Research article

Role of immunological and biochemical markers in bone turnover in type I diabetic patients in Karbala province, Iraq

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(Received: August 2023 Revised: September 2023 Accepted: October 2023)

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ABSTRACT

Introduction & Aim: Long-term diabetes mellitus (DM) is known to have a deleterious impact on bone health, resulting in change in bone mineral density, bone turnover, and bone quality, all of which increase the risk of fractures. The aim of this study was to link immunological and pro-inflammatory cytokine (I L-6, I L-1, and TNF-alpha) markers in patients with type 1 diabetes to Their connection to bones formation (sPINP) and bone resorption parameters (sCTX).

Materials & Methods: This study included 80 patients suffering from T1DM in the age range of 20-45 years. The patients were assayed for their biochemical (Vitamin D and HbA1c), Immunological (IL-6, IL-1 and TNF-alpha) parameters, as well as bone formation and resorption markers.

Results: HbA1c values were observed to be >7.5 in 85% of individuals, while vitamin D levels were 16 ng/mL. Correlation in patients with HbA1c>7.5. The results of a linear regression between I L-6 and sCTX showed that there was an increase in sCTX for each unit of IL-6.

Conclusion: In patients with HbA1c levels >7.5, there is an association between IL-6, TNFα, and the bone resorption TNF-alpha and IL-6 have been linked to metabolic control loss.

Keywords: Diabetes mellitus; pro-inflammatory cytokines; HbA1c; vitamin D; sPINP; sCTX.

INTRODUCTION

Diabetic type on considers as chronic autoimmune diseases characterized by lifetime reliance on exogenous insulin due to immune-T cell recruitment and activation within the pancreatic islets and mediate the autoimmune process (1-3). In response, MHC class I-restricted islet autoantigens of the beta-cell surface are recognized by autoreactive CD8+ T lymphocytes, which then cause cytotoxicity through a number of effector mediators. Th1 cytokines, such as TNF- and IFN-, are some of them (4, 5). Type I diabetes traditionally involved as Th1 plays a role in pathophysiology (5, 6). Furthermore, suppressor T cells (Tregs) with any defect lead to suppress CD4+ and CD8+ T cell activity and proliferation have been reported (7-9). The patients with T1DM are regarded as environmental and genetic variables interacting with triggers. The onset of an auto-immune response against beta cells in this complicated multifactorial disease (10). Despite the specific circumstances that led to T1DM continuing to be unknown, numerous environmental and genetic markers have been identified as contributors to the development of diseases (11-13).

Hence, the purpose of this study was to find a link between immunological and pro -inflammatory cytokine (I L-6, I L-1, and TNF alpha) markers in patients with type 1 diabetes to bone formation (sPINP) & bone resorption parameters (sCTX) (14,15).

MATERIALS AND METHODS

This study included 80 TDM1 patients who have had frequent follow-ups (5 or or more than visits per year) at the diabetes mellitus Type 1 Clinic of AL-Hussein Medical Hospital, Karbala province. Ethical approval was obtained from the Hospitals ethics committee. The patients were made to sign an informed consent letter prior to the study. Blood (5 mL) withdrawn from each of the participants was transferred to sterile tubes, followed by centrifugation at 300 x g for 15 mins. The serum obtained was transferred to two separate tubes for biochemical and immunological assays.

The serum glucose, triglycerides, Total cholesterol, and c-HDL levels was analyzed using commercially available kit (COBAS 2010, Roche Diagnostics) and the analysis performed using photocolorimetry with a spectrophotometer (Roche Modular P800 meter). For HDL-C samples, polyethylene glycol enzymes and dextran-sulfate were used in a modified enzyme procedure, and the same photocolorimetric procedure was followed. The glycosylated hemoglobin (HbA1c) levels were assessed using a turbidimetric immunoassay kit (COBAS 2010, Roche Diagnostics).

kits from Biosciences. The coefficient of variation (CV) set for IL-6 was 8-10%, IL-1 4-7%, IL-10 as 8-

11%, and for TNF-alpha as 8-15%. The limit levels for each cytokine were assessed as 1.6 pg/mL for IL-6, 1.9 pg/mL for IL-1, 2.4 pg/mL for IL-10, and 2.8 pg/mL for TNF-alpha. Markers of bone turnover in serum, specifically the N-terminal propeptide bone formation marker type I collagen (sPINP) and resorption marker bone C-terminal telopeptide of type I collagen (sCTX), were analyzed using the ELISA technique. The restricted level set for sPINP and sCTX was less than 1 ng/mL, with a CV of 2.6-3%. The data was subjected to statistical analysis using SPSS version 20. A $p < 0.05$ value was considered as statistical significance.

RESULTS

The eighty TDM1 patients included in this research, were aged between 20-45 years, with majority (n=61) being females. The mean HbA1c recorded was 8.9%, and 85% of the patients had HbA1c levels above 7.5%. The median level of creatinine clearance recorded was 67.5 mL/min (59.9-90.2), while for vitamin D it was 15.8 ng/mL (11.8- 22.1) (Table 1). No patient showed data of adequacy for vitamin D (>30 ng/mL). The mean enzyme alkaline phosphatase, calcium, phosphorus, and total cholesterol levels among patients was 81U/L (70.1-99.7), 9.1 mg/dL (8.9-9.9), 4.1 mg/dL (3.2-4.5) and 190.1mg/dL (163-240) respectively **(Table 1).** The results showed TNF-α and sCTX to have a significant positive correlation in patients with HbA1c>7.5 ($r = 0.41$, $p = 0.05$). Similarly, a significant positive relationship was also observed between IL-6 and sCTX ($r = 0.43$, $p = 0.035$) (Table 2).

Table 1: Baseline features of diabetes mellitus patients in this study

Markers	**Values**	
	No. (%)	
Males	19 (23.75)	
Females	61 (76.25)	
	Median	IR
Range (years)	31.6	20-45
$HBA1_C$ (%)	8.9	7.6-11.4
Creatinine clearance (mL/min)	67.5	59.9-90.2
Calcium (mg/dL)	9.1	8.9-9.9
Vitamin D (ng/mL)	15.8	11.8-22.1
Phosphorus (mg/dL)	4.1	3.204.5
ALP (U/L)	81	70.1-99.7
Total Cholesterol (mg/dL)	190.1	163-240
HDL-C (mg/dL)	55	41.9-66.8
LDL-C (mg/dL)	101	87.8-140
triglycerides (mg/dL)	1.9	82.1-189.1

Glycosylated hemoglobin HbA1c; IR = interquartile range; ALP: Alkaline phosphatase, HDL-C: High-density lipoprotein, LDL-C: low-density protein-associated cholesterol.

Table 2: Immunological and biochemical parameters levels in T1DM patients

Parameters	**Median**	**IR**
Interleukin-1 (pg/ml)	8.11	5.91-20.2
Interleukin-6 (pg/ml)	6.25	3.1-9.8*
Interleukin-10 (pg/ml)	4.92	1.94-9.21
TNF-a (pg/ml)	4.93	1.96-12.41*
PINP (ng/ml)	985.42	661.5-2011.3
CXT(pg/ml)	421.6	299.5-710.6*

PINP: N-terminal propeptide of type I collagen in serum, CTX: telopeptide C-terminus of type I collagen in serum. IR: interquartile range. IL: interleukin; TNF: tumor necrosis factor.

DISCUSSION

The pathophysiology of bone turnover in type I diabetic patients' remains still unclear. Osteoblasts, which build bone, and osteoclasts, which dissolve bone, work together during the dynamic process of bone remodeling. This mechanism is kept in perfect balance by several signaling pathways with the assistance of both systemic and local humoral components. Human and animal studies have demonstrated that DM1 patients with metabolic dysregulation are more likely to develop significant bone formation impairment, with diminished osteoblast differentiation and function (16). The prevalence of DM1 has increased globally, and this condition has been associated with an increased risk of hip and spinal column fractures, with the latter happening 6.4-6.9 times more commonly in individuals with diabetes than in those without it (17, 18). The average lifespan of DM1 patients has increased, and therefore an increase in the incidence of fractures is projected in the future (19). In this study, we observed TNF-α and sCTX as well as IL-6 and sCTX to be positively correlated in diabetes patients with HbA1c>7.5. Previous studies have demonstrated that DM influences bone matrix and has a direct influence in cells involved in bone modeling due to glycosylation of proteins and lipids (18). In addition, diabetes patients are also at a greater risk of osteoporosis due to intestinal malabsorption disorders, hypogonadism, such as hyperthyroidism and celiac disease (17,20) additionally, hypovitaminosis D hinders the mineralization of healthy osteoid leading to impairment of osteoblast function. In this investigation, diabetes patients displayed vitamin D values that were inadequate or deficient which is in agreement with an earlier report (12). DM1 is linked to a rise in cardiovascular illnesses, regardless of glycemic management. Both cardiovascular disease and changes in bone metabolism may be impacted by the inflammatory state. RANKL expression, osteoclast genesis, and enhanced resorption are all encouraged by IL-6 and TNF-alpha.

CONCLUSION

In this study, we observed that patients with a HbA1c>7 to be positively correlated for the pro- were found linked and the rise of one unit of IL-6 resulted in a 23.8

pg/mL increase in the bone resorption marker sCTX. A positive correlation was also found.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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