The Relationship Between Pro-Inflammatory Cytokines and Growth Factors in Patients with Diabetic Foot Ulcers

Abstract

Several factors are involved in the healing process of diabetic foot ulcers. Thus, the present study was conducted to evaluate the relationship between pro-inflammatory cytokines and growth factors in patients with diabetic foot ulcers. This study was conducted on three groups of subjects. The first group was men and women (n=30) with diabetes and diabetic foot ulcers referring clinical centers in Iran. Another group was including diabetic women and men patients (n=30) without ulcers. Third group was including healthy men and women (n=30). Blood samples were collected and assessed for the serum concentrations of TNF-α, IL-1β, IL-6, bFGF, IFN-γ, selenoprotein, PDGF, VEGF and GM-CSF with the help of specific kits. The results showed significant differences between groups for the serum concentrations of TNF-α (P=0.001), IL-1β (P=0.001), IL-6 (P=0.001), bFGF (P=0.001), IFN-γ (P=0.001), selenoprotein (P=0.001), PDGF (P=0.001), VEGF (P=0.001) and GM-CSF (P=0.001). The results showed the serum concentrations of TNF-α, IL-1β, IL-6, IFN-γ, PDGF, and GM-CSF were significantly higher in the patients with diabetes and foot ulcer, diabetes and healthy subjects, respectively. The results showed a positive correlation between pro-inflammatory cytokines, while a negative correlation was observed between pro-inflammatory cytokines with other variables. In conclusion, pro-inflammatory and inflammatory factors were significantly higher in those with diabetes and especially with diabetic ulcers. The concentration of factors can be considered for the treatment and as markers for the treatment of foot ulcers.

Introduction

A wound is defined as a disruption in the defense function of the skin [1, 2]. It is also defined as the loss of the integrity of the covering tissue, which can be accompanied or without damage to the connective tissue, which is the result of physical or thermal damage to the skin [3, 4]. Diabetes is a type of metabolic disease related to the body's metabolism and it is one of the most common glandular diseases that is associated with high blood glucose levels that is caused by defects in insulin secretion or its function or both [5, 6]. High blood glucose levels (hyperglycemia) can lead to serious health problems. It causes ulcers in diabetic patients. About 25% of people who suffer from diabetes show foot ulcers during their lifetime. Ulcers and other complications are responsible for the hospitalization of 20% of the approximately three million people who refer to hospitals for diabetes treatment every year [7]. Diabetes delays cell infiltration, angiogenesis, coagulation and wound closure [8]. The increase in blood glucose causes an increase in oxidative stress and a decrease in the expression of insulin-like growth factor-1, which in turn affects the proliferation of fibroblasts and keratinocytes and the regeneration of epithelial tissue and the wound healing process [9, 10]. The decrease in endothelial insulin/insulin-like growth factor-1 signaling is a key factor that delays wound healing process [10, 11]. Chronic diabetic wounds are closely related to the state of permanent inflammation, increased pro-inflammatory cytokines and defects in the expression of growth factors [12]. Indeed, in diabetic patients, due to impaired insulin secretion, the cells of the body are not able to use the glucose inside the blood vessels in such a way that the inside of the cells is free of glucose and glucosemia, while the amount of glucose in the blood vessels and capillaries is high [13, 14]. Over time, high glucose can cause damage in the capillaries and nerves in such a way that improper blood supply and nerve damage in the lower limbs make them prone to diabetic ulcers [15]. One of the first symptoms of diabetic foot ulcer is abnormal inflammation, burning, redness and bad smell [16]. Black tissue (dark) around the wound, which is caused due to insufficient blood supply in the area, is one of the visible signs of diabetic foot ulcer, which indicates the death of cellular tissue around the wound due to infection [17]. Diabetic foot ulcer symptoms are not always visible. Sometimes the ulcer is not visible until an infection occurs [13, 18]. Several factors are involved in the healing process of diabetic foot ulcers. In the resent study, we aimed to investigate pro-inflammatory cytokines and growth factors in patients with diabetic foot ulcers. Thus, the present study was conducted to evaluate the relationship between pro-inflammatory cytokines and growth factors in patients with diabetic foot ulcers. The results can help to identify physiological mechanisms and seeking efficient therapeutic strategies for the treatment of diabetic foot ulcers.

Materials and Methods

This study was conducted on three groups of subjects. The first group was men and women (n=30) with diabetes and diabetic foot ulcers referring clinical centers in Iran. Another group was including diabetic women and men patients (n=30) without ulcers. Third group was including healthy men and women (n=30). All the patients had awareness of details of the current study. In each group, we selected an equal ratio of both genders (15 men and 15 women). We also tried to equal ratios for age, BMI, etc. The mean of age was 41.23±7.85 year, 44.56±10.11 year and 42.10±12.10 year in the first to third groups, respectively. All the subjects signed written consents. Blood samples were collected and assessed for the serum concentrations of TNF-α, IL-1β, IL-6, bFGF, IFN-γ, selenoprotein, PDGF, VEGF and GM-CSF with the help of specific kits.

The data were analyzed for normality and the data were normal. Since the data were normal, ANOVA was used to analyze the data. To investigate correlation between variables, Pearson correlation was used.

Findings

Table 1 depicts the results for the serum concentrations of factors. The results showed significant differences between groups for the serum concentrations of TNF-α (P=0.001), IL-1β (P=0.001), IL-6 (P=0.001), bFGF (P=0.001), IFN-γ (P=0.001), selenoprotein (P=0.001), PDGF (P=0.001), VEGF (P=0.001) and GM-CSF (P=0.001). The results showed the serum concentrations of TNF-α, IL-1β, IL-6, IFN-γ, PDGF, and GM-CSF were significantly higher in the patients with diabetes and foot ulcer, diabetes and healthy subjects, respectively. The results also showed the serum concentrations of bFGF, selenoprotein and VEGF were significantly higher in healthy, diabetic and diabetic with foot ulcer subjects, respectively.

Table 1. The results for the serum concentrations of factors

Factors	Foot ulcers	Diabetic	Healthy	P-values
TNF-α (ng/mL)	7.21±1.23	4.23±1.23	1.56±0.23	0.001
IL-1β (ng/mL)	17.23±3.21	12.35±1.56	4.23±0.69	0.001
IL-6 (ng/mL)	10.25±1.26	6.78±0.78	2.36±0.57	0.001
bFGF-2 (pg/mL)	221.23±15.63	251.84±15.23	321.23±14.56	0.001
VEGF (ng/mL)	0.56±0.21	1.21±0.23	3.21±0.21	0.001
IFN-γ (pg/mL)	3.21±0.23	1.89±0.42	0.45±0.21	0.001
Selenoprotein (µg/mL)	2.21±0.25	2.89±0.12	5.12±0.21	0.001
PDGF (ng/mL)	7.21±1.25	5.63±0.21	2.54±0.15	0.001
GM-CSF (pg/mL)	2.85±0.31	1.75±0.25	0.95±0.25	0.001

Table 2 shows the results for correlation between healthy subjects. The results did not show any negative and correlation between variables in healthy subjects.

Table 2. The results for correlation between healthy subjects

Factors	1	2	3	4	5	6	7	8
TNF-α (1)	-							
IL-1β (2)	0.089	-						
IL-6 (3)	0.078	0.58	-					
bFGF-2 (4)	0.045	0.081	0.032	-				
VEGF (5)	0.052	0.101	0.061	0.025	-			
IFN-γ (6)	0.057	0.025	0.052	0.063	0.081	-		
Selenoprotein (7)	0.065	0.063	0.057	0.048	0.026	0.032	-	
PDGF (8)	0.052	0.081	0.069	0.052	0.032	0.032	0.063	-
GM-CSF (9)	0.058	0.075	0.041	0.063	0.041	0.056	0.41	0.052

Tables 3 and 4 show the results for correlation between diabetic subjects. The results showed a positive correlation between pro-inflammatory cytokines, while a negative correlation was observed between pro-inflammatory cytokines with other variables.

Table 3. The results for correlation between diabetic subjects

Factors	1	2	3	4	5	6	7	8
TNF-α (1)	-							
IL-1β (2)	0.356***	-						
IL-6 (3)	0.354***	0.312***	-					
bFGF-2 (4)	-0.341***	-0.331***	-0.44***	-				
VEGF (5)	-0.412***	-0.404***	-0.512***	0.512***	-			
IFN-γ (6)	0.551***	0.375***	0.632***	-0.412***	-0.412***	-		
Selenoprotein (7)	-0.441***	-0.365***	-0.414***	0.514***	0.514***	-0.412***	-	
PDGF (8)	-0.351***	-0.341***	-0.471***	0.415***	0.415***	-0.581***	0.321***	-
GM-CSF (9)	0.412***	0.362***	0.381***	-0.301***	-0.301***	0.389***	0.313***	0.351**

Superscript (***) shows correlation between variables at P<0.0001.

Table 4. The results for correlation between diabetic subjects with foot ulcers

Factors	1	2	3	4	5	6	7	8
TNF-α (1)	-							
IL-1β (2)	0.412***	-						
IL-6 (3)	0.368***	0.341***	-					
bFGF-2 (4)	-0.414***	-0.321***	-0.442***	-				
VEGF (5)	-0.521***	-0.415***	-0.369***	0.371***	-			
IFN-γ (6)	0.365***	0.369***	0.412***	-0.369***	-0.441***	-		
Selenoprotein (7)	-0.402***	-0.412***	-0.512***	0.441***	0.552***	-0.369***	-	
PDGF (8)	-0.326***	-0.371***	-0.441***	0.442***	0.443***	-0.512***	0.341***	-
GM-CSF (9)	0.325***	0.312***	0.369***	-0.369***	-0.371***	0.341***	0.321***	0.341**

Superscript (***) shows correlation between variables at P<0.0001.

Discussion

This study was conducted to evaluate the relationship between pro-inflammatory cytokines and growth factors in patients with diabetic foot ulcers. The results showed higher concentration of TNF-α in those with diabetes and foot ulcer. It shows that foot ulcer provokes TNF compared with diabetes. TNF-α is a pleotropic cytokine that is produced by a group of cells such as keratinocytes, macrophages, and mast cells [19]. This factor acts in mechanisms such as the use of leukocytes,

mainly neutrophils, the control of molecular adhesion, the production of chemokines and matrix metalloproteinases, and also as an inhibitor of metalloproteinases. TNF-α plays a beneficial role in the wound healing process and shows its mechanism by reducing the production of granulation tissue, but reducing the expression and concentration of this factor plays an important role in the production of collagen [20]. This factor regulates the activity of some fibroblasts, keratinocytes, and vascular endothelial cells and plays an important role in the production of metalloproteinases [21]. Thus, TNF increases in the response to the inflammation and exhibits its responses.

Similar to the results for TNF-α, IL-1β concentration was significantly increased in the response to ulcer and diabetes. It was significantly increased in diabetic patients with ulcer foots. Interleukin-1 beta is secreted from keratinocytes, fibroblasts, endothelial cells, nervous system, immune cells such as macrophages and mast cells and glial cells such as Schwann cells, microglia and astrocytes [22]. The expression of interleukin-1beta in the wound area is related to the phenotype of pro-inflammatory macrophages and inhibits the interleukin-1beta pathway in the wound area [23]. Interleukin-1 causes various activities such as neurological, hematological, endocrinological and metabolic system changes and has various effects on wound healing [24]. First, interleukin-1 induces capillary endothelial cells to produce some chemokines such as MCP-1 and increase the synthesis of vascular adhesion molecules [24]. The activities cause mononuclear cells to seep into the injury points and control inflammatory responses. The increase of the factors is a result of response to ulcer. Seemingly, ulcer and diabetes cause major responses.

The results also showed that IL-6 concentration was significantly higher in those in diabetic and ulcers, diabetic patients and healthy subjects. Interleukin-6 plays an essential role in the inflammatory process, especially in the preliminary phase of inflammation [25]. In laboratory studies, it was shown that interleukin-6 has no chemotactic activity for leukocytes, but mice lacking interleukin-6 have lower leukocyte infiltration. These mice showed lower fibrotic changes in liver fibrosis. Interleukin-6 may control the use of leukocytes in inflammatory points and fibrotic changes [26]. The increase of IL-6 in patients with diabetes could be attributed to disorders in metabolic responses. Excessive increase in diabetic patients with foot ulcer could be attributed to severe inflammation in foot.

Inflammatory factor was IFN-γ. Interferon gamma is a type II interferon. This interferon plays an essential role in innate immunity and adaptive immunity against viruses, some bacteria and protozoa. This interferon stimulates macrophages and expression of major histocompatibility complex class II [27]. Thus, it also has a major role in immune responses and its concentration increases in the response to inflammation of ulcer.

The results showed a decrease in concentration of bFGF-2 in patients compared with healthy subjects. The efficiency of this factor is known in the regenerated epidermis, newly created capillaries and cells seeping into the flesh bud tissue. This factor, along with vascular endothelial growth factor, induces angiogenesis, supports cell nutrition, provides oxygen, and helps provide energy [28]. In patient subjects, this factor is degraded and/or consumed. Thus, its serum concentration is lower in patients compared with healthy subjects.

The results also showed a decrease in concentration of VEGF in healthy subjects. Vascular endothelial growth factor is one of the desired genes that intervenes in the wound healing process and acts as an endothelial cell mitogen and chemotactic [29]. The mechanism of this factor is not only by stimulating angiogenesis but also by increasing the permeability of the vessel and facilitating wound healing. VEGF is a cytokine that is responsible for inducing angiogenesis, cell migration, proliferation and synthesis of extracellular fluid proteins [30]. It was expected to be higher VEGF in diabetic patients and those with foot ulcers. However, the results showed lower concentrations in the patients. It could be attributed to selenoprotein concentration. Selenoprotein protects endothelial cells and the decrease in selenoprotein decrease the concentration of VEGF.

The serum concentration of PDGF was significantly higher in patients compared with healthy subjects. Platelet-derived growth factor (PDGF) constitutes a family of dimeric isoforms, acting on connective tissue cells and certain other cell types. PDGF was originally discovered as a constituent of platelets, which are released into serum in conjunction with blood coagulation [31]. It shows that PDGF concentration increases in the response to metabolic disorders of diabetes and also damages in patients with foot ulcers.

GM-CSF was significantly higher in patients compared with those in healthy group. It is a monomeric glycoprotein is secreted by macrophages, T cells, mast cells, natural killer cells,

endothelial cells and fibroblasts and acts as a cytokine [32]. Thus, GM-CSF works parallel with pro-inflammatory cytokines and increases the inflammatory responses.

The results showed a positive correlation between inflammatory cytokines. It is natural that inflammatory factors work in the same way and increase the inflammatory responses. In addition, the increase in inflammatory responses modulates with the expression and concentration of growth factors. It is essential to apply interventions for decreasing the inflammation and increasing the concentration of growth factors. Higher responses in those with diabetes and ulcer could be attributed to bilateral disorders.

Conclusions

In conclusion, pro-inflammatory and inflammatory factors were significantly higher in those with diabetes and especially with diabetic ulcers. Higher inflammatory concentrations is a response for the inflammatory status. The concentration of factors can be considered for the treatment and as markers for the treatment of foot ulcers.

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