes at 20°C), aliquoted, and stored at -20°C. Ethical approval was secured, ensuring compliance with the Declaration of Helsinki.

2.2 Body Mass Index Calculation

Height and weight were measured three times using precision instruments, and the average values were used to calculate Body Mass Index (BMI) (kg/m²) using the standard formula.

$$BMI = \text{Weight (kg)} / [\text{Height (m)}]^2$$

2.3 Bone Mineral Density Measurement

Bone Mineral Density (BMD) was measured using DEXA (Hologic Horizon A) at the lumbar spine (L1–L4) and femoral neck, following ISCD guidelines. Daily phantom calibration ensured accuracy, with CVs maintained below 1.5%.

2.4 T-score and Z-score Derivation

DEXA software (APEX v5.0) automatically calculated T-scores and Z-scores. T-scores compared participants' BMD to a healthy 30-year-old reference, while Z-scores were adjusted for age, sex, and ethnicity using NHANES III data.

2.5 Serum Calcium Quantification

Serum calcium (Ca) was measured using the o-cresolphthalein complexone method (Randox) with absorbance read at 570 nm. Values were adjusted based on albumin concentration.

2.6 HbA1C% Analysis

A hemoglobin A1C (HbA1C) was analyzed using HPLC (Bio-Rad D-10), separating hemoglobin variants *via* cation-exchange chromatography. Intra-assay variability was under 2%, meeting NGSP standards.

2.7 Thyroid-Stimulating Hormone Measurement

Serum Thyroid-Stimulating Hormone (TSH) levels were assessed with chemiluminescent immunoassay (Siemens ADVIA Centaur XP), using anti-TSH monoclonal antibodies. The assay's detection limit was 0.01 mIU/mL, calibrated to WHO standards.

2.8 Vitamin D3 (25-hydroxyvitamin D) Analysis via Colorimetric Assay

Vitamin D3 levels were measured *via* a colorimetric method involving protein precipitation, derivatization with PTAD, and chromogenic reaction. Absorbance at 620 nm was compared to a standard curve (0–150 ng/mL). Lipid-rich samples underwent solid-phase extraction, and results were cross-validated with LC-MS/MS for accuracy.

2.9 Evaluation of Human TGF-β3

The quantification of human transforming growth factor-beta 3 (TGF-β3) in serum, plasma, and cell culture supernatants was performed using the FineTest® Human TGF-β3 ELISA Kit (Catalogue No.: EH0289), a sandwich enzyme-linked immunosorbent assay (ELISA) with a detection range of 15.625–1000 pg/mL and sensitivity of 9.375 pg/mL. The protocol employed biotinylated detection antibodies and horseradish peroxidase (HRP)-conjugated streptavidin, with tetramethylbenzidine (TMB) as the chromogenic substrate. Absorbance was measured at 450 nm, and data were analyzed using a four-parameter logistic curve *via* CurveExpert 1.4 software.

2.10 Evaluation of Human WNT5A

The analysis of serum samples was conducted using the FineTest® Human WNT5A ELISA Kit (Catalogue No. QT-EH1164) following a standardized protocol. The absorbance was measured at 450 nm. Data were analyzed by subtracting the blank OD450 values, and a four-parameter logistic curve was generated using CurveExpert 1.4 software. Final sample concentrations were calculated by interpolating corrected OD values against the standard curve, with adjustments applied for dilution factors. All steps were performed using calibrated pipettes, sterile tips, and adherence to specified storage conditions to ensure reproducibility and minimize cross-contamination.

2.11 Statistical Analysis

Data were analyzed using SPSS v22.0. Results are expressed as mean ± SD. Group differences were evaluated using one-way ANOVA with LSD post-hoc tests. Pearson's correlation assessed relationships between bone health indicators (BMD, T-score, Z-score), biochemical markers (calcium, HbA1C%, TSH, vitamin D3), and molecular biomarkers (WNT5A and TGF-β3). ROC curve analysis determined the diagnostic performance of molecular markers in distinguishing osteoporosis with thalassemia from other groups, reporting AUC, sensitivity, specificity, and optimal cutoffs. Statistical significance was set at $p < 0.05$.

3 RESULTS

3.1 Demographic and Clinical Parameters

Comparative analysis of demographic and clinical parameters across eight groups—Control (C, n= 30), Osteopenia Clean (n= 30), Osteoporosis Clean (n= 30), Osteopenia DM (n= 30), Osteopenia Parathyroide (n= 19), Osteoporosis DM (n= 16), Osteoporosis Thalassemia (n= 11), and Osteoporosis Parathyroide (n= 16) revealed significant intergroup variations (Table 1).

Table 1: Comparison of demographic and biochemical parameters among studied groups.

Groups Parameters	C Mean± SD N=30	Osteopenia clean Mean± SD N=30	Osteoporosis Clean Mean ±SD N=30	Osteopenia DM Mean± SD N=30	Osteopenia parathyroid Mean ±SD N=19	Osteoporosis DM Mean± SD N=16	Osteoporosis thalassemia Mean± SD N=11	Osteoporosis parathyroid Mean± SD N=16	P-value
Age (years)	60.76±8.33	63.26 ±7.48	63.80 ±8.09	61.43 ±7.99	64.57 ±7.99	65.25 ±9.02	51.54 ±15.52	61.12 ±4.68	0.004
BMI (kg/m2)	30.51± 1.86	30.76 ±1.97	30.67 ±2.16	30.70± 1.77	30.29± 1.82	30.13± 2.19	29.72 ±0.64	30.06± 1.91	0.710
BMD	1.12 ±0.15	0.85± 0.05	0.71± 0.07	0.832± 0.05	0.837 ±0.09	0.72± 0.07	0.57 ±0.078	0.70± 0.08	0.0001
Tscore	0.62± 1.00	-1.64± 0.38	-3.07 ±0.45	-1.886 ± 0.34	-1.889± 0.35	-2.85± 0.45	-4.17± 0.39	-3.02± 0.54	0.0001
Zscore	1.59± 0.95	-0.14± 0.65	-1.19 ±0.69	-0.123 ±0.48	-0.121± 0.49	-0.74± 0.71	-2.54 ±0.60	-1.35± 0.80	0.0001
Ca	9.11± 0.65	8.19± 1.41	5.02 ± 1.35	5.96± 1.19	6.44± 1.23	9.10± 0.94	8.95± 1.01	6.04± 2.26	0.0001
HbA1C%	4.88 ±0.56	4.93± 0.722	5.12± 0.729	11.56 ±2.53	5.23 ±0.62	12.54 ±3.44	4.26± 1.58	5.02± 0.70	0.0001
TSH(mIU/mL	2.44± 1.29	2.75± 1.30	3.1± 1.39	17.68 ±6.63	3.04± 1.27	2.28± 1.47	2.28 ±1.47	13.34± 5.30	0.0001
VitD3	32.17 ±11.47	30.26± 9.10	23.95 ±14.73	19.06± 9.02	15.39± 8.03	13.35± 5.28	14.12± 3.49	14.88± 9.06	0.0001
WNT5A	0.127± 0.68	0.207± 0.35	0.14 ±0.17	0.16± 0.09	0.13 ±0.064	0.129± 0.07	0.89± 0.68	0.12± 0.05	0.0001
TGF-β3	0.1367± 0.04	0.149 ±0.16	0.1367±0.116	0.143± 0.06	0.19± 0.36	0.18± 0.07	0.28± 0.18	0.15± 0.05	0.177

Bone mineral density (BMD), T-score, and Z-score exhibited pronounced differences ($p < 0.0001$), with the Osteoporosis Thalassemia cohort demonstrating the lowest BMD (0.57 ± 0.078), T-score (−4.17 ± 0.39), and Z-score (−2.54 ± 0.60). Biochemical markers also varied: Calcium (Ca) was lowest in Osteoporosis Clean (5.02 ± 1.35 mg/dL; $p < 0.0001$), HbA1c% highest in Osteoporosis DM (12.54 ± 3.44%; $p < 0.0001$), and TSH elevated in Osteopenia DM (17.68 ± 6.63 mIU/mL; $p < 0.0001$). Vitamin D3 (VitD3) was lowest in Osteoporosis DM (13.35 ± 5.28 ng/mL; $p < 0.0001$). Signaling molecules (i.e. WNT5A) were markedly elevated in Osteoporosis Thalassemia (WNT5A: 0.89 ± 0.68; $p < 0.0001$), while TGF- β3 showed no significant intergroup difference ($p = 0.177$).

3.2 Correlation Analyze

Osteopenia Clean Cohort. Significant negative correlations included BMD vs. WNT5A ($r = −0.437$, $p = 0.016$), T-score vs. TGF-β3 ($r = −0.376$, $p = 0.040$), Z-score vs. WNT5A ($r = −0.429$, $p = 0.018$), and VitD3 vs. TGF-β3 ($r = −0.393$, $p = 0.031$), Figure 1 A-D. Osteopenia DM Cohort: Positive correlations included WNT5A vs. TGF- β3 ($r = 0.433$, $p = 0.017$), Figure 1 E.

Osteopenia Parathyroide Cohort: TGF-β3 and TSH were negatively correlated ($r = − 0.483$, $p = 0.036$), Figure 1 F. Osteoporosis DM Cohort: HbA1c% and TGF- β3 were negatively correlated ($r = −0.503$, $p = 0.047$), Figure 1 G. Osteoporosis Parathyroide Cohort: Ca and TGF- β3 correlated positively ($r = 0.517$, $p = 0.040$), Figure 1 H.

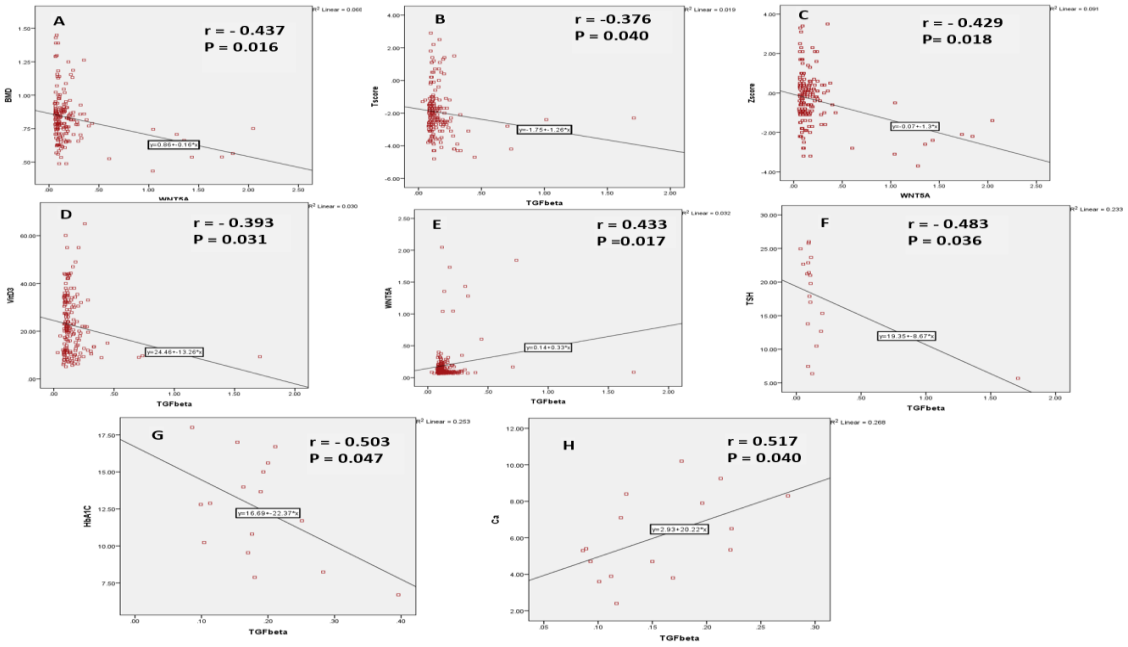

Figure 1: Pearson correlation in osteopenia clean a) negative correlation between BMD and WNT5A, b) Negative correlation between T-score and TGF- β3, c) negative correlation between z-score and WNT5A, d) negative correlation vitD3 and TGF- β3, in osteopenia DM, e) positive correlation between WNT5A and TGF- β3, in osteopenia-parathyroid, f) negative correlation TSH and TGF- β3, in Osteoporosis DM, g) negative correlation between HbA1C and TGF- β3, in osteoporosis parathyroid, h) positive correlation Ca and TGF- β3.

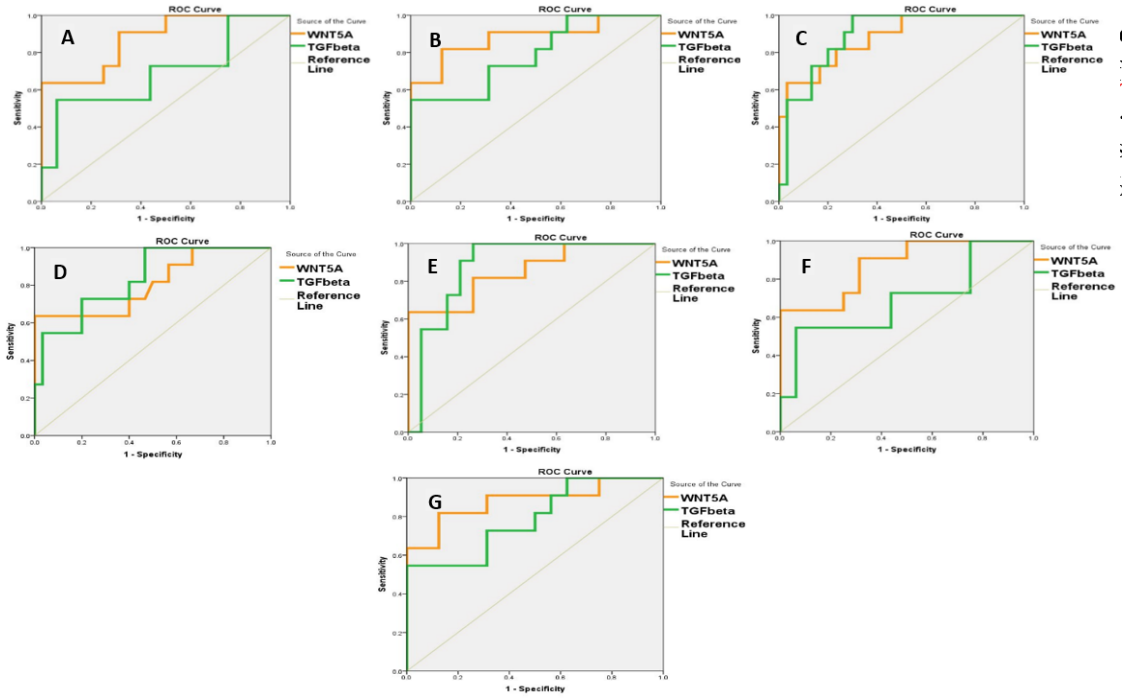

Figure 2: ROC results of WNT5A and TGF- β3 between; a) control and osteoporosis thalassemia; b) osteopenia clean and osteoporosis thalassemia; c) osteoporosis clean and osteoporosis thalassemia; d) osteopenia DM and osteoporosis thalassemia; e) osteopenia parathyroid and osteoporosis thalassemia; f) osteoporosis DM and osteoporosis thalassemia; g) osteoporosis parathyroid and osteoporosis thalassemia.

Table 2: Analysis of ROC data among studied groups.

Parameters	Groups	AUC	SE	P-value	Sensitivity%	Specificity%	Cut-off value
WNT5A	C & osteoporosis thalassemia	0.882	0.059	0.0001	81	73	0.158
	Osteopenia clean &osteoporosis thalassemia	0.821	0.075	0.002	72	70	0.172
	Osteoporosis clean & osteoporosis thalassemia	0.879	0.059	0.0001	81	76	0.1565
	Osteopenia DM & osteoporosis thalassemia	0.808	0.085	0.003	72	66	0.1715
	Osteopenia parathyroid & osteoporosis thalassemia	0.852	0.075	0.002	81	73	0.134
	Osteoporosis DM & osteoporosis thalassemia	0.875	0.068	0.001	81	73	0.154
	Osteoporosis parathyroid& osteoporosis thalassemia	0.881	0.074	0.001	72	68	0.164
TGF- β3	C & osteoporosis thalassemia	0.868	0.061	0.0001	90	60	0.1225
	Osteopenia clean &osteoporosis thalassemia	0.873	0.061	0.0001	81	73	0.1365
	Osteoporosis clean & osteoporosis thalassemia	0.891	0.050	0.0001	81	80	0.1345
	Osteopenia DM & osteoporosis thalassemia	0.833	0.068	0.001	70	72	0.1505
	Osteopenia parathyroid & osteoporosis thalassemia	0.880	0.064	0.001	81	78	0.1305
	Osteoporosis DM & osteoporosis thalassemia	0.693	0.110	0.093	63	56	0.1825
	Osteoporosis parathyroid& osteoporosis thalassemia	0.790	0.091	0.012	72	68	0.1795

3.3 Diagnostic Performance of Biomarkers

Receiver operating characteristic (ROC) analysis evaluated WNT5A, and TGF- β3 in distinguishing Osteoporosis Thalassemia from other groups (Fig. 2, Table 2). Key findings: WNT5A consistently demonstrated high diagnostic accuracy, with AUCs >0.85 for most comparisons (e.g., AUC = 0.882 for Control vs. Osteoporosis Thalassemia; sensitivity 81%, specificity 73%). TGF- β3 showed robust performance (AUC = 0.868–0.891) in distinguishing Osteoporosis Thalassemia from Control, Osteopenia Clean, and Osteoporosis Clean cohorts. Optimal cutoffs varied by biomarker and comparison (e.g., WNT5A cutoff = 0.1580 for Control vs. Osteoporosis Thalassemia; sensitivity 81%, specificity 73%).

4 DISSCUSSION

This study conducted a comprehensive analysis of biochemical profiles in distinct osteoporosis cohorts patients without comorbidities (“osteoporosis clean”) and those with Type 2 Diabetes Mellitus (T2DM), parathyroid disorders, or β-thalassemia compared with healthy controls. Age was similar across osteoporotic/osteopenic and control groups, but all patient categories had BMI values in the overweight range (29.72–30.67 kg/m²). The β-thalassemia subgroup recorded the lowest BMI (29.72 ± 0.64 kg/m²), consistent with chronic undernutrition and disease-related burden in thalassemia-associated osteoporosis [13]. Slightly higher BMIs in the other groups support evidence that excess adiposity may provide partial protection against bone loss; however, this benefit is diminished in conditions such as diabetes or parathyroid dysfunction [14], [15]. Overall, the findings highlight the combined influence of aging and systemic diseases on skeletal health.

Bone mineral density (BMD), T-scores, and Z-scores were significantly reduced in all osteoporosis groups, reflecting disease severity. The “osteoporosis clean” group showed severe bone loss (BMD: 0.71 ± 0.07 g/cm²; T-score: −3.07 ± 0.45). Interestingly, the T2DM group demonstrated slightly higher BMD (0.72 ± 0.08 g/cm²) and less negative T-scores (−2.85 ± 0.45), aligning with reports that diabetics may present preserved BMD despite higher fracture risk

from poor bone quality and advanced glycation end-product accumulation [16], [17]. The parathyroid disorder group exhibited marked cortical bone loss (BMD: 0.70 ± 0.08 g/cm²; T-score: −3.02 ± 0.54), while β-thalassemia patients had the most severe deficits (BMD: 0.57 ± 0.078 g/cm²; T-score: −4.17 ± 0.39), reflecting the skeletal consequences of ineffective erythropoiesis, marrow expansion, and iron overload [18]. These results reinforce the value of DXA-derived BMD and T/Z-scores in fracture risk assessment across varied osteoporosis subtypes.

Vitamin D deficiency was present in all osteoporosis cohorts, with the lowest levels observed in β-thalassemia (14.12 ± 3.49 ng/mL), followed by parathyroid disorder (14.88 ± 9.06 ng/mL) and T2DM (13.35 ± 5.28 ng/mL), linked to limited sun exposure, hepatic impairment, and defective hydroxylation [19], [20]. Serum calcium patterns differed markedly: parathyroid disorder patients had profound hypocalcemia (6.04 ± 2.26 mg/dL), whereas thalassemia patients maintained near-normal levels (8.95 ± 1.01 mg/dL), suggesting compensatory regulatory mechanisms. Additional parameters further distinguished groups T2DM patients showed poor glycemic control (HbA1c: 12.54 ± 3.44%), intensifying fracture risk *via* osteoblast dysfunction and advanced glycation end-product (AGE) accumulation. Elevated TSH in T2DM (13.34 ± 5.30 mIU/mL) and parathyroid disorder (17.68 ± 6.63 mIU/mL) indicated hypothyroidism, a factor known to impair bone turnover. In contrast, β-thalassemia patients displayed artifactually low HbA1c (4.26 ± 1.58%) due to transfusion-related red cell turnover, highlighting the importance of context-specific biomarker interpretation.

Serum WNT5A levels exhibit significant disease-specific dysregulation across osteoporosis subtypes (p-value = 0.0001), characterized by marked elevation in thalassemia-associated osteoporosis (0.89 ± 0.68 vs. controls) compared to reductions in DM-associated (0.129 ± 0.07) and parathyroid disorder-associated (0.12 ± 0.05) cohorts. This divergence underscores WNT5A's context-dependent role in skeletal homeostasis. While WNT5A can potentiate osteoblastogenesis *via* canonical Wnt/β-catenin signaling enhancement (upregulating Lrp5/6), it concurrently exhibits pro-osteoclastogenic activity, particularly under inflammatory conditions where its upregulation exacerbates bone resorption [21]. In DM-associated osteoporosis, dysregulation involves suppressed WNT5A expression (evidenced by reduced mRNA in clinical bone samples) and disrupted signaling due to hyperglycemia-induced reactive oxygen species (ROS) [22], [23], AGEs accumulation, and insulin resistance, impairing both canonical and non-canonical pathways [21], [24]. In osteoporosis, oxidative stress is a major contributor to the dysfunction of osteoblasts and osteocytes. The accumulation of ROS can lead to the denaturation of proteins and activation of apoptotic pathways [25], [26]. Several studies reported that oxidative stress plays a significant role in the development and progression of many types of diseases [27]-[30]. Parathyroid disorder-associated suppression correlates with chronic PTH exposure downregulating WNT5A/ROR2 signaling in osteoblasts, disrupting osteoblast-osteoclast crosstalk [31], [32]. Conversely, thalassemia-associated elevation aligns with iron chelation therapy (e.g., deferoxamine) inducing robust WNT5A upregulation (2–3.7-fold) in osteoprogenitors, promoting osteogenic differentiation and mineralization as a countermeasure to iron overload-induced bone loss [33]-[35]. Collectively, these findings position WNT5A as a critical, albeit complex, modulator of bone remodeling whose therapeutic targeting requires precise contextual understanding.

Serum TGF-β3 quantification across osteoporosis cohorts revealed context-dependent alterations compared to controls, demonstrating no significant change in uncomplicated osteoporosis. While osteopenic individuals without comorbidities exhibited levels (0.149 ± 0.16 ng/mL) consistent with reports of elevated TGF-β3 positively correlated with bone mineral density and osteoblast activity [8], [36], the osteoporosis with DM cohort showed stable levels (0.14 ± 0.09 ng/mL), potentially reflecting compensatory mechanisms counteracting metabolic bone disruption despite TGF-β3's known promotion of osteogenic differentiation in mesenchymal stem cells [9], [37]; this group further displayed significant negative correlations between TGF-β3 and HbA1c (indicative of hyperglycemia potentially impairing TGF-β3 function). In parathyroid disorder-associated osteoporosis, a slight elevation (0.15 ± 0.05 ng/mL) was observed, congruent with studies indicating PTH-induced reduction in TGF-β receptor signaling *via* internalization, yet permitting residual pathway activity [38]; a positive correlation with serum calcium aligned with TGF-β3's role in modulating intracellular calcium in bone cells and its association with bone mineral density. Strikingly, thalassemia-associated osteoporosis exhibited significantly elevated TGF-β3 (0.28 ± 0.18 ng/mL), likely representing a compensatory osteogenic response to the high-turnover bone loss driven by marrow

expansion and iron overload. Collectively, these findings underscore TGF-β3's integral role, shared amongst TGF-β superfamily members, in regulating osteoprogenitor proliferation, osteoblast differentiation, and bone remodeling dynamics. The observed mild variability most pronounced in thalassemia contrasts with stable or slightly elevated levels in DM and hyperparathyroidism, suggesting context-specific, often insufficient, reparative signaling. Consequently, TGF-β3 emerges as a potential biomarker indicative of bone remodeling status, particularly in complex or secondary osteoporosis etiologies, further supported by its high discriminatory power (AUC, sensitivity, specificity) in ROC analyses alongside WNT5A for osteoporosis diagnosis or fracture prediction.

5 CONCLUSIONS

This study highlights how comorbidities modulate signaling (WNT5A/TGF-β3) pathways in osteoporosis, identifying unique biomarker patterns that extend beyond conventional bone mineral density evaluations. In β-thalassemia-related osteoporosis, marked increases in WNT5A (0.89 ± 0.68) together with its strong correlations ($r = 0.763–0.961$, $p \leq 0.006$) indicate a compensatory response to persistent oxidative stress and iron overload, yet may paradoxically intensify bone deterioration through chronic inflammation and disrupted remodeling. In contrast, diabetes and parathyroid disorders suppressed this molecular network, compromising proteostasis and Wnt-driven bone formation. Notably, WNT5A (AUC ≤ 0.882) and TGF-β3 (AUC ≤ 0.891) outperformed traditional biomarkers in distinguishing thalassemia-associated osteoporosis. These results suggest that TGF-β3–WNT5A interplay represents a potential therapeutic target for comorbidity-tailored treatment and support incorporating WNT5A/TGF-β3 into precision diagnostic strategies for high-risk groups, particularly in resource-limited healthcare settings—linking mechanistic insights with more equitable osteoporosis care.

ACKNOWLEDGMENT

Authors would like to acknowledge all healthy individuals, patients with osteoporosis and all the medical staffs of Alyarmouk Teaching Hospital who participated in this study for their contribution.

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