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Effects of High-Rate Frequency Modulation Treatment on Malondialdehyde in Diabetic Polyneuropathy

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This study is planned to investigate the effects of transcutaneous electrical nerve stimulation (TENS) treatment on patients which have diabetic polyneuropathy. Fourteen diabetic polyneuropathy patients suffering ischemic pain were examined during the cure. Malondialdehyde (MDA) and glycemia levels were determined by collecting 5 mL blood samples from 14 patients 24 h before beginning the treatment. In case 50 Hz signals as high-rate frequency modulation (HRFM; continuous pulses changed from 90 Hz to 55 Hz over 90 msec, 1.3 times a second), were applied to patients as long as 20 d for 20 min a day. Transcutaneous electrical nerve stimulation (TENS) treatment increased significantly free oxygen radicals ($p < 0.05$). The levels of MDA before TENS were compared to the levels of MDA after TENS and the end of the following term of 20 days by paired sample test and a meaningful increase was seen significantly ($p < 0.01$). Besides, glycemia levels were decreased significantly before TENS-after TENS treatment ($p < 0.01$) and not changed before TENS and the end of the following term of 20 d ($p > 0.05$). Moreover, it was observed that MDA levels were decreased significantly between in the final of the treatment and the end of the following term of 20 d ($p < 0.01$). Glycemia levels were not changed significantly after TENS and the end of the following term of 20 d ($p > 0.05$). According to this results, it is observed that TENS treatment has been positive effect on polyneuropathy.

Key Words: Diabetes, Polyneuropathy, Transcutaneous electrical nerve stimulation, Malondialdehyde.

INTRODUCTION

Diabetes is recognized as one of the leading causes of morbidity and mortality in the World. Type 2 diabetes occurs predominantly in adults over than 30 years old. About 2.5-3.0 % of the words population suffers from this disease[1]. Peripheral neuropathy is the most common complication of type 2 diabetes, occurs in the distal extremities and typically affects the sensory, motor and autonomic systems[2]. In diabetic patients, chronic hyperglycemia can produce neuropathic changes that affect peripheral nerve function and produce extremity pain[3].

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Peripheral neuropathy is a common complication of diabetes, affecting nearly one of every three patients with type 2 diabetes and increasing in incidence with the duration of diabetes[2]. Advanced neuropathic deficits underlie most food ulcers and amputations[3]. Today diabetic neuropathy remains untreatable except by palliative measures. For symptomatic relief, various analgesics, anticonvulsants and tricyclic antidepressants have been tried with variable success[4,5]. New drugs[6] and non-pharmacological therapies such as transcutaneous electrical nerve stimulation (TENS)[7,8], acupuncture[9,10] and spinal cord stimulation[11] are being explored to alleviate the pain and discomfort associated with peripheral neuropathy. Prompted by the beneficial effects of electrotherapy in alleviating pain associated with arthritis and rheumatological conditions[12].

Chronic hyperglycemia in diabetes mellitus is an oxidative stress created by an imbalance of prooxidants over antioxidant defenses. Glycemic equilibrium plays a very important role in the prooxidant/antioxidant balance[13].

The present study was designed to investigate the effect of TENS treatment on diabetic polyneuropathy patients. With this aim, before and after TENS were determined serum malondialdehyde (MDA) that an oxidative stress marker and blood glucose levels.

EXPERIMENTAL

Selection of patients: Fourteen diabetes patients, which have applied to internal diseases polyclinic in Yuzuncu Yil University, Medical Faculty, Research and Practice Hospital, are volunteers to this study. Seven of these patients were women and the other 7 were men. The average of their age were 51 (the youngest: 33, the oldest: 60). Illness duration is changed between 4 and 16 years, the average is estimated 8.8 years. 6 patients treated with crystalline insulin, 8 patients don't use insulin. All subjects the study voluntarily and gave informed written consent. The protocol of the study was approved by our local ethics committee.

Electrical stimulation: Peripheral or central electrical stimulation of nerve system is a tool, which is used to control chronic pain for a long time[14]. Neurostimulator apparatus, which indicatives a rapid stage for technologic improvements are very portable and popular. For local pain, TENS is adequate and favourable. To the TENS (HRFM; continuous pulses changed from 90 Hz to 55 Hz over 90 msec, 1.3 times a second) electrodes, which are accommodate to the aching region, performed electrical treatment signals. These electrical signals are also proved as neuropsychological security and efficiency. On functional electrical stimulation, the neurons, which has lost its functional, are stimulated with electrical signals that are favourable to nerve system characteristics to get them once more functional.

Physical effect of neurostimulation; we can put in order as: remove pain or diminish pain increase blood cycle, decrease muscle atrophy, diminish edema and effusion and diminish in muscle spasm.

The contraindications are rarely skin allergy against TENS electrodes and shah vessel sinuses on patients carrying pace maker implants and pregnant women.

Blood samples collection and preparation: Before and after TENS treatment, in the morning 5 mL blood were collected from each patient with empty stomach. Blood samples were put to straight tube and centrifuged 2500 rpm (cycle/min). On obtained serum, glucose and malondialdehyde levels were observed.

200 µL samples and 800 µL phosphate buffer, which are collected from serum, are mixed with 25 µL BHT and 500 µL 30 % TCA in tube. After tubes in which there are plasmas are frozen in defreeze (2 h), these was thawed in 25 ºC. After that it is centrifuged 2000 rpm during 15 min in 4 ºC. Afterward 1 mL samples were taken from supernatant in the tubes, 75 µL from EDTA (0.1 mol) and 250 µL from TBA (1 %) were added on the samples. After that the samples were kept in bath with hot water (in hot water bath) during 15 min and than these was thawed to refrigerate in 25 ºC. The absorbance of these samples were recorded on spectrophotometer that was regulated to 532 nm. The result was multiplied by coefficient of extension (64,7636 nmol/mL).

This study was applied to blood samples from the patients 20 d after following final period.

Application of TENS treatment method: To determine MDA and glycemia levels, blood samples were collected from each patient before 24 h beginning TENS treatment. Each patient were applied TENS treatment as séance once a day during 20 days. It was used 4 pairs of self-jealous electrodes. To increase the electrode's conductivity, after shutting off equipments, the finger tap was wet to alter polarity *via* little water before and middle the treatments. Two pairs of these electrodes were settled on median nerves in the interior part of wrists as parallel by 2 cm intervals. The other two pairs electrodes were settled on talus and tibial anterior, respectively, onto the anterior of the foots. Each séance took 20 min and polarity was changed to minimize the effect of electrolysis. So, negative and positive polarities were applied by 10 min. To avoid the effect of electrical shock, before changing polarity, TENS system were turned off. After that, the position of electrodes was reversed and by turning on the system, current value was increased over again from 0 to treatment levels. In the following minutes of the treatment, the patients were asked whether current value were diminishing or not. According to the answers, if necessary, current values were increased.

Statistical analysis: The experimental parameter values were expressed as mean ± SD. Statistical analysis was performed using the statistical package SPSS version 10.0. Paired t-test was used to analyze the differences between the control and the corresponding values the experimental groups. Values of $p < 0.01$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The effects of transcutaneous electrical nerve stimulation (TENS) treatment on the diabetic polineuropaty are shown in Figs. 1 and 2. Glucose levels of diabetic patients were compared with healthy young control subjects (n = 14). It is very

usual for the high level of the glucose to be found in the diabetes compared to the following term. After the TENS done for a month, it was observed that the glucose level decreased (from 217 to 175 as average) significantly in the patient group compared to the previous one. At the end of the fallowing term the average glycemia level increased to the value 198 again (Fig. 1).

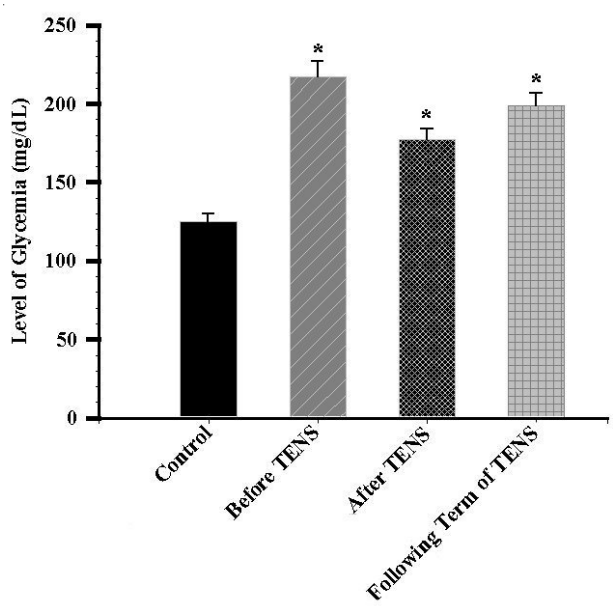

Fig. 1. Change of serum glucose levels in diabetic patients before, after and following term of TENS-treatment. Results are expressed as means ± SD; n = 14, *p < 0.01; Control group was compared with before, after and end of the following term of TENS

It was aimed to study with respect to the effects of the TENS treatment yet not an elucidated subject on the balance of the oxidant-antioxidant in the diabetics. MDA levels of diabetic patients were compared with healthy young control subjects (n = 14). Average MDA levels of the patients in the results obtained from the tests done on the blood samples; 4.94 before TENS, 10.07 after TENS and 7.40 at the end of the following term were found. When the levels of MDA before TENS-after TENS by paired sample test were compared to, statistically a meaningful increase is seen ($p < 0.01$; Fig. 2). As for in the comparison of MDA levels obtained just after the treatment TENS and the values at the end of the following term of 20 days, statistically a meaningful decrease was determined ($p < 0.01$; Fig. 2). MDA value increased just after the treatment but it was seen that this value decreasing approached to the level before the treatment when checked at the end of following term of 20 d ($p < 0.01$). In this study, while the MDA value in the serum compared to the previous value was found higher before the TENS treatment and just after the treatment of 20 days in the diabetic patients, it decreased rather on the control (Fig. 2).

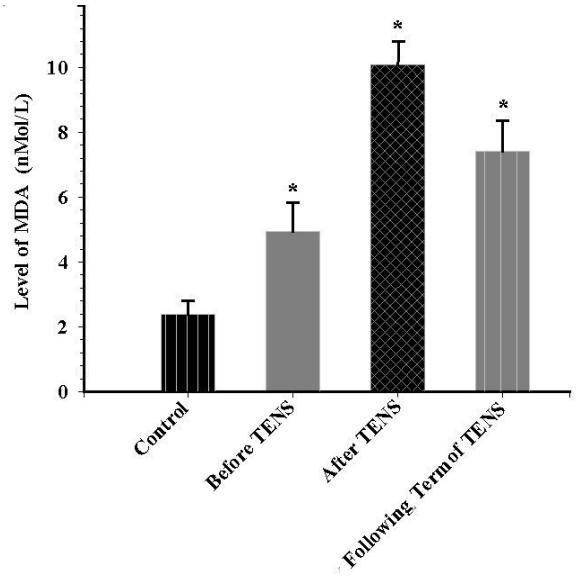

Fig. 2. Basal formation of MDA in diabetic patients before, after and following term of TENS-treatment. Results are expressed as means ± SD; n = 14. *p < 0.01; Control group was compared with before, after and end of the following term of TENS

Chronic hyperglycemia in diabetes mellitus is an oxidative stress created by an imbalance of prooxidants over antioxidant defenses. The pathogenesis would involve several mechanisms including glucose autoxidation, protein glycation, the polyol pathway and overproduction of superoxide radicals in mitochondria and *via* NAD(P)H oxidase. Glycemic equilibrium plays an important role in the prooxidant/antioxidant balance. Macromolecules such as found in the extracellular matrix, lipoproteins and deoxyribonucleic acid also constitute targets for free radicals in diabetes mellitus.

Free radicals are considered to have a great role in the pathogenesis of many diseases such as cell damage, diabetes mellitus, cancer, myocardial infarct, atherosclerosis, hemolytic and immune disease. The levels of these reactive oxygen species are controlled by antioxidant enzymes that is to say, malondialdehyde (MDA), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), calatase (CAT) and nonenzymatic scavengers like reduced GSH, selenium, vitamin E, coenzyme Q, β-carotene. The reports indicate increased or decreased lipid peroxidation, MDA, GSH-Px, SOD and CAT activity in various tissues; like liver, kidney, blood vessels, heart, lymphoid organs, uterus and lungs[13,15,16].

Malondialdehyde is one of the lipid peroxidation products frequently used to determine the oxidant/antioxidant balance in diabetic patients[17,18]. However, there are contradictory results in literature about serum MDA levels in patients with diabetes. Véricel *et al.*[19] and Sundaram *et al.*[20] studied 467 cases of type 2 diabetics and found that lipid peroxidation was significantly raised in plasma and erythrocytes.

In present study, a significantly increasing MDA levels in serum diabetic patients is noted. On the other hand, normal MDA level was found in diabetic patients[21-23]. High levels of glucose can produce permanent chemical alterations in protein and increase lipid peroxidation in a variety of experimental models of hyperglycemia[24,25]. Hyperglycemia, itself, may stimulate platelet aggregation[19,26-28] and autooxidation of glucose may also lead to free radical production in diabetics[29,30].

The most important problem in the diabetic patients is the hyperglycemia[31]. Moreover, the resistance development against insulin is the most important reasons of hyperglycemia, too. Due to the fact that the patients involved in the study have Type II DM, here we can mention about the resistance against the insulin. It is very usual for the high level of the glucose to be found in the diabetes compared to the following term. After the TENS done for a month, it was observed that the glucose level decreased (from 217 to 175 as average) significantly in the patient group compared to the previous one. This case may result from the weight lose of the individuals. So, it can be said that the decrease of serum glucose level may result from the acceleration of the blood circulation by the TENS treatment, the decrease of the resistance against the insulin depending upon the lose of the muscles and the increased glucose uptake by the cells. At the end of the fallowing term the average glycemia level increased to the value 198 again. It is shown that glucose levels decrease in present diabetic patients parallel to the increase of MDA at end of the following term of TENS. Any study investigating the effect of the oxidant-antioxidant on the balance and the investigation of the effect of MDA of the treatment TENS in the diabetics has not been met in the current literature. In a study[24], both lipid peroxidation and the antioxidant capacities in the serum of the diabetics were compared to the healthy controls and it was reported that the diet treatment in the diabetic patients affected the lipid peroxidation and non-enzymatic antioxidant capacities in a positive way. The oxidative stress, diabetes and following complications play on important role in pathogenesis. Non-enzymatic glycolysis, auto oxidative glycolysis, metabolic stress resulting from the changes in the antioxidant defense system and the levels of the inflammatory mediators, local tissue damage occurring as a result of hypoxia and ischemic reperfusion are the mechanisms increasing the oxidative stress in the diabetes[24].

Besides the problems of peripheric nerve transport in the diabetic patients[32,33], vascular problems leading to ischemic pains in lower organs (in legs and feet) take place, too[34,35]. The possibility of providing of vasodilatation by the TENS treatments in those patients was shown before[24,36]. It was reported also that there was a correlation between the stimulation treatment and biochemical parameters (such as serotonin, noradrenalin)[35,36]. On the other hand, it was proved that again HRFM style TENS treatment in the previous comparable studies was good enough in the control of the pain[14]. The increase of the MDA value just after the treatment and the rapid decrease on the control at the end of the resting period in this study can be interpreted as passive exercise effect took place in the person treated by TENS and so it may cause the transient stress.

In conclusion, the authors showed that after transcutaneous electrical nerve stimulation (TENS) and end of the following term of TENS had elevated of malondialdehyde as a marker of oxidative stress.

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