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Relationship between Osteocalcin and Bone Alkaline Phosphatase in Patients with Diabetic Neuropathy.

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Abstract

The current study aimed to determine bone formation markers (osteocalcin and bone alkaline phosphatase) as biomarkers for diagnosis the patients with diabetic neuropathy (DN) and to find the relationship between these markers and dyslipidemia in Iraqi patients with diabetic neuropathy, and if these patients are more prone to the risk of atherosclerosis disease.***Subjects & Methods*:** in this study, blood were obtained from 25 healthy individuals as a control group (G1), 25 diabetic patients with dyslipidemia as a group (G2), and 25 diabetic patients without dyslipidemia as a group (G3). Age range (45-65) years for all subjects.Patients were attended in the National Diabetes Center / AL-Mustansiriya University/Baghdad. Serum was frozen until used for the analysis of fasting blood glucose (FBG), osteocalcin (Ocn) , bone alkaline phosphatase (BALP), triglyceride (TG), cholesterol (Ch), high density lipoprotein-cholesterol (HDL-ch), low density lipoprotein-cholesterol (LDL-ch) , and very low density lipoprotein-cholesterol (VLDL-ch). Glycated haemoglobin (HbA1C) is determined in all blood.***Results**:* the results showed a highly significant increase in FBG,HbA1C, Ocn and BALP in G2 and G3 when compare with G1, and a significant difference in lipids levels between two patients groups and control. There is a significant negative correlation between serum Ocn and BALP. Serum Ocn and ALP level concentrations were independently and highly positively correlated with TG,VLDL-ch, and AIP ratio. ***Conclusions:*** Ocn and BALP were further increased in DN with complications and poor glycemic control. The AIP ratio was highly significant increased in DN patients with and without dyslipidemia comparing with control ,therefore, these patients are more prone to the risk of atherosclerosis disease.

Keywards: *Diabetes mellitus , Diabetic neuropathy, Osteocalcin, Bone alkaline phosphatase, lipids.*

Introduction

Diabetic neuropathy (DN) is the most common long-term diabetic complication. This complex category of diseases encompasses various parts of the nervous system and has a broad variety of clinical symptoms [1],and it is a clinical or subclinical manifestations of complication diabetes mellitus(DM) that affect the peripheral nervous system (PNS). It can have a range of clinical signs and symptoms, as well as various pathophysiologic mechanisms, onset, and progression [2]. The number of metabolic syndrome components, such as hypertriglyceridemia, hypertension, abdominal obesity, and low HDL levels, is consistently associated with diabetic neuropathy in patients with type two diabetes mellitus (T2DM), regardless of HbA1c levels [3].

Furthermore, Excessive protein C kinase activation was promoted by high glucose levels, which controls the synthesis of nitric oxide, resulting in ischemic peripheral nerve injury. Due to the strong relationship rate between dyslipidemia and DM (DLD)[4], it has been observed the contribution of excessive lipids as a cofactor in DN pathogenesis.In addition, systemic dyslipoproteinemia (DLP) effects facilitate the development of pro-inflammatory compounds and oxidative stress[5]. The activation of the hexosamine pathway, which is caused by hyperglycemia, results in changes in the expression of certain genes and the functioning of intracellular proteins, is linked to all of these metabolic pathways. The polyol pathway, as well as final advanced glycosylation products (FAGP) and protein kinase C, increase the production of free

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radicals in response to oxidative stress. When mytochondrial dysfunction occurs, it triggers the apoptosis cascade in the cells [6].

Bone formation markers such as serum bone-specific alkaline phosphatase (BALP) and osteocalcin (Ocn) are released in the bloodstream during bone formation [7]. Ocn seems to play a part in energy metabolism as well. When Ocn is in carboxylated form, it promotes insulin secretion and improves insulin sensitivity in both adipose and muscle tissue. Levels of Ocn have been reduced by an inverse association between Ocn and metabolic syndrome, which may affect the pathophysiology of T2DM [8]. Also, several researchers observed that bone ALP raised in diabetic patients [9]. The most sensitive indicators for assessing bone formation are bone alkaline phosphatase and osteocalcin, which are both synthesized by osteoblast. Total alkaline phosphatase was measured rather than individual bone alkaline phosphatase, and it was found to be higher in all diabetic patients compared to the control group, with a positive association with glycemic indices [10].

Diabetic neuropathy is caused by dyslipidemia, which is a major contributor. In type 2 diabetes mellitus, metabolic lipid abnormalities in terms of atherogenicity coexist with neuropathy despite the disease period and are a valuable marker for preclinical atherosclerosis[11]. As a result, each diabetic neuropathy patient must be thoroughly investigated [12]. Arterial dysfunction is caused by the irregular metabolic syndrome associated with diabetes. Chronic hyperglycemia, dyslipidemia, and insulin resistance are all relevant disorders. As a result of these causes, arteries are vulnerable to atherosclerosis[13]. Diabetes affects the role of a variety of cell types in this condition, including endothelium, platelets, and smooth muscle cells, suggesting the severity of vascular dysfunction [14].

Aim of the study to find the relationship between bone formation markers (bone alkaline phosphatase and osteocalcin) with dyslipidemia in Iraqi patients affected with diabetic neuropathy, and if these patients are more prone to the risk of atherosclerosis disease.

Subjects & Methods:

Subjects:

Blood was obtained from 25 healthy individuals as a control group (G1),25 diabetic patients with dyslipidemia as a group (G2), and 25 diabetic patients without dyslipidemia as a group (G3).Age range (45-65) years for all subjects. These patients were diagnosed by clinical examination, medical history, and nerve response testing. Patients were attended at AL-Mustansiriya University/ the National Diabetes Center in Baghdad. Serum was frozen until used for the analysis of fasting blood glucose (FBG), osteocalcin (Ocn), bone alkaline phosphatase (BALP), triglyceride (TG), cholesterol (Ch), high density lipoprotein-cholesterol (HDL-ch), low density lipoprotein-cholesterol (LDL-ch) , and very low density lipoprotein-cholesterol (VLDL-ch). While glycated haemoglobin (HbA1C) was determined in whole blood.

All patients signed an informed approval after explaining the aim of the study, and a protocol approval from our local ethically and scientifically institution was obtained.

Methods

Fasting blood glucose, triglyceride, cholesterol, and HDL-ch were determined using standard biochemical techniques by Hitachi auto-analyzer, in addition to LDL-ch [15], VLDL-ch [16], and AIP is calculated according to Khazaál, M. S.[17]**.** Serum BALP and Ocn were assessed by enzyme-linked immune sorbent assay (ELISA) kits from Cusabio-China, which uses based on biotin double antibody sandwich technology

Statistical analysis: The statistical analysis determined by Microsoft excel 2013. The data were confirmed as Mean ± SD. The variations between patients and normal groups were evaluated by using a t-test, P-value of < 0.001, and < 0.05 that were considered highly significant and significant respectively. Pearson's connection coefficient (r) is used for clarification the relationship between all studied parameters [18].

Results

This study involved 50 diabetic neuropathy patients and 25 healthy control; their ages between (45-65) years. FBG, HbA1C%, Ocn , and BALP levels in patients and control groups are summarized in Table 1. The results in the current study display a highly significant *($P<0.001$)* increase in FBG, HbA1C, Ocn, BALP levels in G2 and G3 when compared with control (G1), while, there was no substantial *($P \geq 0.05$)* variance in these parameters between two patients groups (G2 and G3).

In the current study, the levels of lipids, and AIP ratio for patients groups and control were illustrated in Table2, which show a highly significant *($P<0.001$)* raise in levels of TG, Ch, LDL-ch, VLDL-ch, and AIP ratio in G2 and G3 as compared to G1, also, there were highly significant *($P<0.001$)* differences in concentrations of TG, Ch, and AIP ratio, and a significant *($P<0.05$)* variance in LDL-ch and VLDL-ch levels between two patients groups (G2 and G3). While there was no significant *($P \geq 0.05$)* decrease in HDL-ch level in G2 and G3 as compared to G1, also no significant difference was found in the HDL-ch level between G2 and G3.

Furthermore, correlation coefficients (r) and p- values between serum Ocn and BALP with FBG, HbA1C,

TG, Ch, HDL-ch, LDL-ch, VLDL-ch, and AIP ratio in all diabetic neuropathy patients groups are shown in Table 3. There were highly significant positive correlations between serum Ocn and both of FBG, HbA1C, TG, VLDL-ch, and AIP ratio, also a highly significant negative correlation between Ocn and both of Ch, HDL-ch, and LDL-ch, and a significant negative correlation between Ocn and BALP in the Iraqi patients with diabetic neuropathy.

The data in Table 3. Show a highly positive correlation between BALP and both of TG, Ch, LDL-ch, VLDL-ch, and AIP ratio, while a highly negative correlation with FBG, HbA1C, and HDL-ch in Iraqi diabetic neuropathy patients.

Table 1: Mean ± SEM of FBG, HbA1C, Ocn, and BALP levels in control (G1), diabetic neuropathy patients with dyslipidemia (G2) and without dyslipidemia (G3).

Groups / Parameters	G1 No.(25)	G2 No.(25)	G3 No.(25)
FBG (mg/dl)	82.95± 1.78	240.45± 22.77 a^{**}	267.65± 19.85 b^{**} c^{NS}
HbA1C%	4.62±0.27	9.86 ±1.56 a^{**}	8.93±0.71 b^{**} c^{NS}
Ocn. (ng/ml)	6.58 ± 0.40	22.82± 0.92 a^{**}	20.73± 0.62 b^{**} c^{NS}
BALP (IU/L)	10.35± 0.45	27.45± 1.08 a^{**}	28.79± 1.03 b^{**} c^{NS}

**** (P<0.05), ** (P < 0.001), NS: Non-Significant (P ≥ 0.05).***

a: t-test between G1 and G2, b: t-test between G1 and G3, c: t-test between G2 and G3

Table 2: Mean ± SEM of lipid profile and atherosclerosis index of plasma (AIP) levels in control (G1), diabetic neuropathy patients with dyslipidemia (G2) and without dyslipidemia (G3).

Groups / Parameters	G1 No.(25)	G2 No.(25)	G3 No.(25)
TG (mg/dl)	61.40 ± 4.53	227.15 ± 13.65 a^{**}	119.90 ± 7.97 b^{**} c^{**}
Ch (mg/dl)	157.20 ± 3.97	244.35 ± 6.79 a^{**}	178.15 ± 5.16 b^{**} c^{**}
HDL-ch (mg/dl)	50.82 ± 1.87	46.55 ± 2.96 a^{NS}	47.11 ± 2.83 b^{NS} c^{NS}
LDL-ch (mg/dl)	95.85 ± 4.21	147.40 ± 8.82 a^{**}	116.25 ± 3.71 b^{**} c^{*}
VLDL-ch (mg/dl)	10.11 ± 0.70	43.50 ± 3.81 a^{**}	27.15 ± 3.07 b^{**} c^{*}
AIP ratio	0.155 ± 0.017	0.682 ± 0.040 a^{**}	0.392 ± 0.047 b^{**} c^{**}

**** (P<0.05), ** (P < 0.001), NS: Non-Significant (P ≥ 0.05).***

a: t-test between G1 and G2, b: t-test between G1 and G3, c: t-test between G2 and G3.

Table 3: Pearson's correlation analysis between serum Ocn and BALP with studied parameters in all diabetic neuropathy patients.

Parameters	Ocn		BALP	
	r	p-value	r	p-value
BALP	-0.015	*S*	-	-
FSG	0.147	*HS*	-0.289	*HS*
HbA1C%	0.228	*HS*	- 0.049	*HS*
TG	0.090	*HS*	0.479	*HS*
Ch	- 0.423	*HS*	0.316	*HS*
HDL-ch	-0.078	*HS*	- 0.144	*HS*
LDL-ch	- 0.438	*HS*	0.421	*HS*
VLDL-ch	0.047	*HS*	0.361	*HS*
AIP ratio	0.087	*HS*	0.428	*HS*

HS: Highly significant (P < 0.001)

Discussions

Nerve damage is most likely caused by a combination of factors, containing metabolic factors like high blood glucose, long-term diabetes, elevated blood lipid levels, and probably low insulin levels; and neurovascular factors, which cause damage to the blood vessels that transport oxygen and nutrients to nerves; nerve inflammation caused by autoimmune factors; mechanical damage to nerves, such as carpal tunnel syndrome; inherited traits that increase susceptibility to nerve disease; Smoking and alcohol intake are examples of lifestyle causes [19].

In this study, there was a highly significant increase in Ocn level in DN patients,this result is in agreement with Maghbooli et al.[20] and El-Kafrawya N. et al.[21] studies who found that Ocn level was elevated in diabetic patients with microvascular complications in the form of peripheral neuropathy than patients without complications [20,21].

Osteocalcin regulates the alignment of BALP parallel to collagen fibrils in bone, and it is necessary for bone strength in the longitudinal direction of the long bone[22]. Ocn is also involved in muscle-bone cross-talk, which contributes to mechanical load, which promotes the growth of muscle mass and function [23].There is evidence that osteocalcin regulates insulin secretion, insulin resistance, and energy expenditure in addition to its role in bone metabolism[24]. It's probable that the frequency of ALP reactions, irrespective of the involvement of the fatty liver, plays a direct role in the pathogenesis of atherogenic dyslipidemia and impaired glycemic control, likely through the activation of chronic inflammation and insulin resistance [25].

Bad metabolic regulation in type 2 diabetes contributes to increased bone resorption, which leads to increased bone formation markers(Ocn and BALP), according to a limited number of cross-sectional studies. In a prospective study, it was discovered that as glycaemic regulation increased, in addition to decrease all biochemical parameters of bone turnover . It can thus be inferred from results by Capoglu *et al*.[26] that poor glycaemic control increases bone turnover. It's also likely that some patient groups will find bone biomarkers less useful. Diabetes mellitus has been related to changes in bone metabolism[26]. Furthermore, prolonged hemodialysis has been related to improvements in bone metabolism. The majority of diabetics have impaired glucose regulation, which can impair bone metabolism. Also after beginning hemodialysis, diabetic patients with good control of hyperglycemia have lower bone turnover rates [27].

Dyslipidemia leads to the evolution of diabetic neuropathy by stimulating oxidative stress in sensory neurons in the root ganglia[28]. Short-term glycemic regulation can enhance vibratory sensation in patients with type 2 diabetes mellitus, according to Fujita et al.[29], with metabolic changes in glucose and lipids (total cholesterol, triglyceride, and free fatty acids) being the factors responsible for peripheral nerve function impairment. According to Ibrahim GA. et al.[30] research, diabetic patients have a high prevalence of dyslipidemia, which can play a major role in the development of cardiovascular diseases and cerebrovascular accidents [30]. The considered significant risk factors for atherosclerosis are age, diabetes, elevated cholesterol, and LDL levels, low HDL levels, hypertension, cigarette smoke, obesity, and an inactive lifestyle [31].

The relationship between cardiac autonomic neuropathy (CAN) and the development of atherosclerosis in diabetic patients was the subject of a study by Mala S. et al.[32]. While certain atherosclerosis risk factors correlate with CAN risk factors, the pathophysiology of CAN tends to be more complex and multifactorial [32].

Some studies have discovered a link between circulating autonomic tissue autobodies and diabetic autonomic neuropathy[33]. Besides, the function of genetic polymorphisms and epigenetic changes in several genes linked to autonomic function is discussed [34]. Long-term hyperglycemia is thought to

be a significant contributor to micro and macrovascular problems in people with diabetes. Hyperglycemia causes endothelial dysfunction, reduced neuronal blood flow, and nerve fiber damage by altering multiple metabolic pathways [35].

Future studies

Much remains to be further investigated to confirm such a relationship between Ocn and BALP, in addition, to clarify their role in DN. Changes in bone biomarkers can reflect these underlying changes ahead of time, enabling clinicians to respond fast and effectively, which allows bone biomarkers to be used as markers for DN diagnosis.

Conclusions

In conclusion, the current research shows that serum Ocn and ALP levels in diabetic neuropathy patients are significantly higher compared with control, in addition to complications and impaired glycemic regulation. A significant negative correlation between levels serum Ocn and BALP was found in this study. Serum Ocn and ALP level concentrations were independently and highly positively correlated with TG,VLDL-ch, and AIP ratio. The AIP ratio was highly significant elevated in DN patients with and without dyslipidemia comparing with control ,therefore, these patients are more prone to the risk of atherosclerosis disease.Ocn level was highly positively correlated with FBG and HbA1C(markers of glycemic control). BALP level was highly positively correlated with Ch and LDL-ch . All these finding suggesting a link between atherosclerosis disease, Ocn, BALP and glycemic control in DN patients.

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