Serum Interleukin-18 Levels are Not Increased in Type 2 Diabetic Patients

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Keywords: interleukin 18, type 2 diabetic patients, diabetic retinopathy

Posted Date: December 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-134807/v1

Abstract

Background: To investigate the effects of interleukin 18 (IL-18) on diabetic retinopathy (DR) of type 2 diabetic patients, the contents of IL-18 were measured in serum of 206 case subjects with type 2 diabetes and 40 case subjects without diabetes as control.

Methods: According to the degree of DR, the diabetic patients were further divided into three groups: non-diabetic retinopathy (NDR, n=69), non-proliferative diabetic retinopathy (NPDR, n = 52) and proliferative diabetic retinopathy (PDR, n=85).

Results: Unlike previous reports, we didn't found a significant increase in serum IL-18 level in diabetic patients (mean ± SD are 107.4±36.6 and 112.5±32.0 pg/ml for control and type 2 diabetes patients respectively, $p > 0.05$). Further analysis also failed to found any significant increase of serum IL-18 in patients with NDR, NPDR or PDR (113.0±32.1, 110.8±31.4 and 114.5±33.4 pg/ml respectively) when compared with control (for all values, $p > 0.05$). Real-time qPCR suggests that the expression of IL-18 mRNA in type 2 diabetic patients with DR was comparable to that of controls ($p>0.05$). Interestingly, there was a significant positive correlation between levels of serum IL -18 and the amount of fasting blood glucose (FBG, $r=0.15$ $p=0.03$) and that Hemoglobin A1c (HbA1c) was relatively higher in diabetic patients than in control subjects ($p<0.05$). These results suggest that the levels of serum IL -18 in diabetic patients are within the normal range. Even in patients with diabetic retinopathy, the levels of serum IL -18 were only slightly increased in type 2 diabetic patients and was not statistically different from control subjects.

Conclusion: these data suggest that the serum IL -18 levels are not associated with the severity of type 2 diabetic patients.

1. Introduction

Recently, there is a great debate about the effect of Interleukin 18 (IL-18) on the retinopathy including diabetic retinopathy (DR) and age-related macular degeneration (AMD). IL-18, as proinflammatory cytokines, is a member of IL-1 cytokine superfamily and plays an important role in immune regulation and immune pathological lesion. Serum IL-18 levels were upregulated in a variety of inflammatory diseases and acute injury such as Acute Kidney Injury, Acute hepatic necrosis, Rheumatoid Arthritis and so on [1–3]. In the past decade, it has been reported that the serum IL-18 levels were increased in type 1, 2 diabetic patients with retinopathy, and speculated that IL-18 may be associated with the pathogenesis of DR [4–6]. However, bioactivity assay indicates that IL-18 not only inhibits the formation of new blood vessels but also promotes the maturity of new blood vessels in vivo and in vitro[7–10]. Since then, the role of IL-18 in DR and AMD has drawn a great amount of attention. One interesting function of IL-18 is that it specifically inhibits the proliferation of capillary endothelial cell and neovascularization in cornea [11]. IL-18 gene knockout results in retinal vascular expansion and leakage, as well as overexpression of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), bFGF, and pigment epithelium-derived factor (PEDF) in newborn mice, suggesting that IL-18 has an inhibitory role in choroidal

neovascularization (CNV) [12]. Animal experiments have shown that IL-18 inhibits angiogenesis and vascular leakage by suppressing the activity of VEGF. In a mice CNV model, intraocular injection of VEGF and VEGF caused obvious retinal vascular leakage, whereas the mice received recombinant IL-18 led to a significant reduction in the amount of neovascularization [13.14]. A more recent study suggests that recombinant human IL-18 is safe and can reduce the development of choroidal neovascular lesion in cynomolgus monkeys [7]. The studies have shown that serum IL-18 level was upregulated in type 2 diabetic patients. The present study analyzed the expression level of IL-18 in patients with type 2 diabetic retinopathy to investigate the safety of IL-18 in the treatment of diabetic retinopathy.The other study IL-18 can reduce choroidal neovascular lesion development in cynomolgus monkeys [7]. Surprisedly, our results showed that serum IL-18 levels in type 2 diabetic patients were not significantly increased. However, this is consistent with reports revealing that IL-18 has no therapeutic effects on neovascular AMD [15].

2. Materials And Methods

2.1 Subjects

In total, 246 subjects consisting of 206 patients with type 2 DR were used in the current study. Patients with diseases like acute infection, hypertension, atherosclerotic heart disease, hypothyroidism and malignancy were excluded. Subjects in the control group were not receiving any medication. Type 2 DR was diagnosed according to WHO standard in 1999. The diabetic patients were further divided into three groups according to diabetic retinopathy lesions: non-diabetic retinopathy (NDR, n = 69), non-proliferative diabetic retinopathy (NPDR, n = 52), proliferative diabetic retinopathy (PDR, n = 85). All of the subject characteristics were summarized in Table 1.

2.2 Methods

Fasting blood glucose (FBG), Hemoglobin A1c (HbA1c), and spontaneous fluorescence value (AGEs) levels. For measuring IL-18, samples were centrifuged and stored at -80℃ until the day of analysis. Before the day of analysis, serum samples were transferred to -20℃ and were dissolved at room temperature.

Quantification of IL-18 with Enzyme-linked immunosorbent assay(ELISA)

Serum IL-18 levels were measured with ELISA kit (TaKaRa Biotechnology, Shenyang, China). Results were expressed as pg/ml.

Real-time quantitative PCR

The expression of IL-18 messenger RNA (mRNA) was examined by real-time quantitative PCR detecting system (qPCR). Total RNAs were separated from people peripheral blood using RNAiso reagent (TaKaRa Biotechnology, Shenyang, China). The total RNAs were treated with DNase I (TaKaRa Biotechnology, Shenyang, China) and then subjected to reverse transcription using PrimeScript™ RT Reagent Kit (Perfect

Real Time) (TaKaRa Biotechnology, Shenyang, China). The qPCR experiments were performed with a TaKaRa TP800Real Time PCR System (TaKaRa Biotechnology, Shenyang, China) using 2 μl cDNA with 10 μl SYBR green PCR mastermix (TaKaRa Biotechnology, Shenyang, China) and 0.4 μl of each specific primer. The glyceraldehyde-3-phosphatedehydrogenase (GAPDH) of human was used as an internal control to normalize the starting quantity of RNA.

3 Statistical analysis

All statistical analyses were performed using IBM SPSS statistics software (version 22; SPSS). Data were analyzed using parametric statistics with two-tailed Student's test, bivariate correlation test, or X^2 –Test, as appropriate. All data are expressed as mean ± SEM.

3. Results

3.1 Serum IL -18 levels are comparable between diabetic patients and control subjects

The clinical and biochemical features of the subjects included in the study are summarized in Table 1. The levels of FBG, HbA1c and AGEs in type 2 DR were significantly higher than those in control group. However, the levels of serum IL-18 were comparable between type 2 DR patients and control subjects (Table 1). It can be concluded that the IL-18 levels was security treatment in type 2 DR.

3.2 Diabetic retinopathy was related to IL-18 levels

Type 2 diabetes patients were divided into three groups according to diabetic retinopathy lesions: NDR, NPDR, and PDR. As shown in Table 2, the expression level of IL-18 in three groups of type 2 DR were slightly upregulated than that in controls, respectively, but none of them were significantly different. Thus, the level of serum IL-18 was not associated with the severity of type 2 diabete.

3.3 Duration of diabetes were related to IL-18 levels

Type 2 diabetes patients were further divided into four groups according to duration of diabetes: 0-5years,5-10years,10-15years,15 years. As shown in Table 3 the duration of diabetes were not associated with the security of type 2 DR.

3.4The relative expression of IL-18 mRNA

The IL-18 mRNA levels were examined by a realtime quantitative PCR detecting system (qPCR). The IL-18 mRNA level was detected in 4 control subjects and 7 type 2 DR patients. As shown in Fig. 1, the expression of IL-18 was slightly upregulated in diabetes patients but not significantly when compared to control groups ($p = 0.07$).Type 2 diabetes patients were divided into three groups according to serum IL-18 levels: ≤ 80 pg/ml, 80–120 pg/ml, and > 120 pg/ml. As the increase of IL – 18 levels, the patient's vision

had no obvious change. Thus, the serum IL-18 levels were not affected by the vision of patients. From the above results, it can be inferred that IL-18 plays an important role in security treatment that has no side effect.

3.5FBG was related to IL-18 levels

In diabetic patients, we found a significant correlation between the level of serum IL-18 and FBG ($r = 0.15$, $p = 0.03$) and then HbA1c ($r = 0.15$, $p = 0.02$) was relatively high.

These results suggest that IL-18 expressed in normal human and diabetic patients were within the normal range. Although with diabetic retinopathy level aggravating and serum IL − 18 with increased slightly in type 2 diabetic patients, the difference was not statistically significant. Thus, IL-18 level and type 2 DR were no correlation.

Table 1
Clinical and biochemical features of the subjects

	Control group	**Diabetic patients**	**p**
	n = 40	**n = 206**	
Age(years)	61.5 ± 5.0	57.8 ± 7.9	0.861
Duration of diabetes (years)	-	9.4 ± 5.5	-
FBG(mmol/L)	5.6 ± 0.9	9.7 ± 2.7	0.021
HbA1c (%)	5.2 ± 0.7	8.1 ± 1.8	0.026
AGEs	0.1 ± 0.04	0.3 ± 0.06	0.041
IL-18 (pg/ml)	107.4 ± 36.6	112.5 ± 32.0	0.463
Abbreviations: FBG, fasting blood glucose; AGEs, spontaneous fluorescence value; IL-18, interleukin-18.			

Table 2
The serum IL-18 levels of diabetic retinopathy

	Control group	**Diabetic patients**		
	n = 40	NDR	NPDR	PDR
		n = 69	n = 52	N = 85
IL-18 (pg/ml)	107.4 ± 36.6	113.0 ± 32.1	110.8 ± 31.4	114.5 ± 33.4
p		0.897	0.428	0.608
p values were obtained from t test between diabetic patients and control subjects				

Table 3
The serum IL-18 levels of duration of diabetes

	Control group	Diabetic patients (years)			
	n = 40	≦ 5	5–10	10–15	≧15
		n = 53	n = 94	n = 25	n = 31
IL-18 (pg/ml)	107.4 ± 36.6	113.9 ± 31.3	113.6 ± 33.1	109 ± 30.7	112.7 ± 33.5
p		0.341	0.266	0.942	0.873
p values were obtained from t test between diabetic patients and control subjects					

4. Discussion

With the extension of life expectancy of Chinese population and the changes in dietary habits and structure, the incidence of diabetes is increasing year after year. According to the latest statistics, currently about 50 million people are facing the threat of diabetes in China, and diabetes has become a common disease affecting human health. In addition, DR is one of the leading causes of blindness. The pathogenesis of type 2 DR is still unclear, thus the study of pathogenesis, prevention and therapy of DR has become urgent in medical science. The cytokine plays an important role in mediated inflammatory mechanisms in type 2 diabetic patients. The studies have shown that, IL-6,TNF-α and other proinflammatory cytokine levels are significantly increased in type 2 diabetic patients, suggesting that these proinflammatory cytokines have promoted inflammatory response in the pathogenesis of type 2 diabetic patients[16]. IL-18 is a multifunctional cytokine in the inflammatory cascade and is critical in promoting inflammation pathogenesis in type 2 diabetic patients [17]. Multiple reports have indicated that the serum IL-18 levels in type 2 diabetic patients were elevated in varying degrees[18]. In contrast, there are studies showing that in AMD patients, the IL-18 level in the aqueous humor was decreased and that the IL-18 level was positively correlated with vision and the therapeutic efficacy of monoclonal antibody. Thus, the increase of serum IL-18 level in type 2 diabetic patients and its role in the pathogenesis of DR are still a debate.

4.1 The severity of diabetes was not related to the change of IL-18 level

To clarify the changes of serum IL-18 levels in type 2 diabetic patients and its relationship with the DR, the current study found that the protein levels of serum IL-18 was comparable between diabetic patients and control subjects, although the mRNA level of IL-18 was slightly higher in serum from type 2 diabetic patients compared to that of control group (Table 1 and Fig. 2).The further analysis of IL-18 levels of diabetic patients' serum revealed that the severity of diabetes was also not associated with the change of

IL-18 levels (Table 2). In addition, there were no significant correlations between the serum IL-18 levels and medical history as well as vision among diabetic patients.

4.2 IL-18 mRNA was not significantly increased in patients with type 2 diabetes mellitus

As an endogenous multifunctional regulator cytokine, IL-18 is first synthesized in the cells as a biologically inactive precursor protein Pro-IL-18, which is then digested by caspase-1 and become active mature IL-18[19]. However, partial of IL-18 is inhibited by binding to IL-18 binding protein. In normal circumstances the total mature IL-18 levels in blood is about 80–120 pg/mL [20]. However, ELISA only measures the free IL-18. To reflect the expression level of IL-18 in type 2 diabetic patients, the current study applied the quantitative PCR (RT-qPCR) method to determine the expression amount of peripheral blood cell IL-18 mRNA and found that there was no significant increase in the expression of IL-18 mRNA in type 2 diabetic patients compared with normal control subjects (Fig. 2).

4.3 IL-18 plays a critical role in inhibiting the formation of new blood vessels

In a CNV model, intraocular injection of VEGF and VEGF induced obvious retinal vascular leakage, and the mice with recombinant IL-18 injection had significant reduction in the area of new blood vessels, and the blood seeping area were also significantly reduced [13.14]. The latest studies suggest that recombinant human IL-18 is safe and can reduce choroidal neovascular lesion development in cynomolgus monkeys [7]. Thus, IL-18 plays a critical role in inhibiting the formation of new blood vessels. The current study suggest that the levels of serum IL-18 in diabetic patients were not significantly different compared to control group by ELISA and RT-PCR, suggesting that the functioin of IL-18 in DR needs to be further studied. The capability of IL-18 to inhibit angiogenesis indicates that IL-18 is a good candicate for DR treatment.

In conclusion, we found that the serum IL-18 levels were not significantly increased in the patients with type 2 diabetic patients. The phase II clinical trial has proved that the recombinant IL-18 is safe in human subjects, and the results from the study of recombinant IL-18 in the Department of Ophthalmology have indicated that IL-18 has therapeutic value in pathological neovascularization.

Declarations

Ethical Approval and Consent to participate

The serum of patients with diabetes mellitus in this study was from the hospital directly under the group (He Eye Specialist Hospital), and the patients were informed and agreed, and were approved by the ethics committee.

Consent for publication

All authors have read and approved the final manuscript.

Availability of supporting data

Not applicable.

Competing interests

The authors declare that there are no competing interests.

Funding

This work was supported by the Science and technology foundation of Liaoning Province（2015010568-301）.

Authors' contributions

All corresponding and first authors contributed to the study concept and design.SZ and CL wrote the manuscript. ZW performed the experiments PZ analyzed the data. All authors reviewed and approved the final manuscript.

Acknowledgements

Not applicable

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Figures

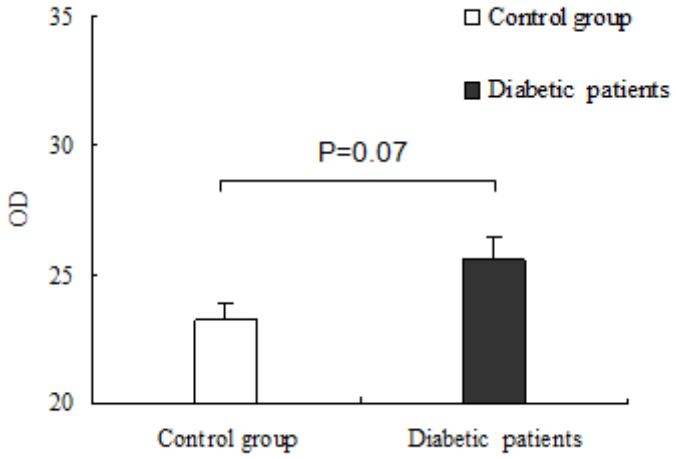

Figure 1

The serum IL-18 mRNA expression levels in control and diabetic patients.