Journal of Mind and Medical Sciences

Volume 6 | Issue 1 Article 14

2019

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Recommended Citation
Dănciulescu Miulescu, Rucsandra; Guja, Loreta; Ochiana, Lavinia Claudia; Ungurianu, Anca; Șeremet, Oana Cristina; and Ștefănescu, Emil (2019) "Serum markers of bone fragility in type-2 diabetes mellitus," *Journal of Mind and Medical Sciences*: Vol. 6 : Iss. 1 , Article 14.
DOI: 10.22543/7674.61.P7885
Available at: https://scholar.valpo.edu/jmms/vol6/iss1/14

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https://scholar.valpo.edu/jmms/
https://proscholar.org/jmms/
ISSN: 2392-7674

J Mind Med Sci. 2019; 6(1): 78-85
doi: 10.22543/7674.61.P7885

Received for publication: April 11, 2018
Accepted: July 14, 2018

Review

Serum markers of bone fragility in type-2 diabetes mellitus

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Abstract

Patients with type-2 diabetes mellitus (T2DM) have normal or increased bone mineral density (BMD) but despite that, they are characterized by an increased hip and vertebral fracture risk that involves the alteration of bone quality and not the reduction in bone mass. BMD is utilized for the diagnosis and evaluation of osteoporosis, but BMD itself cannot provide an accurate diagnosis of the individuals at increased risk of fracture and, therefore, studies have focused on identifying other risk factors that are partially or fully independent of BMD.

The fracture risk score tool-FRAX® models provide information about a 10-year probability of osteoporotic fractures, but do not include risk factors specific to illness such as diabetes duration, diabetes drug therapy, glycemic control, or the presence of micro-vascular complications. Multiple markers have been investigated to provide information on the risk of fractures in patients with T2DM such as: advanced glycation end products (AGEs), insulin-like growth factor-I (IGF-I), osteocalcin (OC), adiponectin, and sclerostin, but epidemiological studies did not provide homogeneous information regarding the link between these markers and bone fragility in T2DM subjects. Markers that increase the accuracy of fracture risk estimation in patients with T2DM need to be identified and employed in current medical practice.

Keywords : diabetes mellitus, bone fragility, markers of fracture risks

Highlights

- ✓ Patients with type 2 diabetes mellitus have significantly higher scores in over-vigilance and inhibition schematic domains.
- ✓ Epidemiological studies do not provide unitary information on the association between markers of bone fragility and fracture risk in T2DM.

To cite this article: Miulescu RD, Guja L, Ochiana LC, Ungurianu A, Șeremet OC, Ștefănescu E. Serum markers of bone fragility in type-2 diabetes mellitus. *J Mind Med Sci*. 2019; 6(1): 78-85. DOI: 10.22543/7674.61.P7885

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Introduction

According to the American Diabetes Association, diabetes is a heterogeneous chronic disease which includes the following categories: type-1 diabetes (T1DM) generated by beta-cell destruction; T2DM due to a progressive loss of beta-cell insulin and insulin resistance; and gestational diabetes and other types of diabetes caused by other problems. T2DM represents 90-95% of all types of diabetes (1). The International Diabetes Federation (IDF) estimates the global prevalence of diabetes in the age group of 20–79 years in 2015 at 415 million, with an uncertainty range of 340–536 million. For the same group, IDF predicted 642 million with an unclear range: 521–829 million for 2040 (2). After the analysis of the published studies, Kaiser AB and co-workers estimate that in 2018 there are more than 500 million cases of T2DM worldwide and this prevalence will increase (3). In 2018, in Romania the number of patients with diabetes who underwent treatment was 823,280, out of which 241,600 following insulin therapy and 581,680 non-insulin agents (4).

Individuals with T2DM present normal ranges or increased ranges of BMD in comparison with subjects without diabetes. Despite that, they are characterized by increased fracture risk caused by the alteration of bone quality (5). The Rotterdam study evaluated the correlation between T2DM and fractures in 6,655 men and women aged 55 years or older compared to subjects without diabetes. The data from the study show that diabetic patients had high BMD and increased fracture risks compared to the control group (6). The observational study conducted by Leslie WD and co-workers, in which 6,455 diabetic patients and 55,958 subjects without diabetes were included, has revealed diabetes as an independent risk factor for major osteoporotic fractures (7).

The association between the duration of the disease, glycemic control, the presence of microvascular complications, and the risk of fracture have been investigated in several other studies. The Manitoba cohort which included 57,938 women, 8,840 with diabetes and 49,098 without diabetes, aged 40 years or more, had highlighted that diabetes is a risk factor for major osteoporotic fractures only in women who have had the illness for a long period of time (more than 10 years) (8). Another study conducted by Leslie WD and collaborators in which 82,094 diabetic patients and 236,682 non-diabetic subjects were enrolled highlighted that long-term diabetes is correlated with an increased risk of fractures. Newly diagnosed patients have a low risk of fractures (9). The Blue Mountains Eye Study showed that the duration of diabetes for 10 years or more is associated with proximal humeral fracture (10). Concerning the glycemic ranges and fracture risk, in a meta-analysis published in 2007 in *Osteoporosis International*, Vestergaard P found no correlation between glycated hemoglobin (HbA1) and BMD (11). In contrast, other studies support the association between increased fracture risk and poor glucose control (12, 13). Patients with T2DM and microvascular disease have a compromised cortical bone microarchitecture via an increase in cortical porosity (14).

BMD is utilized in the diagnosis and evaluation of osteoporosis, and the most used and validated technique for determining BMD is dual energy X-ray absorptiometry (DEXA). The international reference for the diagnosis of osteoporosis is a T-score equal to or lower than -2.5 standard deviation (SD) at the lumbar spine or femoral neck and osteopenia as a T-score between -1.0 SD and -2.5 SD (15, 16). BMD itself cannot provide a good estimation of the individuals at higher risk of fracture. Therefore studies have focused on identifying other risk factors that are partially or fully independent of BMD. The FRAX® models provide information about a 10-year probability of osteoporotic fractures, with and without BMD at the femoral neck (16).

There is a variant in the Romanian language version, which includes 12 items: age/sex/height(cm) /weight(kg), fracture history, parental hip fracture, active smoking, glucocorticoid therapy, rheumatoid arthritis, secondary osteoporosis, minimal alcohol 3 U/day, and BMD at the femoral neck. The item on secondary osteoporosis only mentions insulin-dependent type-1 diabetes mellitus (T1DM) and not T2DM. T2DM represents a risk factor that is independent of conventional risk factors. For this reason Ferrari SL and collaborators proposed the following risk factors specific to the illness: diabetic age > 5 years, diabetic drug therapy (insulin, thiazolidinedione, sodium-glucose co-transporter 2-SGLT2 inhibitors), HbA1c >7%, micro-vascular complications (17). The results of two studies published in 2012 in the *Journal of Bone and Mineral Research* suggest the inclusion of diabetes in future iterations of FRAX® (18, 19).

Serum potential markers for bone fragility in patients with T2DM

Multiple serum markers have been investigated to provide information on the risk of fractures in patients with T2DM. Serum indicators of bone turnover include bone formation markers produced by direct or indirect

osteoblasts activity, resorption markers which are derived from the skeletal collagen and other indicators.

Bone formation indicator consists of osteocalcin, alkaline phosphatase, bone-specific alkaline phosphatase, procollagen type 1 amino terminal pro-peptide (P1NP), procollagen type 1 carboxy-terminal pro-peptide (P1CP), and osteoprotegerin (20).

Osteocalcin is a protein secreted by osteoblasts. It is considered a marker of bone formation, but osteocalcin also has metabolic effects. Studies on animal models have shown that osteocalcin has a positive impact on insulin expression in beta-cells and adiponectin in adipocytes (21, 22). Clinical trials confirmed the correlation between osteocalcin, glucose and adiponectin levels - osteocalcin was negatively correlated with plasma glucose levels and positively correlated with serum adiponectin and insulin sensitivity [23, 24, 25]. Hyperglycemia alters osteocalcin secretion and glycemic control improves the osteocalcin level [26]. Adiponectin is expressed in bone marrow fat. Osteoblastic cells have adiponectin receptors and adiponectin participates in the differentiation, proliferation and mineralization of osteoblasts (27, 28).

Alkaline phosphatase is an enzyme highly expressed in bone, liver, and kidney. Increased serum alkaline phosphatase levels are found throughout bone growth, in the liver and in bone diseases. Alkaline phosphatase is an inflammatory mediator and previous works have shown an association between alkaline phosphatase levels and cardiovascular events (29, 30). The results of studies on alkaline phosphatase levels in diabetic patients are contradictory. Studies highlighted that diabetic patients have a lower, similar or an increased serum alkaline phosphatase level compared to the control group. In a prospective study, Dutta MK and collaborators have evaluated alkaline phosphatase levels in 67 patients with T2DM and 137 nondiabetic subjects. The mean alkaline phosphatase levels were lower with statistical significance in diabetic patients compared to the control group (88.5 ± 33.3 U/l *vs* 214.7 ± 59.7 U/l, $p < 0.00001$) and the authors suggested that the low levels of alkaline phosphatase reflect a decreased bone turnover and increased osteoclast genesis (31). Akin O *et al* report similar values of alkaline phosphatase concentration in a study group and a control group, while other researchers have highlighted that the mean serum level of alkaline phosphatase in diabetic patients with poor glycemic control is significantly higher compared to healthy controlled subjects (29, 32). Bone alkaline phosphatase is one of the isoforms of the alkaline phosphatase on the surface of osteoblasts and it is an indicator of osteoblast metabolism (33). Insufficient studies are available to establish the correlation between bone alkaline phosphatase and the risk of fractures in T2DM patients. The "*Japanese Population-based Osteoporosis Cohort Study*" evaluated bone alkaline phosphatase in 522 postmenopausal women without illness or treatment affecting bone metabolism. The results of the study revealed that bone alkaline phosphatase was correlated with a risk of vertebral fractures in the patients included in the survey (34). In a cross-sectional study in which 143 diabetic patients and 4.054 subjects without diabetes were enrolled no differences were found in bone alkaline phosphatase between the two groups (33).

Type 1 pro-collagen is synthesized and secreted in the bone matrix. At this level, pro-collagen peptidases cut P1NP from the amino-terminal end and P1CP from the carboxy-terminal end, resulting in mature type 1 collagen. (35). P1NP and P1CP are standard bone formation markers but not currently included in the assessment of fracture risks as well as other markers of bone turnover due to the lack of conclusive information (36). In a cross-sectional study in which 155 healthy subjects were included, P1NP was inversely correlated with insulin and glucose levels (37). In another study in which 183 postmenopausal females were included, 93 with osteoporosis (the study group) and 90 without osteoporosis (the control group), the mean P1NP level was higher, with statistical significance in the study group compared to the control group (38). Liu S *et al* analyzed the level of P1NP in 76 patients with T2DM (19 with osteoporosis, 25 with osteopenia, and 32 with preserved bone mass). The level of P1NP was significantly higher in T2DM patients with preserved bone mass and osteopenia than in diabetic patients with osteoporosis ($p < 0.05$) (39). A review published in 2017 in *the European Journal of Endocrinology*, which included the results of 66 studies, showed that P1NP levels were lower in patients with diabetes compared to control groups (40). The assessment of P1CP levels is increased in subjects with T2DM and it is an indicator for the diastolic dysfunction and progression of diabetic nephropathy (41, 42).

Bone resorptive markers are represented by amino-terminal cross-linked telo-peptide of type-I collagen (NTX), carboxy-terminal cross-linked telopeptide of type-I collagen (CTX), tartrate-resistant acid phosphatase 5b (TRAP), receptor activator of nuclear factor kappa beta ligand (RANKL), pyridinoline, deoxypyridinoline, hydroxyproline (20).

The evaluation of bone resorptive markers provides inconsistent results about the association of these markers

with diabetes. The "*Fremantle Diabetes Study*" revealed higher CTX levels in T1DM male patients (43). A cross-sectional study evaluated bone resorption markers (CTX and TRAP) in 78 T2DM patients compared to 55 subjects without diabetes. T2DM patients have decreased levels of bone resorptive markers compared to control patients (44). Osteoblast cells express RANKL which bind to their RANK receptor on the surface of osteoclasts and stimulate the differentiation of precursors in mature osteoclasts. Osteoprotegerin is secreted by osteoblasts and protects the skeletal system from exacerbated bone resorption by interacting with RANKL and preventing it from binding to RANK (45). Alteration of the RANKL/osteoprotegerin pathways have been involved in metabolic bone disease, vascular calcification and atherogenesis (46). Epidemiological studies do not provide homogeneous information on RANKL and osteoprotegerin levels in diabetic patients. A case-control study evaluated RANKL, osteoprotegerin levels in 42 women (21 women with T2DM and 21 women without diabetes). The authors found decreased levels of RANKL in the study group compared to the control cohort; the osteoprotegerin levels were not significantly different between the groups (47). Lappin DF *et al* evaluated plasma levels of bone markers in 63 patients with T1DM and 38 control subjects. T1DM patients had significantly decreased RANKL levels and increased osteoprotegerin compared to the control group (48). In contrast, increased levels of RANKL and increased osteoprotegerin were found in 40 young patients with T1DM compared to 40 healthy control subjects (49).

Other studied serum markers of bone fragility in diabetes are AGEs, IGF-I, and sclerostin.

AGEs are formed by the non-enzymatic glycoxidation of the protein-amino group. AGEs accumulate in tissues and are involved in the occurrence of the chronic complications of diabetes. The receptors for AGEs are expressed in the human bone and their activation generates synthesis inhibition of type 1 collagen and osteocalcin (50, 51). Thus, AGEs generate osteoblastic dysfunction and increased osteoclast activity (52, 53). One of the AGEs is characterized by pentosidine. Pentosidine content in cortical or trabecular bone was associated with the deterioration of bone quality (54, 55). Yamamoto T and coworkers have investigated the correlation between serum pentosidine concentration and vertebral fractures in 77 men and 76 postmenopausal women with T2DM. The authors conclude that pentosidine concentration is correlated with vertebral fractures in postmenopausal diabetic women, independent of BMD (56). In a multi-center study in which 271 nondiabetic patients with osteoporosis were included, urinary pentosidine levels at baseline were increased and predicted vertebral fractures under bisphosphonate therapy (57). A cohort study conducted by Schwartz AV highlighted that urine pentosidine concentrations were higher in older individuals with T2DM and predict future facture (58).

Bone mass is regulated by hormones and local factors; IGF-I is synthesized in the liver and osteoblasts and exerts the anabolic effects of bone. Experimental studies highlighted that IGF-I stimulates the synthesis of deoxyribonucleic acid, collagen, and non-collagenous protein; reduce collagen degradation in bone cell cultures; and is essential in bone matrix mineralization (59, 60). Increased levels of glucose and AGEs decrease the proliferative response of osteoblastic cell to IGF-I and secretion of IGF-I by osteoblasts (61, 62). Clinical studies show that free and total IGF-I are lower in patients with osteoporosis compared to controls (63, 64). Few clinical trials have investigated the relationship between IGF-I levels and bone metabolism in patients with T2DM. Miyake H and collaborators followed the association between IGF-I levels and the occurrence of non-vertebral osteoporotic fractures in 356 patients with T2DM (168 postmenopausal women and 188 men). Their results showed that decreased IGF-I concentration is correlated with an increased occurrence of non-vertebral osteoporotic fractures in postmenopausal women with T2DM (65). In 2007 and 2011, Kanazawa I *et al* published two studies about the association between IGF-I concentration and occurrence/severity of vertebral fractures in postmenopausal women with T2DM in *Osteoporosis International*. In the first study, they included 131 postmenopausal women with T2DM and the results showed that IGF-I levels were significantly decreased in women with vertebral fractures compared to women without fractures (66). In the second study, 813 patients with T2DM (334 postmenopausal women and 479 men) were recruited. In postmenopausal women, decreased IGF-I levels have been confirmed to be correlated with the number of vertebral fractures; in men, no association between IGF-I level and vertebral fractures was observed (67). The mechanism that associated changes of IGF levels in osteoporosis remains unclear, but IGF-I levels may predict fractures.

Sclerostin is a tiny protein produced by osteocytes and it has an anti-anabolic effect on bone formation (68). A study that included 74 patients with T2DM highlighted that circulating concentrations of sclerostin are higher in

individuals with T2DM than in the control group (50 subjects) and sclerostin levels were positively correlated with the duration of diabetes and HbA1c (69). Similar results, respectively significantly increased sclerostin levels in patients with T2DM versus control groups, have been reported by Khalek MAA and collaborators (70).

Conclusion

Subjects with T2DM have normal or increased BMD compared to subjects without diabetes, yet despite this, they are characterized by increased fracture risk that involves the alteration of bone quality. BMD is used in the diagnosis and evaluation of osteoporosis, but BMD itself cannot provide an optimal diagnosis in the detection of patients with high-risk fractures; therefore, surveys have focused on identifying other risk factors that are partially or fully independent of BMD. The FRAX® models provide information about a 10-year probability of osteoporotic fractures, but do not include risk factors specific to the illness such as: diabetes duration, diabetes drug therapy, glycemic control or the presence of microvascular complications. Epidemiological studies do not provide unitary information on the association between markers of bone fragility and fracture risk in T2DM. Markers that increase the accuracy of fracture risk estimated in patients with T2DM must be identified and used in current medical practice.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

Compliance with ethical standards

Any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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