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Levels of Serum Procollagen Type-1 Amino Terminal Propeptide and C-terminal Cross-linked Telopeptide of Type-1 Collagen in Children and Adolescents with Type 1 Diabetes Mellitus

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ABSTRACT

Background: In patients with type 1 diabetes mellitus (T1DM), accelerated bones turnover and bone loss are caused by increased inflammation. The serum levels of the bones turnover markers (BTM), serum procollagen type 1 N-terminal propeptide (P1NP) and serum C-terminal telopeptide of type I collagen (CTX), can help identify increasing bones fragility in T1DM earlier on. **Objective:** The aim of the study is to measure the levels of BTM in children with T1DM, specifically serum P1NP and serum CTX, and to look into the relationships between BTM and indicators of glycemic control and diabetics micro-vascular problems.

Patients and methods: A total of 40 pediatric and adolescent T1DM children were compared with a control group of healthy control, age- and sex-matched participants. HbA1c, fasting lipids profiles, microalbumin in urine, fasting serum P1NP, and serum CTX concentrations were measured in the laboratory.

Results: P1NP and CTX concentrations in the sick group were statistically significantly higher than those in the control group ($p<0.001$), according to the findings of our research. LDL-C and TDD of insulin, which rose in the uncontrolled sample, were determined to differ significantly between participants with HbA1c ≤7% and participants with HbA1c ≥7% ($p<0.001$ and $p=0.008$, respectively), although there was no differences in the levels of serum P1NP and CTX between the two samples. **Conclusion:** In younger T1DM children, serum concentrations of P1NP and CTX may offer predictive information on bones health. To demonstrate that oxidative stress causes major changes in the activities of bone production and resorption, more research should be conducted.

Keywords: BTM, P1NP, CTX, bone turnover, diabetes complications, type 1 diabetes, case control study, Ain Shams University.

INTRODUCTION

A metabolic disorder known as type 1 diabetes mellitus (T1DM) is defined by the autoimmune destruction of pancreatic beta-cells, which results in a lack of insulin release and the emergence of hyperglycemia [(1)].

It is one of the most prevalent autoimmune illnesses among children and teenagers, and its prevalence is growing globally [(2)].

Patients with diabetes either type 1 or 2 diabetes were found to have an increased bone fragility despite higher or even normal bone mineral density (BMD) [(3)]. For type 1 Diabetic patients, the higher risk of fractures has been attributed to a number of factors, notably alterations in bones microarchitectures, bones marrow features, and the balancing of bone turnover. In order to calculate bone production and/or resorption, bones turnover indicators are examined. Serum procollagen type 1 N-terminal propeptide (P1NP) is a significant marker included in our analysis that is recognized to be effective for estimating bones turnover, while serum C-terminal cross-linked telopeptide of type 1 collagen (CTX) is a marker for bones resorption [(4)].

Chronic inflammation and increased levels of pro-inflammatory cytokines are responsible for bone turnover acceleration and bone loss in patients with T1DM.

Another possible mechanism is that poorly controlled T1DM is associated with ongoing cellular oxidative stress. Previous studies have shown that exposure to chronic state of oxidative stress due chronic hyperglycemia could result in tissue damage and abnormal responses in the body's antioxidant defenses [(5)]. Free oxygen radicals in diabetes namely; malondialdehyde, high total serum cholesterol, and reactive hydrogen peroxides are responsible for the pathophysiology of several micro-vascular complications of T1DM through lipid peroxidation, DNA damage, and mitochondrial malfunction. Presence of free oxygen radicals result in oxidation of proteins, lipids, and nucleic acids and production of toxic products leading to tissue damage and dysfunction. Moreover, oxidative stress can produce an unbalance between proteolytic enzymes and their inhibitors that results in progressive osteoporosis in patients with T1DM. Previous studies on bone function in the presence of diabetes have shown that osteoblastic differentiation was inhibited and osteoblasts damage and apoptosis were evident. Eventually therefore, oxidative stress may be related to the pathogenesis of bone disorders associated with diabetes [(6)].

In earlier clinical trials on bones turnover, fractures risks, and osteoporosis, the usage of P1NP and CTX was advised. Variations in serum P1NP and serum

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CTX are as effective as BMD in determining the decrease of fractures risks in children receiving anti-resorptive and bones-strengthening medication, according to studies evaluating both BMD and BTM in relation with fractures risks in osteoporotic patients [7].

As a result, employing BTM during bones treatment may also be advantageous; nevertheless, the variance of BTM is affected by a number of pre-analytical parameters. While CTX is a suggested biomarker for bones resorption, a number of factors affect its level. Among those considerations is the circadian rhythm of CTX, which has a concentration peak at about 5 a.m. and a lower point at about 2 pm [8].

The levels of CTX are also influenced by other variables such as dietary intake, renal dysfunction, age, and activity. In order to reduce pre-analytical fluctuation as much as feasible, assessments of serum CTX must be performed on individuals who are fasting and earlier in the morning too though. In contrast, because P1NP has a weak circadian rhythms, the timing of serum P1NP withdrawals can be disregarded if it is the only BTM being examined. Similar to CTX, particular dietary intake can affect the serum level of P1NP, albeit to a smaller extent. The day before blood sample, avoid engaging in strenuous exercise to reduce fluctuations in its level [9].

PATIENTS AND METHODS

Study design

A total of 40 adolescents and children with T1DM, who were in the paediatric age (range 8-18 years), and T1DM for more than a year participated in this case control study. They were chosen from the Ain Shams University Hospital's Pediatric and Adolescent Diabetes Clinic. According to glycemic control, the participants' sample was further separated into two groups: a controlled group with HbA1c of less than <7% and an uncontrolled group with a HbA1c of more than >7%. Ain Shams University Hospitals' Children Hospital gathered 40 normal participants with similar age and gender characteristics from its outpatient department.

Inclusion Criteria

The International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines for diabetic identification in children, 2018, were used to make the T1DM diagnosis.

- Individuals who satisfied the requirements of fasting for 8 hours prior to sample collection and halting basal and bolus insulin prior to their visit's morning appointment.
- Children and teenagers who have had T1DM for further than one year.

Exclusion Criteria

The following patients were excluded from the study:

- Children with T1DM duration less than 1 year.
- Children who are taking medication for vitamin D (25(OH)D <30 ng/ml), bisphosphonates, or calcium deficiencies.
- Patients with history of diseases affecting bone turnover markers other than diabetes e.g. chronic inflammation, chronic diseases, and recent bone fractures.
- Patients taking medications affecting bone mineral density such as corticosteroids.

All participants in this study were subjected to the following:

1. Full history taking was conducted laying stress on; duration of diabetes, symptoms of complications such as neuropathy as burning sensation, numbness, tingling, ulcers, and pain. Symptoms of nephropathy such as headache, low urinary output, and presence of lower limb edema. Symptoms of retinopathy such as blurring of vision and decreased visual acuity. Total daily dose (TDD) of insulin was calculated for each patient.
2. Physical Examination: All survey respondents got thorough physical examinations, included neurological, chest, and abdominal checks as well as assessments of height and weight. Body mass index (BMI) percentile graphing based on the proper age and sex for height, weight, and BMI. Muscles tones and strength, tendon reflexes, vibrating sensitivities, and the existence of edema in the lower extremities. A qualified ophthalmologist used direct ophthalmoscopy to do a fundus evaluation for the purpose of assessing retinopathy. Using the Bedside Neuropathy Disability Scoring (NDS) as a screening instrument, diabetic peripheral neuropathy was identified. The big toe's ability to feel pinpricks and temperature changes, as well as the existence or absence of ankles reflexes, were all tested to determine this level. The sensory symptoms were noted and evaluated, and clinical diabetic peripheral neuropathy was identified with a score of 2 or above.
3. Laboratory Investigations

Sampling method: A clean venipuncture was used to acquire 10mL of fasting venous blood, of which 2mL were deposited in an EDTA tubes for further HbA1c testing and the other 8mL were evacuated into two standard test tubes. Centrifugation was used to isolate the serum (1000x g for 15 minutes). While the serum obtained from the second tube was separated into three replicates and kept at −20 °c for later testing, the serum from the first tube was directly analysed for its lipids profile.

A. On the Bio-Rad d-10 hemoglobin testing machine, the higher performance liquid chromatography (HPLC) method was used to measure HbA1c (Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drives, Hercules,

California 94547, USA). On the Beckman AU-680 equipment auto-analyzer (Beckman Coulter, Inc. Diagnostics Division Headquarters250 South Kraemer Boulevard Brea, California 92821-6232 USA), serum specimens were tested for lipids profile applying company-supplied reagents.

B. Higher density lipoprotein cholesterols (HDL-C) test has been focused on precipitations of lower density lipoprotein cholesterol (LDL-C) and very lower density lipoprotein cholesterol (VLDL-C), and then the cholesterol in the HDL cholesterol fractions which stays in the supernatant is analyzed by a scheduled endpoint technique. Triglycerides (TG) and total cholesterol were evaluated on AU680 is focused on enzymatic colorimetric technique. Using the "Friedwald formula," LDL cholesterol is computed as follows: LDL-cholesterol = Total cholesterol-(HDL-C+TG/5).

C. AER (albumin elimination rates); Immune turbidimetric techniques. It is employed to determine whether nephropathy is present. Individuals who had AER >30 mg/mg creatinine at the time of initial detection were requested to complete two further urine collections at intervals of three to six months. When two out of three specimens displayed an outflow ratio of 30 to 300 mg/mg creatinine, persistent microalbuminuria was considered to exist [(10)].

D. At the beginning of the trial, serum levels of vitamin 25OHD3 were measured using a Micromass Quattro Ultima Pt mass spectrometer in a confirmed NIST traceable LC-MS/MS assays (Waters Corp., Milford, MA, USA). Patients with 25OHD3 levels less than 30 ng/ml were not included in the research.

E. Procollagen type 1 amino terminal propeptide (P1NP) and the bones resorption indicator C-terminal cross-linked telopeptides of type 1 collagens (CTX) were measured in blood samples using an ELISA kits.

Before serum P1NP and serum CTX removal, take the following precautions:

- Before collecting the sample, participants fasted for at least 8 hours.
- Participants should refrain from strenuous activity 24 hours before sample.
- Twelve hours prior to the morning of the blood samples collection, participants were stopping their basal and bolus insulin.
- At 8 am, participants withdrew blood samples (to prevent diurnal changes in serum CTX concentrations).

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Ethical considerations:

The Ethics Committee of the Faculty of Medicine at Ain Shams University gave its approval to the research. Each individual that was included in the research provides written consent, which was acquired from their parents/guardians. The conduct of this study was guided by the Helsinki Declaration, the World Medical Association's rule of ethics for human studies.

Statistical Analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 25.0 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages.

Chi square test ($\chi 2$) and Fisher's exact test to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean and standard deviation (SD). Independent samples t-test or Mann-Whitney U test was used to compare between two independent groups.

Both serum PINP and serum CTX were modelled separately using regression analysis, and the independent variables included (Age, sex, disease status, lipids profile and BMI). P-value $\leq$0.05 was considered significant.

RESULTS

A total of 40 children and adolescents with T1DM participated in our research; there were 26 females and 14 boys, with a mean age of 12 (SD 3.34) years.

The average illness lasted 7.08 (SD 3.05) years. They were contrasted with a 40-person, gender- and age-matched healthy group of controls. Tanner stage for pubertal evaluation revealed no pubertal delays in any of our participants. Regarding anthropometric measurements, there was no statistically significant distinction between the two samples.

Regarding the clinical and laboratory results, serum concentrations of triglycerides and low-density lipoprotein cholesterol (LDL-C) were found to be significantly greater in patients than in controls ($p<0.001$ and $p=0.043$, respectively), while HDL-C was found to be less in the patient population compared to the control group ($p<0.001$), as demonstrated in **Table 1**.

Table (1): Descriptive and comparative statistics of lipid profile parameters between Patients with T1DM and control group

Variable		Control group No. = 40	Patients group No. = 40	Test value	P-value	Sig.
Cholesterol mg/dL	Mean ± SD	172 ± 15	181 ± 30	1.580•	0.117	NS
Triglycerides mg/dL	Mean ± SD	141 ± 12	101 ± 3	7.257•	<0.001	HS
HDL mg/dL	Mean ± SD	58 ± 5	44 ± 10	8.315•	<0.001	HS
LDL mg/dL	Mean ± SD	75 ± 9	82 ± 19	2.051•	0.043	S

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant. •: Independent t-test.

Compared to the controls, there was a substantially significant rise in the concentrations of the BMT indicators PINP and CTX in the patients' sample (p<0.001) (**Table 2**).

Table (2): Descriptive and comparative statistics of serum CRP, P1PN and CTX between Patients with T1DM and control group.

Variable		Control group No. = 40	Patients group No. = 40	Test value	P-value	Sig.
CRP mg/L	Mean ± SD	3.5 ± 0.37	7.1 ± 1.28	5.205•	<0.001	NS
PINP ug/L	Mean ± SD	54 ± 12	447 ± 9	7.635•	<0.001	HS
CTX ng/mL	Mean ± SD	2.2 ± 0.7	16.8 ± 3.9	9.228•	<0.001	HS

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant. •: Independent t-test.

According to glycemic control, the patients' group was further categorized into two parts: a controlled group with a HbA1c of less than 7% and an uncontrolled group with a HbA1c of more than 7%. A significant difference was found between the two groups in terms of LDL-C and TDD of insulin (p<0.001, p = 0.008, respectively), which rose in the uncontrolled group (HbA1C >7%). With a p-value of <0.001, HDL-C was, though, lower in the uncontrolled group (HbA1C >7%). However, there was no statistically significant change in the two groups' serum P1NP and CTX levels, which are BTM indicators (**Table 3**).

Table (3): Descriptive and comparative statistical analysis of descriptive and laboratory data between controlled and uncontrolled patients with T1DM.

Variable		Patients with HBA1c =<7 N 12 (30%)	Patients with HBA1c >7 N 28 (70%)	*Test Value	P value	Sig.
Age (in years)	Mean ± SD	11±4	13± 2	1.424	0.163	NS
Disease duration (in years)	Mean ± SD	6.50±2.71	7.32± 2.56	0.912	0.368	NS
BMI Z score	Mean ± SD	0 ± 0.5	0 ± 1.5	0.947	0.397	NS
Microalbumin/creatinine ratio (mg/g)	Mean ± SD	10.36± 2.8 7	62.61± 4.94	1.125	0.286	NS
CRP mg/L	Mean ± SD	9± 2	22± 3	1.217	0.231	NS
TDD (Units/Kg/day)	Mean ± SD	1± 0.16	2± 0.43	5.124	<0.001	HS
Cholesterol mg/dL	Mean ± SD	172± 25	184± 32	1.246	0.220	NS
TG mg/dL	Mean ± SD	97± 18	102± 9	0.410	0.684	NS
HDL mg/dL	Mean ± SD	33± 5.32	48± 9.29	5.420	<0.001	HS
LDL mg/dL	Mean ± SD	93± 19	77± 16	2.822	0.008	HS
PINP ug/L	Mean ± SD	553± 37	402± 7	1.325	0.198	NS
CTX ng/ml	Mean ± SD	17.2± 3.1	16.6± 4.5	0.182	0.856	NS

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant. •: Independent t-test.

According to the results of the multiple linear regression analysis for factors determining P1NP concentration, CTX's effect on P1NP level remained significant and was unaffected by the other factors, as illustrated in **Table 4** with p value <0.001.

/Table (4): The multiple linear regression analysis for factors affecting P1NP level.

Independent variables	Unstandardized Coefficients		Standardized Coefficients	T	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Age	-11.518	12.181	-0.106	-0.946	0.348	-35.838	12.802
Gender(Male)	9.708	57.548	0.016	0.169	0.867	-105.190	124.606
Groups (case)	143.607	102.181	0.237	1.405	0.165	-60.404	347.617
BMI	-0.503	9.917	-0.008	-0.051	0.960	-20.303	19.297
C peptide	145.281	83.198	0.152	1.746	0.085	-20.829	311.391
Cholesterol	-0.660	1.130	-0.053	-0.584	0.561	-2.916	1.596
TG	-1.118	1.102	-0.119	-1.014	0.314	-3.317	1.082
HDL	-2.008	4.001	-0.072	-0.502	0.618	-9.996	5.981
LDL	-0.112	2.239	-0.006	-0.050	0.960	-4.581	4.358
CRP	-3.206	6.066	-0.056	-0.529	0.599	-15.317	8.905
CTX	15.074	3.558	0.507	4.237	<0.001*	7.970	22.177

Tanner staging is shown in **Table 5**; in our sample, the vast majority of boys (about 70%) were all in stages 1 and 2, whereas almost half of girls (45%) were in stages 1 and 2**.**

Table (5): Tanner staging in patients with Type 1 Diabetes Mellitus

Variable		Gender				Chi-square test	P value
		Female		Male			
		N	%	N	%		
Tanner Staging	1	5	10.90%	12	35.30%	4.8	0.308
	2	6	13.00%	12	35.30%		
	3	21	45.70%	8	23.50%		
	4	11	23.90%	2	5.90%		
	5	3	6.50%	0	0.00%		

There were no statistically significant distinctions between the sexes when P1NP and CTX serum concentrations were compared in males and females with T1DM, as indicated in **Table 6**.

Table (6): Comparison of serum levels of P1NP and CTX between males and females with T1DM.

Gender			Mean	SD	T test	P value
CTX ng/ml	Female	46	10.539	1.7041	1.107	0.27
	Male	34	7.994	1.3689		
PINP ug/L	Female	46	277.07	31.083	0.917	0.326
	Male	34	214.26	26.082		

According to **Table 7**, P1NP and CTX serum concentrations in the patients' group at various stages of puberty were not statistically significant (p>0.05).

Table (7): Serum P1NP and CTX in patients with T1DM in different stages of puberty according to Tanner staging of puberty.

Variable		Tanner Staging					ANOVA	P value
		1	2	3	4	5		
CTX ng/ml	Mean	9.6	7.6	9.6	11.6	9.2	0.284	0.887
	Standard Deviation	2.4	1.5	2.1	2.2	1.8		
PINP ug/L	Mean	335.56	183.32	211.64	312.28	293.5	0.821	0.516
	Standard Deviation	59.9	48.3	32.5	47.5	43.83		

DISCUSSION

Given the peculiar characteristics of skeletal development throughout growth, it is crucial to comprehend the process of bones turnover in children and adolescents with T1DM. Peak amounts of bone turnover occur throughout puberty and the development spurts, and this process is ongoing [(11)]. In our study, serum levels of BMT markers (P1NP, CTX) demonstrated a statistically significant increase in patients' group in comparison to controls' group ($p<0.001$).

The increased levels of P1NP would simply represent an enhanced osteoblastic function. Furthermore, there could be malfunctioning osteoblast activities, which is primarily brought on by the adverse effects of other variables such chronic hyperglycemia, lower levels of IGF-1 and insulins, lower levels of IGF-1, and a buildup of advanced glycation outcomes [(12)]. It is more plausible, in our view, elevated bones turnover is the cause of the P1NP concentration increases. The bones deficiency (both for bones architecture and bones strength) linked to insufficient osteoblastic activities may be expressed by this trait in the future [(13)].

Furthermore, there was no statistically significant distinction between participants with managed T1DM (HbA1c <7%) and those with uncontrolled diabetes (HbA1c >7%) in our investigation in terms of serum concentrations of P1NP. These long-term micro-vascular problems of T1DM, like as micro-vascular and neurological issues, may be linked to these changes in the bones. Additionally, it has been hypothesized that hyperglycemia, as found in T1DM, reduces the anabolic effect of physical activities, specifically mechanical stimulating on bones, perhaps accentuating the damaged bones seen in diabetic patients [(14)].

According to earlier research by ***Khoshhal et al.,*** kids with T1DM had elevated serum concentrations of CTX, Tartrate-Resistant Acids Phosphatases (TRAP), and Pyridinoline (PYD) than did kids without the condition [(15)]. Serum CTX concentrations and insulin sensitivity were found to be correlated, and an elevation in CTX may serve as a compensatory mechanism to enhance glucose homeostasis [(16)]. This hypothesis corroborates our observations of elevated CTX levels. As a defense mechanism against insulinopenia, prior research hypothesized that an increase in CTX Z-score would lead to a rise in insulin levels. A 4-year follow-up revealed that normoglycemic individuals who had the greatest tertile of CTX initially had the greatest incidence of dysglycemia 4 years later (46.5%), providing additional evidence for the idea that CTX mediates compensatory mechanisms [(17)].

In our study, serum CTX levels was not statistically significant on comparing controlled and uncontrolled young patients with T1DM. Unfortunately, also CTX Z-score charts are not available for growing children with T1DM. Further studies are needed on large number of patients to record CTX in different sex and age in growing children as well as different levels of glycemic control. In our study, serum levels of P1NP and CTX were statistically not significant in different stages of puberty nor with sex differences ($p>0.05$), this can be attributed to the small size of our sample, and further studies on large number of patients are needed to confirm this finding.

As per glycemic controls, our participants' sample was further separated into two groups: a controlled group with a HbA1c of less than 7% and an uncontrolled group with a HbA1c of more than 7%. Regarding LDL-C and TDD of insulins, there was a statistically significant distinction between the two groups ($p<0.001$ and $p=0.008$, in both), which enhanced in the uncontrolled group (HbA1C >7%). Individuals with uncontrolled diabetes and chronic hyperglycemia typically have increased liver synthesis of very lower-density lipoproteins (VLDL) and ApoC-III, as well as increased intestinal uptake of chylomicrons. As a result, participants with concomitant insulin resistance may experience extended dyslipidemia, which is a relatively common outcome [(18)]. Due to competition between VLDL and chylomicrons on the lipoprotein-mediated pathways to remove TG from the circulatory, postprandial hyperlipidemia impairs the elimination of both VLDL and TG [(19)].

Cholesteryl-ester-transfer proteins (CETP), which controls the exchanging of VLDL or chylomicrons for LDL cholesteryl esters that would eventually generate tiny dense LDL, regulates the prevalence of smaller and dense LDL under settings with high serum insulin. In addition, low concentrations of HDL and apoA-I cholesterols, higher rates of TG hydrolysis, higher levels of hepatic lipase, and higher levels of lower HDL are linked to higher TDD of insulin and related insulin resistance. The hepatocytes would prefer to clean smaller, denser HDL particles more quickly than bigger HDL particles, which will additionally aid in the reduction in HDL cholesterol [(20)].

CONCLUSION

Serum levels of P1NP and CTX may provide prognostic information about bone health and bone fracture risk in young patients with T1DM. However, more studies should be done on BTMs to prove that oxidative stress induces significant alterations of the differentiation process and activity of bone formation and resorption. Further studies are recommended on large number of patients to be able to evaluate BTMs in different stages of puberty and in both sexes as proper Z-scores are needed. It's probable that a number of pathways, particularly chronic hyperglycemia and/or insulinopenia, affect the procedures that affect bones turnover in T1DM. In T1DM teenagers who switch

to a more physiological insulin supplementation using continuous subcutaneous insulin infusion (CSII) via insulin pumps, prior literature have shown a notable decrease in diabetes complications [(21)]. Hence, additional research on BTM and BMD in diabetic adolescents receiving insulin pumps treatment is advised.

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REFERENCES

1. **Clark M, Kroger C, Tisch R (2017):** Type 1 Diabetes: A Chronic Anti-Self-Inflammatory Response. Front Immunol.., 8:1898. doi: 10.3389/fimmu.2017.01898.
2. **Kimbell B, Lawton J, Boughton C *et al.* (2021):** Parents' experiences of caring for a young child with type 1 diabetes: a systematic review and synthesis of qualitative evidence. BMC Pediatr., 21:160.
3. **Sealand R, Razavi C, Adler R (2013):** Diabetes mellitus and osteoporosis. Curr Diab Rep., 13(3):411-8. doi: 10.1007/s11892-013-0376-x.
4. **Starup-Linde J, Vestergaard P (2016):** Biochemical bone turnover markers in diabetes mellitus - A systematic review. Bone, 82:69-78. doi: 10.1016/j.bone.2015.02.019.
5. **Khoshhal K, Sheweita S, Al-Maghamsi M *et al.* (2015):** Does type 1 diabetes mellitus affect bone quality in prepubertal children? Journal of Taibah University Medical Sciences, 10(3):300-5.
6. **Sandukji A, Al-Sawaf H, Mohamadin A *et al.* (2011):** Oxidative stress and bone markers in plasma of patients with long-bone fixative surgery: role of antioxidants. Hum Exp Toxicol., 30(6):435-42.
7. **Langdahl B, Ferrari S, Dempster D (2016):** Bone modeling and remodeling: potential as therapeutic targets for the treatment of osteoporosis. Ther Adv Musculoskelet Dis., 8(6):225-35. doi: 10.1177/1759720X16670154.
8. **Chubb** S **(2012):** Measurement of C-terminal telopeptide of type I collagen (CTX) in serum. Clinical Biochemistry, 45(12):928-35. doi: 10.1016/j.clinbiochem.2012.03.035.
9. **Szulc P, Naylor K, Hoyle N *et al.* (2017):** Use of CTX-I and PINP as bone turnover markers: National Bone Health Alliance recommendations to standardize sample handling and patient preparation to reduce pre-analytical variability. Osteoporos Int., 28:2541-56.
10. **Donaghue K, Marcovecchio M; Wadwa R *et al.* (2018):** ISPAD Clinical Practice Consensus Guidelines 2018: Microvascular and macrovascular complications in children and adolescents. Pediatric Diabetes, 19(27):262-74.
11. **Jürimäe J (2010):** Interpretation and application of bone turnover markers in children and adolescents. Current Opinion in Pediatrics, 22:494-500. doi: 10.1097/MOP.0b013e32833b0b9e.
12. **Fowlkes J, Nyman J, Bunn R *et al.* (2013):** Osteo-promoting effects of insulin-like growth factor I (IGF-I) in a mouse model of type 1 diabetes. Bone, 57. doi: 10.1016/j.bone.2013.07.017.
13. **Florencio-Silva R, Sasso G, Sasso-Cerri E *et al.* (2015):** Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. Biomed Res Int., 2015:421746. doi: 10.1155/2015/421746.
14. **Parajuli A, Liu C, Li W *et al.* (2015):** Bone's responses to mechanical loading are impaired in type 1 diabetes. Bone, 81:152-60. doi: 10.1016/j.bone.2015.07.012.
15. **Khoshhal K, Sheweita S, Abp M*et al.* (2015):** Does type 1 diabetes mellitus affect bone quality in prepubertal children? doi: 10.1016/j.jtumed.2015.03.004.
16. **Liu T, Liu D, Xuan Y *et al.* (2017):** The association between the baseline bone resorption marker CTX and incident dysglycemia after 4 years. Bone Res., **5**:17020.
17. **Madsen J, Herskin C, Zerahn B *et al.* (2019):** Bone turnover markers during the remission phase in children and adolescents with type 1 diabetes. doi: 10.1111/pedi.12963.
18. **Artha I, Bhargah A, Dharmawan N *et al.* (2019):** High level of individual lipid profile and lipid ratio as a predictive marker of poor glycemic control in type-2 diabetes mellitus. Vasc Health Risk Manag., 15:149-157. doi: 10.2147/VHRM.S209830.
19. **Feingold K (2021):** Introduction to Lipids and Lipoproteins. In: Feingold KR, Anawalt B, Boyce A, et al., Editors. https://www.ncbi.nlm.nih.gov/books/NBK305896/
20. **Gregg E, Li Y, Wang J *et al.* (2014):** Changes in diabetes-related complications in the United States, 1990-2010. N Engl J Med., 370(16):1514-23. doi: 10.1056/NEJMoa1310799.
21. **Downie E, Craig M, Hing S *et al.* (2011):** Continued reduction in the prevalence of retinopathy in adolescents with type 1 diabetes: role of insulin therapy and glycemic control. Diabetes Care, 34(11):2368-73. doi: 10.2337/dc11-0102.