Bone Markers (BMKs) and its Relation with Peripheral Neuropathy (PNP) in Type 2 Diabetic Patient (T2DM) in Basrah

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Abstract

The aim of this study was to evaluate differences in bone metabolism by calculating markers of bone turnover (C-terminal telopeptide and osteocalcin) with type 2 diabetes and patients in diabetic peripheral neuropathy patients compared with those without diabetic peripheral neuropathy.

Key Words: BMKs, PNP, T2DM, Basrah.

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Introduction

Diabetes mellitus is a chronic metabolic disorder accompanied by microvascular complication due to tiny blood vessels hurt like diabetic peripheral neuropathy, osteoporosis, retinal (D'Silva et al., 2016) and macrovascular complication as cerebrovascular disease, peripheral artery disease, coronary heart disease, arrhythmias, sudden death (Viigimaa et al., 2019) and renal disease (Cianflone et al., 2019).

Diabetic peripheral neuropathy mean nerve damage due to hyperglycemia, these nerves located at the periphery (upper and lower limbs, more in lower limbs, can be sensory, motor or both, sensory nerve affected are more (A. Hamdan et al., 2018), also can be mononeuropathy, mononeuropathy multiplex, or polyneuropathy (The British Diabetic Association, 2021).

Diagnosis of Diabetic peripheral neuropathy: done by symptoms, neurological examination and diagnostic strategy.

The symptoms of sensory peripheral neuropathy are tingling, numbness and prickle or sharp stabbing sensation especially in the foot mostly at night these occur in the early stages of disease, while unpleasant event which may occur in the last stage of disease are loss of capability to recognize pain or extreme temperature, and loss feeling the position of joints at moment of burn or gunshot pains (Editor, 2021), while the symptoms of motor peripheral Neuropathy are uncontrollable twitching of muscle commonly arm or hand, muscle weakness and spasm, feeling sensation is robust (Nowacek & Teener, 2012).

The neurological examination: power of muscle, co-ordination and sensation (Romberg, 2019).

The diagnostic strategies are:

1. Rating of neuropathic signs and symptoms: the patients are estimated by the Toronto Clinical Scoring System for Diabetic Polyneuropathy because patient complain from pain, numbness, weakness in the foot or hand and ataxia (Bril & Perkins, 2002).

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2. Quantitative sensory examination as response to light touch, painful stimulus, vibration sense, the Semmes-Weinstein monofilament examination (SWME), thermal sensory testing, pain-related evoked potentials, vibration sense and position sense (Cheng et al., 1999).
3. Examining muscle power and deep tendon reflexes: especially ankle reflexes are examined at both ankles (Ohno & Suganuma, 2006).
4. Nerve conduction studies (NCSs) and Electromyography (EMG): are the most accurate diagnostic procedure (Koçer, 2018).
5. Corneal confocal microscopy : It is sensitive non-invasive examination, benefit to detect small nerve fiber damage (Tavakoli et al., 2012).
6. Skin biopsy: it is the recent diagnostic technique (Devigili et al., 2008).
7. Laser-Doppler-imager (LDI) flare: this technique easily reveal small-fiber dysfunction before the happening of irreversible structural deprivation of nerve fibers (Feng et al., 2011).

Pathophysiology of DPN

DPN is a multifactorial complication with an sprouting schedule of hazardous agents, at the top of list hyperglycemia and dyslipidemia which are crucial for beginning and progression of DPN, these two factors with others result either in acute stage (metabolic, reversible), or chronic stage (structural, irreversible) (Pham et al., 2019).

Acute DPN: In the acute stage elevated intracellular glucose will metabolized through either one of 3routes: glycolysis, hexosamine, or polyol pathways, motivation of these routes results in beginning of inflammatory processes and the formation of reactive oxygen species(ROS), this prompt oxidative stress and interrupt nerve energy supply, polyol cascade stimulation leads to decreased activity of Na /K-ATPase, this lead to piling up of sodium in the axon, harming nerve excitability and deceleratin nerve conduction velocity (NCV) (Arnold et al., 2012).

Chronic DPN: In chronic stage of DPN, small and large nerve axons will be degenerated, this can be seen in the peripheral nervous system through sural nerve biopsies, provoked fiber analysis, and skin punch biopsies (Biessels et al., 2010).

Axonal swelling from sodium aggregation disturbs connective linkage between Schwann cells and their related axons, also advanced glycation end (AGE) products in neurons and Schwann cells will be formed due to chronic hyperglycemia, this worsen and cellular function and protein, induce inflammatory consecutions, and consequent extra oxidative stress (Vincent et al., 2009).

Oxidation of lipoproteins and cholesterol can also exacerbate oxidative stress and promote neuronal apoptosis, free fatty acids also injury Schwann cells directly and stimulate insulin resistance (Almaguel et al., 2010), the mechanisms above also participate in the development of microvascular disease (MVD) that may contribute to neural ischemia (Tesfaye et al., 1993).

The peripheral nervous system is crucially participated in osteogenesis, bone metabolism, and bone remodeling, synovial tissue and subchondral bone of diathrodial joints are innervated by sympathetic sensory nerve fibers, these secret neurotransmitters necessary for bone formation after fracture, also involved in the pathology of inflammatory and degenerative diseases this what occur in adults, while during embryonic skeletal growth these neurotransmitters have vital trophic effects which are very important for appropriate limb formation, several cells of the musculoskeletal system show receptors for sensory and sympathetic neurotransmitters, also these cells synthesize neuropeptides such as calcitonin gene-related peptide and substance P (Grässel, 2014) (Jones *et al*., 2004).

Bone Turn over Markers

Bone remodeling is a continuous and balanced process through which bone formation and resorption occur, and some bone turn over will released as:

C-terminal Telopeptide (CTx)

The organic matrix of bone constitute from around 90% of the organic matrix of bone is type I collagen, a helical protein that is cross linked at the C-and N-terminal ends of the molecule, during resorption, a mixture of acid and neutral proteases secreted from osteoclasts that destroy the collagen fibrils into molecular fragments containing C-terminal telopeptide (CTx), as bone ages, the alpha type of aspartic acid present in CTx converts to beta type, during bone resorption beta-CTx is released into the blood stream and considered a specific bone marker for the dissolution of mature type I collagen, so raised concentrations of serum

beta-CTx reflect increased bone resorption which occur in osteoporosis, osteopenia, hyperthyroidism, hyperparathyroidism and Paget disease (Christgau *et al*., 2000).

Osteocalcin

Osteocalcin is a protein produced by osteoblasts, it is calcium and phosphate binding,which adjust the deposition of mineral by regulating a number of hydroxyapatite crystals, osteocalcin and other growth factors like osteonectin, and bone sialoprotein have a vital roles in mineralization, osteoid formation and bone remodeling (Caetano-Lopes *et al.*, 2007).

Methods

This research work was designed as a case-control study and conducted in Basrah governorate, southern Iraq and was accomplished in the period from November 2020 to April 2021, the patients attended the consultant unit of Rheumatology/ Al-Sader Teaching Hospital. The enrolled cases in the study about 117 persons in the age group between 35 and 75 years and were chosen as T2DM according to all these factors which include history and past report of HbA1C, and these patients divided into 2 groups according to NCS and EMG report, group with peripheral neuropathy as a case study and other group those without neuropathy as a control study.

Blood samples collection: after an overnight fasting, blood samples were getted from T2DM patients (cases) and non diabetic(control) group by venipuncture using disposable syringe while the person in the sitting position, blood samples allowed to clot for 2 hours at room temperature, then centrifuge for 15 min at 2000 rcf at 2~8℃. Then we collect the supernatant to carry out the assay and stored in the eppindorf tube in deep refrigerators (minus 80℃), then serum level of osteocalcin and C- terminal telopeptide of type 1collagen were measured by Enzyme linked immune sorbent assay method (ELISA) using kit supplied by Elabscience, USA.

All patients and control persons with their relatives were informed about this study and taken their promises for sharing by taking oral approval.

Statistical Analysis

Statistical calculations were done using Statistical Package for the Social Sciences version 25 (SPSS Inc.). In which categorical data expressed as numbers and percentages, and the differences between the groups were analyzed using Chi-square test (X2). Continuous data expressed as medians or mean ± SD and the differences between the groups were analyzed by non-parametric Mann Whitney U test for non-normally distributed data and student T test for normally distributed data. Kolmogorov Smirnov and Shapiro-Wilk tests are used to test the normality of the data. 95% confidence interval were applied as the dependent interval in statistics and p-values <0.05 were accepted as statistically significant.

Results

Table 1 The enrolled patients with type 2 DM will collected from 35 to 75 years, the median age for all patients was 51.00, while the mean BMI was 30.80 kg/m2, with standard deviation ± 5.17, 28 (23.9%) of them male and 89 (76.1%) was female. There are 90 (76.9%) had PNP diagnosed by NCS_EMG, while 27 (23.1%) did not had PNP. The median for Human CTX type 1 (ng/ml) was 1.31, while minimum value was 0.000 and the maximum value was 5.300. The median for Human OC/BGP was 67.36 ng/ml, minimum value was 0.00 and maximum value was 205.

Table 1. Anthropometric and investigational characteristics analysis of the participants

Variables		Count	Percentages
Age (year) (median) (min-max)		51 35-75	
BMI(kg/m2) (mean SD)		30.96 ± 5.17	
Gender	**Male**	28	23.9 %
	Female	89	76.1 %
Groups of the study	**Cases (+ve PNP)**	90	76.9 %
	Control (-ve PNP)	27	23.1 %
Human CTX type 1 (ng/ml) (median) (min-max)		1.31 0.000 - 5.300	
Human OC/BGP (ng/ml) (median) (min-max)		67.36 0.00 – 205	

There is no significant differences between patients with peripheral neuropathy and patients without peripheral neuropathy in the form of anthropometric parameters as age, BMI and gender (p-value= 0.854, 0.023 and 0.206) respectively. Also no significant differences in investigational

parameters as Human CTX type 1 and Human OC/BGP (p-value= 0.061and 0.068) respectively.

Table 2. Comparison between control (-ve PNP) and cases (+ve PNP) regarding the anthropometric and investigational parameters.

Variables		Control(-ve PNP) (n=27)	Cases(+vePNP) (n=90)	p-value****
Age(year) (median) (min-max)		51 35-75	52 35-75	0.854*
BMI(kg/m2) (mean SD)		32.94 4.5	30.37 5.23	0.023***
Gender	Male	4 (14.8%)	24 (26.7%)	0.206**
	Female	23 (85.2%)	66 (73.3%)	
Human CTX type 1 (ng/ml) (Median) (min-max)		1.51 0.320-5.100	1.21 0.000-5.300	0.061*
Human OC/BGP (ng/ml) (Median) (min-max)		26.93 0.00-205	70.320 0.00-205	0.068*

* Mann Whitney U test
** X^2 test
*** Independent t-test
**** Significant at p-value < 0.05

There is no significant differences between patients with peripheral neuropathy and patients without peripheral neuropathy in form of bone markers as Human CTX type 1 and Human OC/BGP (p-value= 0.061 and 0.068) respectively.

Table 3. Comparison between control (-ve PNP) and cases (+ve PNP) regarding bone markers

Variables		Control (-ve PNP)	Cases(+ve PNP)	p-value****
Human CTX type 1 (ng/ml) (Median)	Valid	22	71	0.061*
	Missing#	5	19	
Human OC/BGP (ng/ml) (Median)	Valid	22	71	0.068*
	Missing#	5	19	

* Mann Whitney U test
**** Significant at p- value < 0.05
# Missing value due to blood hemolysis.

Discussion

Many studies found that the predominance of positive NCS was 58% in asymptomatic DM patients, and 100% for symptomatic DM patients (Shabgah et al., 2021).

Our study elucidated that increasing age is unconstrained risk factor for developing DPN in patients with T2DM, this is same finding as (Mao et al., 2019) which found that the age is non-linearly linked with odd ratio of DPN.

Another study show contraverse of our study and the prevalence of DPN of sensory type was elevated from 26% (for 65- to 74-year age group) to 54% (for age group equal to or more than 85), so this deficit is more in old age (Mold et al., 2004).

Another study found that diabetic peripheral neuropathy which diagnosed according to Michigan Neuropathy Screening Instrument (MNSI) was significantly and positively related with increasing age (65 vs 59 years; p-value = 0.001) (Popescu et al., 2016).

Also our study found no relationship between DPN and BMI as (Popescu et al., 2016) showed no significant correlations were observed between the severity of DPN which diagnosed according to MNSI and body mass index (BMI) (31.9 vs 29.9 kg/m^2; p-value = 0.003). While (Oh et al., 2019) disagree with our study, they performed study on people with T2DM was analyzed into two groups (with DPN and without DPN) showed that BMI was raised in people with DPN than in people without DPN (26.5±4.3 vs.24.1±3.2 kg/m^2, p value=0.011)

Also our study found no relationship between DPN and gender as (Javed et al., 2014) they found no statistical significant (p<0.324) between gender and DPN with consideration to other factors like frequency of diabetes, age at diagnosis of DPN, duration of DM before beginning of DPN and electrophysiological patterns, Same finding seen by (Abosrea et al., 2020) also found no statistical significant between male and female gender (p-value=0.911) and DPN with regard to age at time of diagnosis of D.M, duration of DM, HbA1c level, electrophysiological and MNSI finding.

Relationship between PNP and bone turn over markers is a recent subject, so few studies found about this association, our study found no relationship between PNP and bone turn over markers as a follow-up study done to check if a previously acute Charcot foot has any chronic effects on bone mineral density (BMD) or local or systemic bone metabolism that may change bone markers they found no persistent effect of an acute Charcot neuropathy on bone metabolism and bone markers (Jansen et al., 2018).

Another two studies contradict to our study done, first on 120 elderly of type 2 diabetic patients patient (mean age 62) consisting from male and

postmenopausal women, grouped into PNP and non PNP patients, they concluded that men patients with PNP had a greater rate of bone turnover (as osteocalcin and CTX and others) than men without PNP, so expected to be more osteoporotic than patients without PNP (Rasul et al., 2012).

Second study which compare bone turnover markers between patients with type 1 diabetes mellitus (T1DM) with and without distal symmetrical sensorimotor polyneuropathy and control, found CTX was lower in subject with T1DM compared with controls (Vilaca et al., 2021; Tahmasebi et al., 2021).

Conclusion

This study found no relationship between PNP and bone turn over markers in term of osteocalcin and CTX in T2DM patients.

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