Original Article

The immunomodulatory effect 1,25 (OH)2 D3 on TLR 2 and TLR4 expression on monocytes of patients with type II diabetes mellitus

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Abstract

Background: Recent studies have shown the immunomodulatory effect of vitamin D3 through down-regulation of Toll-like receptor (TLR) expression in human monocytes. In this study, the effects of vitamin D treatment on TLR2 and TLR4 expression on monocytes derived from type 2 diabetes was investigated. **Materials and Methods:** To assess the influence of vitamin D3 on expression of TLR2 and TLR4 on monocytes from patients with type II diabetes, peripheral blood sample was taken of 30 patients. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifuge and then monocytes were isolated from these cells with using the magnetic activated cell sorting (MACS). To investigate the effect of vitamin D3 on the expression of TLR2 and TLR4, monocytes were cultured in the presence of vitamin D3 (10^{-9} M) for 48 hours. Then the expression of TLR2 and TLR4 was determined by Real-time PCR. **Results:** We found that vitamin D3 suppresses the mRNA expression of TLR2 and TLR4 in patients with type II diabetes. TLR2 and TLR4 expression in the patients exposed to vitamin D3 were significantly decreased in comparison with patients who were not treated with vitamin D3. **Conclusion:** It can be concluded that vitamin D3 supplements may be further analyzed as a therapeutic option by reducing TLR2 and TLR4 expression in patients with type II diabetes.

Keywords: Type II diabetes, Toll like receptor, Monocytes, Vitamin D3

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Introduction

Diabetes is the most common chronic and endocrine diseases. It affects over 150 million people worldwide and the total number of diabetic patients is projected to reach 300 million by 2025 [1]. Type II diabetes consisted of a group of metabolic disorders such as hypoglycemia, inflammation, and insulin resistance can lead to increased risk of cardiovascular diseases [2]. Recent studies have been demonstrated that inflammation plays a crucial role in the development of both insulin resistance and type 2 diabetes[3]. Activation of the innate immune system via toll-like receptors(TLRs) is implicated in the pathogenesis of insulin resistance and type2 diabetes [4]. TLRs were expressed on several cell type such as monocytes, predominant cells of the innate immune system that are pivotal in diabetes [5]. Activation of these cells through TLRs induces an immune response by increasing the expression of molecules MHC-I, MHC-II and stimulatory molecules on the surface of antigen-presenting cells and subsequent activation of T cells which in turn lead to the production of inflammatory cytokines such as interleukin-6 (IL-6), interleukin-18 (IL-18), tumor necrosis factor-α (TNF-α), interleukin-12 (IL-12), and interferon-γ (IFN-γ) [6]. TLRs recognize pathogens via the pathogen-associated molecular patterns (PAMPs). Among the

TLRs, TLR2, TLR4 plays an important role in the pathogenesis of type2 diabetes and its related complications [4]. TLR4 acts as a pattern-recognition receptors (PRRs) for lipopolysaccharide (LPS), was signals by both MyD88-dependent and MyD88-independent pathways and form acomplex with MD-2 for activation of signaling pathways. Additionally, TLR2 and TLR4 recognize various damage-associated molecular patterns(DAMPs) such as heat shock protein 60 and 70, fatty acids, modified low-density lipoproteins (LDLs) serum amyloid A, β-defensin, hyaluronan, high-mobility group box 1 (HMGB1) and extracellular matrix degradation [7, 8].

Based on animal studies, identification of vitamin D3 receptor in pancreatic tissue and also the effect of vitamin D3 deficiency on insulin secretion, it has been suggested that there is a possible association between vitamin D3 and diabetes. In addition,it was reported that vitamin D3 deficiency can lead to glucose intolerance suggesting a plausible role of vitamin D3 in the etiology of diabetes [9]. Based on evidence-above mentioned, we tried to explore the possible association between TLR2 and TLR4 expression with vitamin D3 in patients with type II diabetes.

Methods

Patients. The patients consisted of 30 subjects with type II diabetes (13 women and 17 men; mean age: 42±8.2years) recruited from Diabetes Society. This study was approved by the ethics committee of Tehran University of Medical Sciences and informed consent was obtained from patients prior to the study .Then, 15 ml of peripheral blood samples was taken from patients following an overnight fast and collected in tubes containing 5% EDTA.

Isolation of subpopulations of PBMCs and monocyte isolation. Mononuclear cells were isolated from blood by density-gradient centrifugation over Ficoll Hypaque, then monocytes were isolated by magnetic cell sorting using the Monocyte Isolation Kit II (Miltenyi Biotech). The purity of positively selected monocytes was determined by flow cytometry.

Cell culture. Functional assessments were performed on the cultured monocytes from diabetic patients and $CD14^+$ monocytes (5×10^6 cells) were resuspended in culture medium (RPMI 1640 medium supplemented with 2mM L-glutamine, 100 U/ml penicillin and 100 g/ml streptomycin) with 10% FBS. Then, adherent monocytes were incubated in 10% FBS culture medium in the presence of vitamin D3 (10^{-9} M) for 48 h. Non-treated cells was considered as controls.

Real-time PCR. Total RNA was extracted from $CD14^+$ monocytes (1×10^6) by using RNX Plus Kit (CinnaGen) according to the manufacturer's instructions. Real-time-PCR was performed by using primers specific for TLR2, TLR4 and β-actin as control. The sequences of the forward and reverse primer used for amplification were as follows:
TLR2 F: 5'-GGAGTTCTCCCAGTGTTTGGT-3' and R: 5'-GCAGTGAAAGAGCAATGGGC-3' TLR4 F: 5'-AGAATGCTAAGGTTGCCGCT-3' and R: 5'-CTATCACCGTCTGACCGAGC-3', β-actin F:5'-GACCCAGATCATGTTTGAGACC-3' and R: 5'ATCTCCTTCTGCATCCTGTCG-3'. The TLR2 and TLR4 mRNA expression was expressed as a ratio to β-actin.

Statistical analysis. Data were expressed as mean standard deviation (S.D). To compare variables between diabetic patients, we used paired t-test analysis. A $P<0.05$ was considered statistically significant.

Results

Percent of purified monocytes. After isolation of monocytes from PBMCs by using the Monocyte Isolation Kit II and an MS column, cells are fluorescently stained with CD14-FITC and Mouse IgG1 kappa isotype control. The purity of positively selected monocytes was determined by flow cytometry. The purity was 90.8 % (figure1).

In vitro effect of vitamin D3 on TLR2 and TLR4 expressions. To assess the effect of vitamin D3 on TLR2 and TLR4 expression, human monocytes were exposed to 10^{-9} M of vitamin D3. We found that vitamin D3 suppresses the mRNA expression of TLR2 and TLR4 in patients with type II diabetes. TLR2 and TLR4 expression in the monocytes exposed to vitamin D3 are significantly decreased in comparison with monocytes not treated with vitamin D3 ($P<0.05$) (Figure 2).

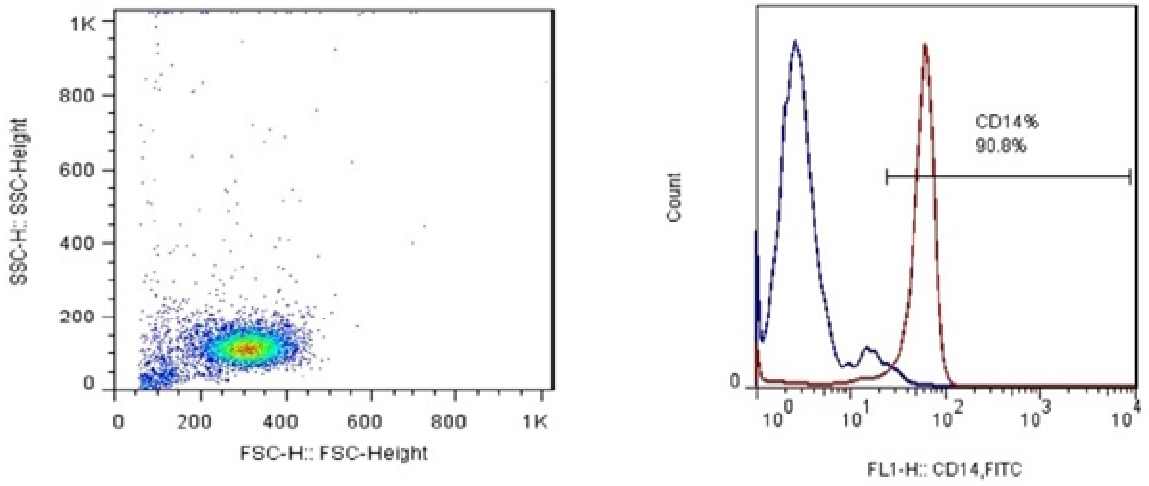

Figure 1. Percent of purified monocytes based on Flow cytometric analysis. Monocytes separated from PBMCs by using the Monocyte Isolation Kit II and an MS Column. Cells are fluorescently stained with CD14-FITC and Mouse IgG1 kappa isotype control. The purity was 90.8%.

Discussion

Several studies conducted on the pathophysiology of type II diabetes have shown that diabetes is a group of metabolic disorders such as hyperglycemia, inflammation, and insulin resistance that result to increased risk of cardiovascular diseases [10]. Based on studies it has been suggested that, activation of the innate immune system by TLRs plays a pivotal role in the pathogenesis of insulin resistance, diabetes and atherosclerosis [4, 11]. Genetic studies on TLR4 and TLR2 polymorphisms linked with diabetes

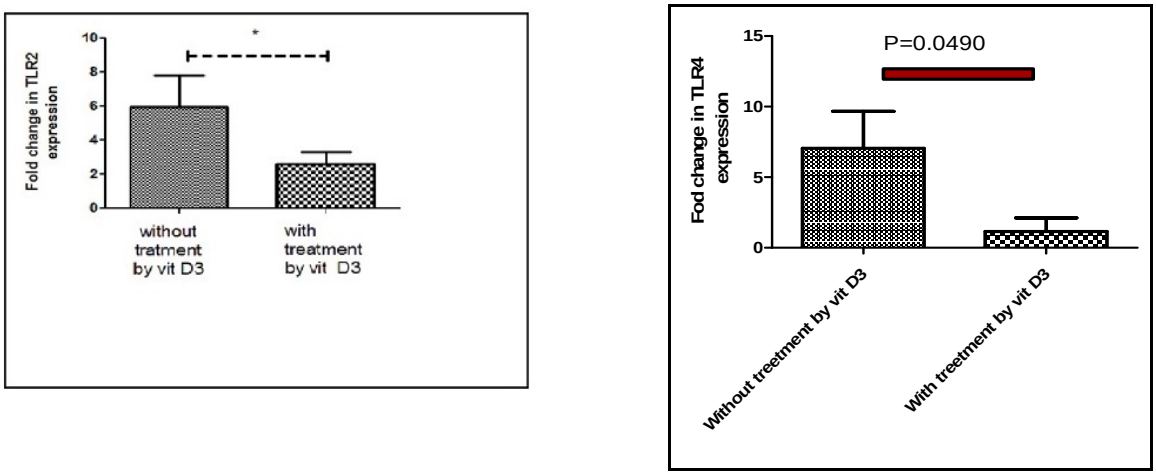

Figure 2. Decreased mRNA expression of TLR2 and TLR4 in monocytes of patients with type II diabetes after treatment with vit D3.

suggests that there is a connection between the TLRs and diabetes [12]. It has been also demonstrated that the activation of cells TLRs induces an immune response through high production of inflammatory cytokines such as IL-6, IL-18, TNF-α, IL-12 IFN-γ [13]. In a study entitled "increased expression TLR2 in monocytes of patients with type I diabetes" was reported that TLR2 expression levels in diabetic patients is higher than in the control group. In this study, the patients' monocytes were isolated using magnetic beads, and then the monocytes were examined in the presence and absence of LPS [14]. In prospective studies by Liu et al. in 2005 and by Pittas et al. in 2006, about the role of vitamin D3 it was shown that there is an inverse relationship between dietary vitamin D3 and risk of type II diabetes [15, 16]. In a review study conducted by Muscogiuri et al. it was found that vitamin D3 deficiency increases the risk of cardiovascular diseases and it is possible that there is an association between the reduced levels of vitamin D3 and obesity, diabetes mellitus, hyperglycemia, and dysfunction of vascular endothelial [17]. In a study entitled "the role of vitamin D3 in inflammation and Type II diabetes in both normal and diabetic persons" it was illustrated that the levels of vitamin D3 are important in the regulation of pathways related to Type II diabetes [18]. Since the activation of inflammatory pathways interferes with normal metabolism and insulin pathway this concept was reinforced that vitamin D3 can affect glucose homeostasis which were confirmed based on epidemiological and clinical data as well [18]. Accordingly, we concluded that the effect of vitamin D3 primarily related to its effect on insulin secretion and insulin sensitivity, but inflammation is related to the second place which is line with other studies [18]. In this study, we showed that monocytes of diabetic patients cultured in the presence vitamin D3, had increased expression levels of TLR2 and TLR4 in comparison with monocytes cultured in the absence of vitamin D3. These results were in agreement with a study in which the expression of TLR2, TLR4 and CD16 on monocytes from patients with Behcet's syndrome was assessed using flow cytometry and RT-PCR .The results showed that the expression levels of TLR 2 and TLR4 in patients with Behcet's syndrome are higher than the control group and vitamin D3 levels in these patients decreased. Consequently, an inverse association was found between vitamin D3 levels and the expression of TLR 2 and TLR4 [19].

Conclusion

So, it can be concluded that the expression level of TLR2 and TLR4 molecules in monocytes of patients with Type II diabetes was higher than healthy subjects, while the expression of TLR2 and TLR4 was reduced in the presence of vitamin D3. Therefore, it seems likely that the use of vitamin D3 supplements can greatly reduce inflammatory responses in patients with Type II diabetes.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgment

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