

## **Therapeutic effects of ozone in patients with diabetic foot ulcers: review of the literature.**

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### **Abstract**

**The prevalence of Diabetes Mellitus (DM) is estimated to be about 300 million people worldwide. Poor diabetes control may lead to complications such as diabetic foot ulcers or amputations. The high incidence of amputation is still considered as an important socio-economic problem. The increased incidence of diabetes increases the incidence of diabetic foot ulcers. Patients with diabetic foot ulcers may also be at high risks of mortality due to the associated cardiovascular diseases. Foot ulcers are one of the most important chronic complications of diabetes and are regarded as a major problem which significantly affects the quality of life. Diabetic foot ulcers is also the most important cause of morbidity and mortality in DM patients and is considered a major psychological, economical, and social problem which has been resulted in the hospitalization of patients with DM all around the world.**

**The aim of this study is to evaluate the effectiveness of ozone therapy (as a complementary treatment) through different techniques in the management of diabetic foot ulcers.**

**The present study provides clinical evidence which supports and recommends the benefits of ozone therapy in diabetic foot ulcer.**

**Keywords:** Diabetic foot, Ozone, Reactive oxygen species.

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### **Introduction**

Diabetes Mellitus (DM) is a metabolic disorder, caused by impaired carbohydrate metabolism, improper insulin production, or consumptions which lead to glycosuria and hyperglycemia. Long-term complications are characterized by microvascular diseases caused by the thickening of the capillary basement membrane, microvascular disease with an increased risk of arteriosclerosis, neuropathy, and neuromuscular dysfunction with muscular dystrophy and reduced immune system response to infections and chronic disorders of the kidneys, eyes, nerves, heart and blood vessels. Increase risk of atherosclerosis is one of the leading cause of the deaths from diabetes, 75% of these death associated with coronary artery diseases. Neuropathy is often accompanied by distal numbness and development of neurotrophic ulcers especially at the soles of the feet. These changes, along with capillary and microvascular complications are the characteristics that may lead to gangrene following a leg injury [1,2].

In patients with DM, the role of Reactive Oxygen Species (ROS's) has been shown in increasing the oxidative damage in the lipid peroxidation surface though the Deoxyribonucleic Acid (DNA) and protein damage [3].

Four molecular mechanisms are affected in glucose-mediated vascular damage: increased polyol pathway, increased formation of advanced glycation end-product, activation of Protein Kinase C (PKC) isoforms, and increased hexosamines pathway [4].

Ozone can be effective though oxidative preconditioning, stimulation of endogenous antioxidant systems and obstruction of xanthine oxidase pathways for ROS production, as shown in the examinations from the damage induced by Carbon Tetrachloride (CCl<sub>4</sub>) or renal and hepatic ischemia re-flow [5].

In addition, it has been shown that oxidative preconditioning of ozone maintains the glycogen content and reduces the formation of lactate and uric acid through controlling the oxidative stress induced by CCl<sub>4</sub> consumption in rat. It has also been shown that intravenous ozone therapy in patients with myocardial infarction has a beneficial effect on blood lipid metabolism by reducing the blood cholesterol levels and activating the antioxidant protection system [6].

Some epidemiological studies showed that there were 300 million type 2 diabetics in 2005. About 15% of the people with diabetes develop diabetic foot ulcer in their lifetime at a rate of 2% to 3% per year. More than 50% of lower extremity amputations in the United States occur in people with diabetes.

Among these amputations, 25% are below and 20% are above the knee. The three-year mortality after the amputations is 20-50%, while the 5-year mortality rate is 39-68% [7].

### **Why is this Review Important?**

It has been recently shown that ozone therapy may contribute to the treatment of diabetic foot ulcers [8]. There has been no systematic review of the available literatures regarding the effectiveness of ozone in the treatment of foot ulcers in diabetics done yet. Since the socioeconomic impact of diabetes is detrimental for both the patient and the society, any treatment capable of stabilizing the oxygen metabolism and balancing the oxidative stress along with antibacterial properties can improve the patient's quality of life and reduce hospitalization. The present review aimed at evaluating the effectiveness of ozone in the treatment of type 2 diabetics with diabetic foot ulcers and examining its effects on oxidative stress, hyperglycemia, and some signs of endothelial damage.

### **Types of studies**

Randomized Controlled Trials (RCTs) were considered for the inclusion.

### **Types of Subjects**

Adult patients with type 1 or 2 diabetes and active foot ulcers

### **Types of Interventions**

Ozone therapy was compared with any other intervention such as antibiotics, topical agents, or conventional care.

Search methods for identifying studies

Electronic searches

Studies were identified through searching in the following electronic databases:

- Ovid Medline (1980 onwards)
- Ovid EMBASE CINAHL (1982 onwards)
- SCI (1980-present)
- Ovid EMBASE (1982-present)

This approach was approved for searching Ovid Medline, Ovid EMBASE and Ovid EMBASE.

### ***Basic ozone and diabetes studies***

Diabetes makes many changes in the blood vessels affecting the response of endothelium and the smooth muscles. Vascular endothelium seems to be a sensitive target for hyperglycemia-induced metabolic changes [9]. Polyol pathway activation, protein non-enzymatic glycosylation and increased ROSs play an important role in the complications of diabetes. Ozone was used as a therapeutic agent, and its beneficial effects were observed. However, only a few biochemical and pharmacokinetic mechanisms are described so far.

Since diabetes is a condition accompanied by oxidative stress, it was thought that ozone treatment may support the antioxidant systems and maintain other signs of endothelial cell damage and diabetes problems at the physiological level. A study was designed through the administration of Streptozotocin (STZ) to induce diabetes in order to test the supportive effects of ozone. Ozone therapy improved glycemic control, increased the aldose reductase, fructoselysine content, advanced oxidation protein products in the whole pancreas, and prevented the oxidative damage. In addition, nitrite and nitrate levels were increased in the STZ group compared to the unchanged levels in non-diabetic controls. The results of that study showed that repeated applications of ozone in non-toxic doses may contribute to the control of diabetes and its complications [10].

In addition, the antioxidant properties of ozone inhibited the B cell function and reduced hyperglycemia. Overall, these results show that this approach may represent a potential supplement for the treatment of diabetes and its complications [11].

### ***Ozone and clinical studies on diabetic feet***

Since ozone therapy may activate the antioxidant system through affecting the blood sugar levels and some signs of endothelial cell damage in the pre-clinical level, a study was conducted to investigate the effectiveness of ozone therapy in patients with type 2 diabetes and diabetic foot aiming at comparing the effectiveness of ozone with the antibiotic treatment. A randomized controlled clinical trial was conducted in which 101 patients were divided into two groups: a group (n=52) was treated with ozone (topical or rectal), and the other group (n=49) was treated with topical and systemic antibiotics. The effectiveness of treatments was evaluated by comparing the glycemic index by comparing the area and periphery of the damage, biochemical signs of oxidative stress, and endothelial damage in both groups after 20 d of treatment. Ozone improved the glycemic control, prevented oxidative stress, normalized the organic peroxides levels, and activated the superoxide dismutase. The pharmacodynamics effect of ozone in the treatment of patients with diabetic neuro-infectious foot can be attributed to the fact that it is a corrosive superoxide. Superoxide is the interface between the four metabolic approaches along with diabetes pathology and complications.

Injuries were also healed which resulted in fewer cases of amputations compared to the control group. No side effects were observed. These results showed that treatment with ozone can be a complementary treatment for diabetes and its complications [12].

### ***The use of ozone for the prevention of fungal infection in diabetic foot ulcers***

Fungal infections may be involved in the pathogenesis of diabetic foot ulcers. Published evidences about fungal infections in diabetic foot ulcers are very rare. Most studies have reported a low incidence of fungal isolations or ulcers that

may be infected by fungus or a low incidence of wound healing during the systemic antifungal treatment [13-15]. Extensive research has been conducted on the incidence and persistence of bacterial infections and its effect on wound healing. However, fungal infection in diabetic foot ulcer is a topic that has not been addressed enough. Ali showed the high incidence and the global spectrum of fungi (10 species) in the diabetic foot ulcers compared to previous studies [16]. Isolates obtained from diabetic foot ulcers in our study were similar to species isolated from blood samples by Gonzalez et al. [17]. These results were consistent with those obtained by Missoni, who reported the incidence of fungi in tissue biopsy samples from 22 diabetes patients [18]. Inhibition of *Penicillium* mycelial growth on citrus caused by the oxidizing effect of ozone was reported by Harding [19]. The extremely high effectiveness of ozone in the treatment of diabetic foot ulcers is associated with not only its antimicrobial effect but its ability to reduce hyperglycemia [20]. The anti-diabetic effect of ozone therapy may be attributed to its antioxidant properties, increased insulin sensitivity, and prevention of oxidative stress associated with diabetes [21]. Ozone treatment may lead to the oxidative preconditioning or adaptation with oxidative stress and prevent the damage caused by ROS [22,23]. Ozone can be successfully used in the treatment of chronic wounds including nutritional ulcers, ischemic ulcers and diabetic ulcers [24,25]. The use of ozonated oils (olive oil, sunflower oil) can help in the treatment of diabetic foot ulcers [26]. The use of ozonated olive oil reduced the pus excretion without any side effects. The positive effects of ozone may be due to the biological, bio-rheological, and metabolic activities driven by the exposure of blood to ozone. The antifungal treatment in infections is effective but does not affect the colonization and depends on the sensitivity of fungal species (resistance problem). In contrast, ozone therapy is more effective in terms of colonization. In this respect, ozone can inhibit infections. It can also help to support the antifungal treatment during the infection.

### ***Local application of ozonated oil for the treatment of burns***

The skin healing process consists of several stages which include numerous complex processes including bleeding, inflammation, and formation of granulation tissue, re-epithelialization, and the final phase of restoration (reconstruction). Previous studies have shown that the Fibroblast Growth Factor (FGF), Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) and Vascular Endothelial Growth Factor (VEGF) play important regulatory roles in the coordination of the wound healing process [27-29]. Ozone ( $O_3$ ) is considered as one of the best known anti-bacterial, anti-viral and anti-fungal agents and is practically utilized in clinical practice as a therapeutic agent for chronic wounds such as trophic ulcers, ischemic ulcers, and diabetic ulcers [30-32]. The beneficial effects of  $O_3$  in wound healing are believed to be due to the reduced bacterial infection, improved skin wound healing or increased oxygen supply to the wound area [33]. Ozone does not actively

penetrate the cells but immediately reacts with Polyunsaturated Fatty Acids (PUFAs) to produce ROSs, such as hydrogen peroxide, which can induce the synthesis of growth factors and accelerate the cell cycle through the activation of redox transcription factors (such as nuclear factor- $\kappa$ B) [34]. It was recently suggested that ozonated oil may be the source of  $O_3$ , which can be released slowly to the skin [33]. This effect may be due to the ability of oil in stabilizing the  $O_3$  (such as ozonoids between the double bonds of unsaturated fatty acids), which can be used effectively for topical treatment of skin ulcers [31,34].

Management of skin burns is often a difficult therapeutic challenge for physicians. Successful treatments should result in the restoration of physiological function, reduction of symptoms, and finally, cosmetic improvements of skin lesions. The exact mechanism of the healing process is extremely complex. However, it generally begins with the transformation of the keratinocytes (through the induction of signal transduction factor due to injury) to the cell that are capable of replication and migration. After the transformation, the cells express the molecules that attack the damaged epithelial matrix and recreate the epithelial cells in the wound area [33].

According to some researchers, it does not seem logical to use ozone as it eliminates the pathogens in the first stage. It then activates the fibroblasts proliferation by releasing oxygen ( $O_2$ ). Therefore, the intercellular matrix is created by the subsequent proliferation of ceratinoblasts which leads to wound healing [32].

In addition, various studies on animal models have shown that the use of ozonated oils (topical form of  $O_3$ ) can speed up the healing of acute skin wounds in the guinea pig models through increasing the collagen synthesis and fibroblasts proliferation in the wound and by increasing the expression of growth factors such as PDGF, TGF- $\beta$  and VEGF [26]. However, researchers indicated that further research is needed to determine the effect of  $O_3$  on neovascularization in the skin ulcers healing process [26]. According to these studies, the effectiveness of ozonated oil was compared to that of hyaluronic acid in the late stage of the healing of second degree burns (partial to full skin thickness) with a focus on the impact of ozonated oil on neoangiogenesis. Campanati et al. showed that ozonated oil had a similar effectiveness to that of hyaluronic acid in reducing the symptoms of skin burns (erythema, stretch marks, itching and burning sensation reported by patients) after 12 weeks of topical application [35].

Ozonated oil did not show a specific effect on the neoangiogenesis in the wound healing process following second degree burns. In contrast, compared to hyaluronic acid, ozonated oil was found to be highly effective in preventing the hyperpigmentation after the injury and reducing their severity. The mechanism of the action of ozonated oil has always been a question. However, Travagli et al. suggested a logical explanation in this regard. Most likely, when the stable ozonoid gets in contact with the wound, it is slowly dissociated to various peroxides (that are easily dissolved in water) and probably produce hydrogen peroxide that can explain the

specific activity of ozonated oil [36]. Similar to the treatment with hyaluronic acid, the ozone treatment of patients was surveyed as a convenient and effective option, and a similar level of consistency was observed between both methods.

## Conclusions

A new topic was put forward on the application of a complementary approach based on the fact that ozone therapy can reduce the oxidative stress, delay the serious complications, and improve the quality of life of diabetic foot patients. Based on the type of the foot ulcers, ozonated water and different gradations of standard ozonated vegetable oils are topically used until full recovery [37]. It has been recently proved that both ozonated water and ozonated oils are antiseptics and suitable healing stimulators and are much more effective compared to treatments that involve topical antibiotics growth factors, maggot and negative pressure wound therapies [38-42]. As a support for anti-fungal treatment, ozone therapy can also be useful in the treatment of infections. It should be noted that no side effects have been observed in the studies. These results show that medical treatment with ozone can be regarded as a complementary therapy alongside conventional treatments and antibiotic therapy for diabetic foot ulcers and its complications.

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