

Role of TNF- α in Diabetic Ulcer Healing Process

Fahrur Nur Rosyid

Lecturer in Nursing Study Program, Faculty of Health Sciences, UMS

Correspondent: fnr100@ums.ac.id, fahrurnurrosyid@yahoo.com

Abstract

One of the long-term complications of diabetes mellitus is diabetic ulcers (15%) and 85% of the cases are the cause of amputation on patients with diabetes mellitus. Diabetic foot ulcers increase apoptosis and decrease fibroblast proliferation of fibroblasts and inflammatory reactions is elongated, this is as evidenced by the presence of neutrophil granulocytes in large quantities in the wound. Neutrophil granulocytes secrete pro-inflammatory cytokines mainly TNF- α . TNF- α is causing wound healing becomes disconnected and uncoordinated. This paper discussed the role of TNF- α in diabetic foot ulcers.

Keywords: Complications of DM, TNF- α and diabetic ulcer healing

1. Background

Diabetes mellitus (DM) is a group of diseases characterized by insufficient insulin production or failure of adequate response to insulin, which might cause hyperglycemia [1, 2]. One of the long-term complications of diabetes mellitus is diabetic ulcers (15%) [3, 4] and 85% of the cases are the cause of amputation in patients with diabetes mellitus [4].

Diabetic ulcer is an open wound in the skin layer to the dermis, which usually occurs in the feet [5]. The risk of infection and amputation is still quite high; the record showed that 40-80% of diabetic foot ulcers become infected [6], and 14-20% requires amputation [7]. This situation is associated with late diagnosis and consultation, inadequate handling, as well as the extent of tissue damage [8]. Amputation of the leg is more often done on the basis of extensive soft tissue infections or in combination with osteomyelitis, in addition to other factors such as ischemia due to peripheral artery disease (PAD) and neuropathy [8, 9].

Diabetic foot ulcers increase apoptosis and decrease fibroblast proliferation of fibroblasts and inflammatory reactions are elongated, with a proven presence of neutrophil granulocytes in large quantities in the wound. Neutrophil granulocytes secrete pro-inflammatory cytokines mainly TNF- α and interleukin-1 β (IL-1 β). Both of these cytokines will stimulate the synthesis of matrix metalloprotease (MMP) which causes degradation of matrix proteins and growth factors that will make wound healing process becomes disconnected and uncoordinated [10]. Some literature reported elevated levels of TNF- α in the tissue of diabetic ulcer patients and experimental animals [10-13], the increase in TNF- α locally and systemically in patients with type 2 DM [14], decreased levels of VEGF in the tissue of diabetic ulcers [15-16] and in diabetic neuropathy [17]. Environmental pro-inflammatory is increased and prolonged in diabetic foot ulcers and is characterized by an increase in TNF- α and is followed by a decline of VEGF, which occurred due to the degradation process by TNF- α induced by bacterial contamination and repeated trauma, in which the bacterial endotoxin, fragments of extracellular matrix, and cells detritus maintain the inflammation [10]. VEGF becomes one of the growth factors that has an important role in wound healing neovascularization [16].

2. Definition of TNF- α

TNF- α is a pro-inflammatory cytokine produced primarily by monocytes and macrophages. It has a role in many processes in the body including the pathogenesis of various diseases such as septic shock, cancer, rheumatoid arthritis, multiple sclerosis and other autoimmune disorders or inflammation. The latest study showed that TNF- α was involved in insulin resistance in obesity and type 2 diabetes mellitus. Even though the molecular mechanism of TNF- α inducing insulin resistance is still unknown, in vitro studies showed that insulin inhibits TNF- α -mediated autophosphorylation of the insulin receptor and decrease phosphorylation in muscle tissues including adipose tissue, which is mostly bound to the p55 TNF receptor. Levels of TNF- α can be local or systemic increase in insulin resistance both in animal and human fat. Besides, the expression of TNF- α in the muscles of people with type 2 diabetes were significantly higher than those with non-DM. Levels of TNF- α circulating in the circle of people with obesity and glucose intolerance increase and are associated with abdominal fat mass [14].

3. Structure of TNF- α

TNF ligand is associated with type II (intracellular N-terminus) transmembrane protein which contains TNF domain homologous in the C terminal extracellular [18-19]. TNF is synthesized as a monomer in the folds of β -sheet sandwich and assembled into functional trimer. Each has 3 receptors of TNF ligand bonded in place [20]. TNF- α has 212 amino acid type II transmembrane protein that is formed in homotrimers. Membrane belonging forming homotrimeric soluble cytokine (sTNF) is formed by proteolytic by metalloproteases TNF- α that alter enzyme [21]. Oppenheim in 2001 stated that the synthesis of TNF- α is derived from intracellular propeptida and then processed and the influence of TNF- α converting enzyme (TACE) causing it to mature and then secreted. As with other cytokines in the same time to form a 2-3 tie with the active TNF receptor, as a result of cross-links from its receptor, which then sends signals / cues into the cell [22]. The structure of TNF- α can be seen in Figure 1.

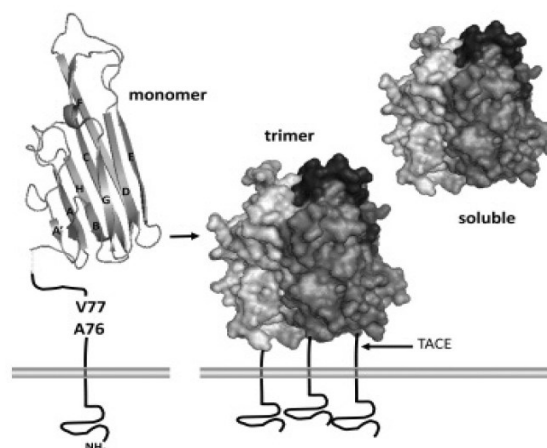


Figure 1. The basic structure of TNF- α [23].

4. Synthesis and biological activity TNF- α

TNF- α has several functions in the inflammatory process such as increases the role of pro trombik and stimulate cell adhesion molecules of leukocytes and inducing endothelial cell; plays a role in regulating the activation of macrophages and the immune response in the tissue,

which stimulates growth factors and other cytokines; functions as a regulator of hematopoietic and komitogen to T cells and B cells as well as cell activation of neutrophils and macrophages [22, 24]. Oppenheim in 2001 stated that the synthesis of TNF- α is derived from intracellular propeptida which is then processed and the influence of TNF- α converting enzyme (TACE) causing it to mature and then secreted [22].

According to Abbas *et al.*, (2000), TNF- α have some effect with the manifestations, which can be described as follows [25]:

- a. TNF as a cytotoxic effect is the effect of some event type of tumor, in which the setback and necrosis are accompanied by bleeding. The mechanism of tumor cell death *in vivo* by TNF is unclear, but it is clear that the death of tumor cells will be accelerated if there is obstacles protein synthesis in tumor cells. The mechanism of tumor cell death *in vivo* is not the direct effect of TNF for tumor tissue necrosis due to vascular disorders. There is evidence, which showed that the activated macrophage cells can kill cells juniors, while TNF is a macrophage cell product.
- b. TNF effect against inflammation is visible at the present time as TNF is considered as the main mediator in inflammation. In the last decade, research showed that the obtained TNF in pure form is biochemically turned out to be responsible for "cahectin" activities which generally work in people who have parasitic infection. Mechanisms at some local inflammatory events are predicted by *in vitro* observations. For example, neutrophil cells reacting with TNF improve binding to endothelial cells, respiratory burst and its degranulation. The pattern of inflammation tissue damage is similar to the damage of IL-1, TNF is considered to be highly important in wound healing process.
- c. TNF effects on hematopoietic are responding activity by inhibiting monocyte colony-granuiosit culture, erythrocytes and multi-potential cell colonies on human bone marrow tissue. However, in contrast, progenitor in the bone marrow tissue is found *in vivo* experiments.
- d. TNF effect on immunologic. Tumor necrosis' factor has activity against multiple stimulations to the activated T lymphocytes, such as proliferative response of T lymphocytes to antigens and increased receptor for IL-2, and IFN- γ production induction. Likewise, specific immunity against the tumor is enhanced by TNF. TNF can also increase the expression of MHC class I antigens in fibroblasts and endothelial cells.

5. Receptor TNF- α

Similar to other cytokines in the same time to form a 2-3 tie with the active TNF receptor, as a result of cross-links from its receptor, which then sends signals / signaling into the cell. There are two TNF- α receptors that have been identified. They are TNFR₁ and TNFR₁₁. In TNFR₁, each receptor on cytoplasmic domain has a large and spacious shape and can send signaling through NF κ B pathway that is important in the field of immunology since TNFR₁ is the major mediator of TNF activity. On the other hand, TNFR₁₁ serves only as a supplement. In addition to the TNFR₁, cytoplasmic portion has a circuit that consists of 80 amino acids, called the death domain; and TNFR₁ and FAS ligand will bind to each of them. Genesis apoptosis that will occur as a result of the bond between TNF and TNFR₁ and FAS with FasL may also occur as a result of activation of caspase-8 with all its cascades. TNF- α will undergo endocytosis after binding to the ligand. TNFR₁₁ binds to TNF- α with the ability of 10-fold compared to receptor TNFR₁.

The expression of receptors TNFR₁ and TNFR₁₁ can be improved through the stimulation of IL-2, whereas IFN- γ will stimulate TNFR₁₁ selectively. Activation of the cell will cause the

cell to immediately release TNF- α receptor to bind to TNF- α during the inflammatory response. TNF- α receptor could be seen in Figure 2 and Figure 3.

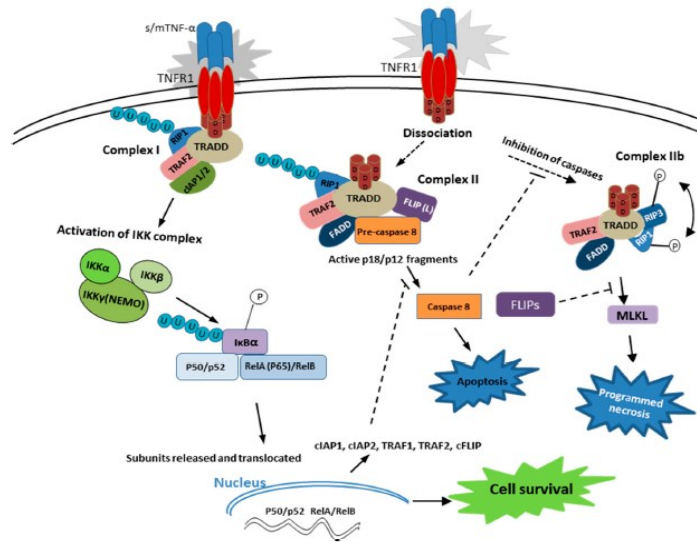


Figure 2. Receptor TNFR₁ [26].

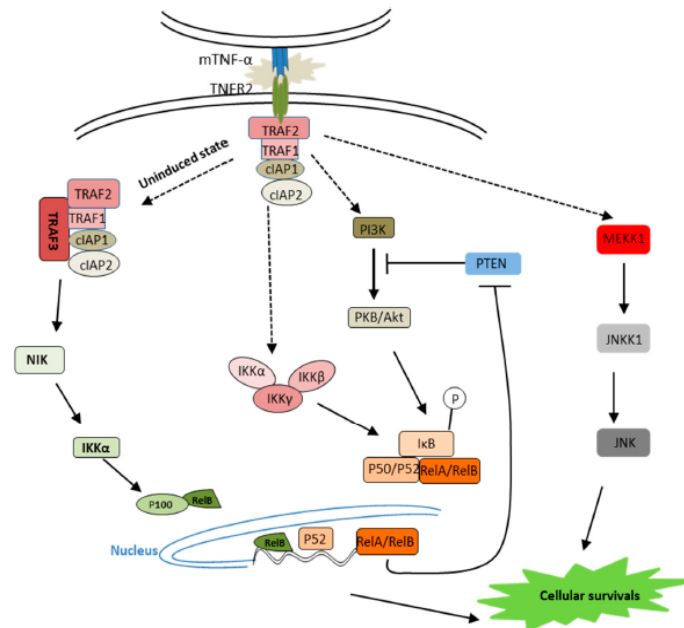


Figure 3. Receptor TNFR₁₁ [26].

6. The role of TNF- α in the process of healing of diabetic foot ulcers

In diabetic foot ulcers, the elevated levels of TNF- α [10-13], increased fibroblast apoptosis, and decreased proliferation of fibroblasts [13] were found, followed by impaired healing process of ulcers. TNF- α is a marker of inflammation in the tissue healing process. Previous studies had shown the relationship of TNF- α on wound healing process. The decreased levels of TNF- α indicates that the control of inflammation and healing progress is inadequate [12] as TNF- α stimulates the synthesis of MMP. With high level of protease in the wound, it causes degradation of matrix proteins and growth factors that are important factors in the wound

healing process. Consequently, healing process becomes disconnected and uncoordinated [10]. In addition, TNF- α suppresses tissue growth factor- β (TGF- β) which induces miofibroblas proliferate to form the proteins that are important in the reorganization of matrix extracellular like α -smooth muscle actin (α -SMA), collagen type 1A, and fibronectin, which will impair wound healing process [11].

7. Factors affecting TNF- α

Most people with risk factors for type 2 diabetes have high levels of TNF. Obesity increased the secretion of TNF- α by adipose tissue as a mediator of insulin resistance [27]. Aging, Black ethnicity and family history of type 2 diabetes are also associated with the increased production of TNF- α [28, 29]. Rash African-American patients with HCV-infected blacks had higher levels of TNF- α compared to non-blacks [29]. Patients with a family with dm sTNFR2 have higher levels compared with non-diabetic patients with a family history [30]. Factors affecting TNF- α could be seen in Figure 4.

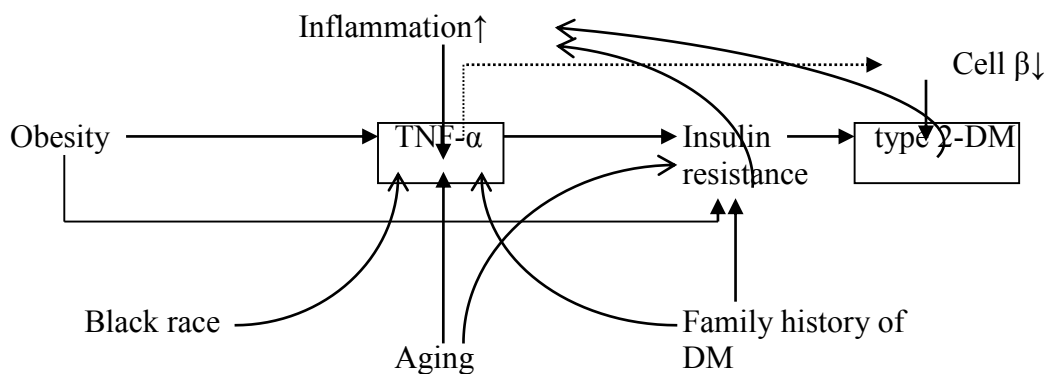


Figure 4. Factors affecting TNF- α [31].

8. TNF- α measurement

Measurement of serum TNF- α could be performed on tissue specimens and plasma. TNF- α levels were measured using the method Sorbant Enzyme-Linked Immuno Assay (ELISA) (R & D Systems, Minneapollis, USA). Siqueira et al. (2010) conducted a measurement of TNF- α from tissue specimens ulcers, tissue was taken by punch biopsy and frozen in a solution of nitrogen, then placed into cytoplasmic lysis buffer containing protease inhibitors (Pierce, Rockford, IL, USA) and destroyed using Fast Prep (Q-Biogene, Solon, OH, USA). Nuclei were separated from cytoplasmic proteins and centrifuged [13]. While Wallace and Stacey (1998) conducted a measurement of TNF- α by ELISA method in patients by taking fluid samples from chronic wounds that were not healed, and the result of a measurement was the median of 2428.5 pg / ml, whereas in patients who recovered the levels of TNF- α was 895.2 pg / ml [32].

9. Conclusion

Diabetic ulcer is an open wound in the skin layer to the dermis, which usually occurs in the feet. The risk of infection and amputation is still quite high. In diabetic foot ulcers, the researchers found elevated levels of TNF- α , increased fibroblast apoptosis, and decreased fibroblast cell proliferation resulting in impaired healing of ulcers. Factors affecting the increase

in the levels of TNF- α is inflammation, obesity, aging, black ethnicity, and family history of diabetes type 2. The increased TNF- α implicated in the impaired wound healing process.

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