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Evaluation of soluble CD200 levels in type 2 diabetic foot and nephropathic patients: Association with disease activity

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
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Background: CD200 (OX-2) is a novel immune-effective molecule, existing in a cell membrane-bound form, as well as in a soluble form in serum (s OX-2), which acts to regulate inflammatory and acquired immune responses.

Material/Methods: We planned this study to evaluate the sOX-2 levels of type 2 diabetic foot (group B), and compare it with that of healthy controls (group A). The patient group had the following values: DM period: 27.9±10.3 year [mean ±SD], HbA1c: 9.52±2.44% [mean ±SD].

Results: Blood samples for sCD200 measurement were always taken in the morning between 8 and 10 A.M.. The results were reported as means of duplicate measurements. Concentrations of sOX-2 in the serum samples were quantified using an ELISA kit. Serum hs-CRP levels were measured using an hs-CRP assay kit. The sOX-2 level in group B was 173.8±3.1 and in group A was 70.52±1.2 [p<0.0001). In subgroup analysis of T2DM-DFI patients, we noticed that sOX-2 levels were higher in WGS (Wagner grading system) I and II patients than in WGS III and IV patients. The HbA1c, BUN, creatinine, hs-CRP levels, and sedimentation rates were higher in the patient group (p<0.0001, p<0.001, p<0.001, p<0.005, and p<0.0001, respectively).

Conclusions: We suggest that there are vascular, immunologic, and neurologic components in DFI, whereas autoimmune diseases and inflammatory skin disorders have only an immunologic component. This is possibly evidence of a pro-inflammatory effect seen in DFI as a vascular complication.

MeSH Keywords: Biological Markers – analysis • Biological Markers – chemistry • Diabetic Foot – blood • Diabetic Nephropathies – blood • Diabetic Nephropathies – diagnosis • Diabetic Nephropathies – immunology

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Background

Diabetic nephropathy (DN) is a progressive kidney disease caused by angiopathy of capillaries in glomeruli secondary to longstanding diabetes. It is the major cause of morbidity and mortality in patients with type-2 diabetes mellitus (T2DM). Diabetic foot infections (DFI) DFI are a serious complication of diabetes, with substantial morbidity and occasional mortality [1–3]. It is estimated that the incidence of diabetic foot infections range from a lifetime risk of 4% in diabetic persons in the community to 7% yearly in patients treated in a diabetic foot center [4]. DFI is a significant complication and sometimes leads to amputation of the lower extremity [1]. It is not well understood what is happening on a physiologic level to increase the risk or severity of infections in persons with diabetes mellitus (DM), but it seems to have multifactorial effects on various parts of the immune system [1,5,6].

The humoral innate immune response consists of local vasoactive cytokines (such as bradykinin, which leads to vasodilatation through a nitric oxide response), the complement system, and proinflammatory cytokines [1]. To produce an appropriately controlled response during infections, the immune system is balanced by the action of activating and inhibitory receptors. Lack of inhibition leads to excessive inflammation and autoimmunity and other severe disease symptoms. One of the receptors regulating this balance is sCD200 (sOX-2) [7].

OX-2 is a member of the immunoglobulin supergene family of receptors [8,9]. Soluble OX-2 (sOX-2) was originally described as a myeloid receptor, being expressed on macrophages, granulocytes and dendritic cells, and also expressed on T cells, B cells, and natural killer cells [10,11]. It displays a restricted tissue distribution, including activated T and B cells. sOX-2 is induced by inflammatory cytokines, including TNF-α, and binds to OX-2 receptor [8,9].

We showed the pro-inflammatory effect of sOX-2 in our previous studies [12,13–17]. A patient of ours with TEN (toxic epidermal necrolysis) also had DM and had higher sOX-2 levels versus a healthy control group, despite TEN treatment [15]. We therefore planned this study to evaluate the sOX-2 levels in patients with DFI as the micro- and macro-vascular complications of type 2 DM (T2DM). We evaluated other biomarkers like high-sensitivity CRP (hs-CRP) in DFI patients to compare with that of healthy controls. Possible correlations were investigated between these markers and creatinine levels, Wagner grading system (WGS), and body mass index (BMI), as well as sedimentation rate, preprandial glucose levels, and age.

Material and Methods

Patients

All patients gave their informed, written consent. The study was approved by the local independent ethics committee and was performed in accordance with the ethics principles of the Declaration of Helsinki. We enrolled 23 healthy controls (group A) and 22 T2DM-DFI patients (group B) . Group B patients had diabetic nephropathy and foot disease. The T2DM-DFI definition was infection, ulceration, or destruction of deep tissues of the foot associated with neuropathy and/or peripheral arterial disease in the lower extremity of people with diabetes. We included patients with DFI but not cancer. Diabetic foot ulcers were graded according to Wagner grading system (WGS) [18]. Healthy volunteers (n: 23) had no history of malignancy or autoimmune disorders, cardiac, liver, renal, or pulmonary diseases.

Experimental procedures

The results were reported as means of duplicate measurements. Blood samples for sCD200 measurement were always taken in the morning between 8 and 10 A.M.. Medical history was taken on the same day. Concentrations of sOX-2 in the serum samples were quantified using an ELISA kit (Sino Biological Inc., Catalog Number: SEK10886).

Serum hs-CRP levels were measured using an hs-CRP assay kit (Behring Latex-Enhanced utilizing Behring Nephelometer BN-100; Behring Diagnostics, Westwood, MA, USA). The sensitivity of the assay ranged 0.04–5.0 mg/L.

Statistical analysis

The data are presented as means ±SEM. Statistical analyses were performed using SPSS software (version 18.0; SPSS, Chicago, IL, USA). Comparison of parameters between the groups was performed using the t-test for independent samples. The correlations between the variables were analyzed using Pearson correlation tests. A p-value of less than 0.05 was considered statistically significant. Data are shown as mean ±SEM.

Results

The demographics of the analyzed patients are shown in Table 1. Group B had the following values: DM period: 27.9±10.3 year [mean ±SD], HbA1c: 9.52±0.44% (normal range: 4–6%) and WGS: 1.61 (5 patients with grade 1, 5 patients with grade 2, 7 patients with grade 3, and 5 patient with grade 4).

The sOX-2 level in the patient group was 173.8±3.1 [mean ±SEM] and in the healthy control group it was 70.52±1.2 [mean ±SEM]

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Table 1. Clinical and laboratory characteristics of the patients (group A) and controls (group B).

	Group A (n=23)	Group B (n=22)
Age (year)	41.64±2.86 (range: 25–87)	57.31±2.66 (range: 29–82)
Sex (female)	14	14
BMI (kg/m^2)	29.20±1.58	32.35±1.26
HbA1c (%)	3.30±0.41	9.52±0.44
Sedimentation (mm/h)	12.42±1.66	66.50±4.48
hs-CRP (mg/L)	3.77±0.82	97.55±9.8
BUN	11.9±0.22	42.56±3.98
Creatinin	0.59±0.31	3.84±0.36
S OX-2 (pg/ml)	70.52±1.2	173.8±3.1

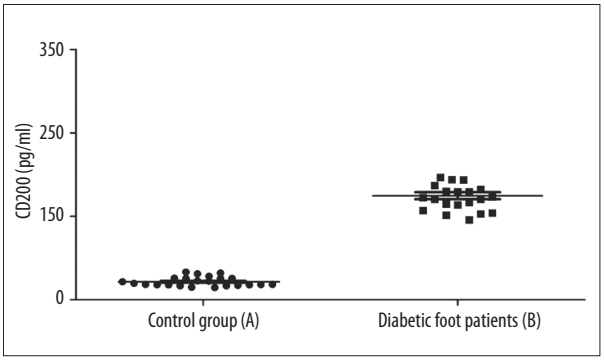

Figure 1. sOX2(CD200)levels. Group A (mean ±SEM): 70.52±1.2. Group B (mean ±SEM): 173.8±3.1.

[p<0.0001) (Figure 1). The HbA1c, BUN, creatinine, hs-CRP levels, and sedimentation rates were higher in the patient group (p<0.0001, p<0.001, p<0.001, p<0.005, and p<0.0001, respectively).

There was a positive correlation between sOX-2 levels and BUN and creatinine rate values (p<0.05), (r1=0.498; r2=0.675). There was a positive correlation between:

HbA1c values and CRP, preprandial glucose, postprandial glucose, and sedimentation rate values (p<0.01, respectively r1: 0.479; r2: 0.549; r3: 0.486, r4: 0.858);

And between **hs-CRP values** and BMI values (p<0.05; r: 0.622).

In subgroup analysis of T2DM-DFI patients we noticed that sOX-2 levels were higher in WGS I and II patients than in WGS III and IV patients.

Discussion

Diabetic foot (DF) is a major complication of long-standing diabetes, accounting for nearly 35% of all hospital admissions in diabetic clinics. It also accounts for nearly 80% of all non-traumatic amputations of the lower limb [19]. The etiopathogenesis of DF is multifactorial and seems to be related to the level of vitamin D [20,21] and neuropeptides [6].

sOX-2 is a novel immune-effective molecule, existing in a soluble form in serum. It regulates inflammatory as well as acquired immune responses through interaction with cell-bound ligands [22]. We previously investigated sOX-2, which can also be linked to apoptosis and is an immuno-effective ligand [15]. Recent studies reported that variable sOX-2 levels have been observed in many diseases like viral or parasitic infections and cancer [23–26].

In the course of our clinical practice we encountered the case of a man with pruritic bullous pemphigoid and very high levels of total IgE (5000 kU/L) who was refractory to standard aggressive immunosuppressive regimens (systemic steroids, daily cyclophosphamide) but responded rapidly to systemic anti-IgE (Omalizumab) and had a higher sCD200 level in the blister fluid (243 pg/mL) than in serum (48.45 pg/mL). During the second month of follow-up, the patient's serum sOX-2 level decreased to 26.7 pg/mL [14]. Reduction of sCD200 level with anti-IgE treatment suggests that sOX-2 could be pro-inflammatory. In another study of our research group, we found that the sOX-2 levels in psoriasis vulgaris (96.7±15.8, [mean +SEM]) and pemphigus vulgaris patients 76.2±14.6, [mean +SEM]) were higher than in healthy controls [13]. Other finding of our research group on a patient with toxic epidermal necrolysis revealed that sOX-2 levels were higher in blister fluid than in serum [15]. This patient also had diabetes mellitus. We noticed that the sOX-2 level was still higher at the end of the treatment. This case is the key point of our present study. In this present study we observed that sTRAIL (soluble TNF-related apoptosis-inducing ligand: APO-2 ligand) levels were significantly suppressed in diabetic nephropathy patients with foot ulcers and also in newly diagnosed type 2 DM compared to healthy

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controls, which suggests a protective role for sTRAIL in the disease setting in our previous studies [12,27].

All these results show that the levels of sOX-2 were higher in macrovascular complication of DM as DFI than in autoimmune diaseases and inflamatory skin disorders. Thus, we suggest that there were vascular, immunologic, and neurologic components in DFI, whereas autoimmune diaseases and inflamatory skin disorders had only an immunologic component. This should be the evidence of has sOX-2 major pro-inflammatory effect in vascular complication. We found a positive correlation between HbA1c and CRP and sedimentation rate. However, there was no correlation between sOX-2 levels and other biochemical biomarkers of HbA1C, blood glucose level, or BMI; therefore, its prognostic value is limited. Our study is limited because the study group was small, obscuring a causal link between sOX-2 and disease perpetuation.

Conclusions

We can suggest that there are vascular, immunologic, and neurologic components in DFI, whereas autoimmune diseases and inflammatory skin disorders have only an immunologic component. This is possibly evidence of a pro-inflammatory effect seen in DFI as a vascular complication.

Declaration of interest

All authors declare that they have no conflicts of interest.

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References:

1. Peters EJG, Lipsky BA: Diagnosis and management of infection in the diabetic foot. Med Clin North Am, 2013; 97(5): 911–46
2. Peleg AY, Weerarathna T, McCarthy JS et al: Common infections in diabetes: pathogenesis, management and relationship to glycaemic control. Diabetes Metab Res Rev, 2007; 23(1): 3–13
3. Kosinski MA, Lipsky BA: Current medical management of diabetic foot infections. Expert Rev Anti Infect Ther, 2010; 8(11): 1293–305
4. Lavery A, Armstrong DG, Wunderlich RP et al: Diabetic foot syndrome: evaluating the prevalence and incidence of foot pathology in Mexican Americans and non-Hispanic whites from a diabetes disease management cohort. Diabetes Care, 2003; 26(5): 1435–38
5. Singh K, Singh VK, Agrawal NK et al: Association of Toll-like receptor 4 polymorphisms with diabetic foot ulcers and application of artificial neural network in DFU risk assessment in type 2 diabetes patients. Biomed Res Int, 2013; 2013: 318686
6. da Silva L, Carvalho E, Cruz MT: Role of neuropeptides in skin inflammation and its involvement in diabetic wound healing. Expert Opin Biol Ther, 2010; 10(10): 1427–39
7. Gorczynski RM: CD200 and its receptors as targets for immunoregulation. Curr Opin Investig Drugs, 2005; 6: 483–88
8. Barclay AN, Wright GJ, Brooke G, Brown MH: CD200 and membrane protein interactions in the control of myeloid cells. Trends Immunol, 2002; 23: 285–90
9. Chen Z, Marsden PA, Gorczynski RM: Role of a distal enhancer in the transcriptional responsiveness of the human CD200 gene to interferon-γ and tumor necrosis factor-α. Mol Immunol, 2009; 46: 1951–63
10. Wright GJ, Cherwinski H, Foster-Cuevas M et al: Characterization of the CD200 receptor family in mice and humans and their interactions with CD200. J Immunol, 2003; 171: 3034–46
11. Rijkers ESK, De Ruiter T, Baridi A et al: The inhibitory SCD200 is differentially expressed on human and mouse T and B lymphocytes. Mol Immunol, 2008; 45: 1126–35
12. Arik HO, Yalcin AD, Gumuslu S et al: Association of circulating sTRAIL and high-sensitivity CRP with type 2 diabetic nephropathy and foot ulcers. Med Sci Monit, 2013; 19: 712–15
13. Karakas AA, Yalcin AD, Koc S et al: There might be a role for CD200 in the pathogenesis of autoimmune and inflammatory skin disorders. Med Sci Monit, 2013; 19: 888–91
14. Yalcin AD, Genc GE, Celik B et al: Anti-IgE monoclonal antibody (omalizumab) is effective in treating Bullous Pemphigoid and effects on soluble CD200 (OX-2). Clin Lab, 2014; DOI: 10.7754/Clin.Lab.2013.130642
15. Yalcin AD, Karakas AA, Soykam G et al: A Case of Toxic Epidermal Necrolysis with Diverse Etiologies: Successful Treatment with Intravenous Immunoglobulin and Pulse Prednisolone and Effects on sTRAIL and sCD200 Levels. Clin Lab, 2013; 59: 681–85
16. Yalcin AD, Ucar S, Gumuslu S, Strauss L: Effects of Omalizumab on Eosinophil Cationic Peptid, 25-Hydroxyvitamin-D, IL-1β, and sCD200 in a cases of Samter's syndrome: 36 Months follow-up. Immunopharmacol Immunotoxicol, 2013; 35: 524–27
17. Yalcin AD, Cilli A, Bisgin A et al: Omalizumab is effective in treating severe asthma in patients with severe cadiovascular complications and effects on sCD200, d- dimer, CXCL8 and IL-1β levels. Expert Opin Biol Ther, 2013; 13(9): 1335–41
18. O'Neal LW, Wagner FW: The diabetic Foot, Mosby, St Louis 1983; 274
19. Gupta SK, Singh SK: Diabetic foot: a continuing challenge. Adv Exp Med Biol, 2012; 771: 123–38
20. Zubair M, Malik A, Meerza D, Ahmad J: 25-Hydroxyvitamin D [25(OH)D] levels and diabetic foot ulcer: is there any relationship? Diabetes Metab Syndr, 2013;7(3): 148–53
21. Tiwari S, Pratyush DD, Gupta B et al. Prevalence and severity of vitamin D deficiency in patients with diabetic foot infection. Br J Nutr, 2013; 109(1): 99–102
22. Gorczynski RM: CD200: CD200R-Mediated Regulation of Immunity. ISRN Immunology 2012, doi: 10.5402/2012/682168
23. Karnam G, Rygiel TP, RaabenM et al: CD200 receptor controls sex-specific TLR7 responses to viral infection. Plos Pathogens, 2012; 8(5): e1002710
24. Rygiel TP, Karnam G, Goverse G et al: CD200-SCD200 signaling suppresses anti-tumor responses independently of CD200 expression on the tumor. Oncogene, 2012; 31(24): 2979–88
25. Snelgrove RJ, Goulding J, Didierlaurent AM et al: A critical function for CD200 in lung immune homeostasis and the severity of influenza infection. Nat Immunol, 2008; 9: 1074–83
26. Caserta S, Nausch N, Sawtell A et al: Chronic infection drives expression of the inhibitory receptor SCD200, and its ligand CD200, by mouse and human CD4 T cells. PLoS One, 2012; 7(4): e35466
27. Bisgin A, Yalcin AD, Gorczynski RM: Circulating soluble tumor necrosis factor related apoptosis inducing-ligand (TRAİL) is decreased in type-2 newly diagnosed, non-drug using diabetic patients. Diabetes Research and Clinical Practice, 2012; 96: e84–e86

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