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Evaluation of the effect of electron beam therapy on oxidative stress and some minerals in patients with type 2 diabetes mellitus

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ABSTRACT

Background: The study on the control and treatment of type 2 diabetes mellitus, as a growing metabolic disease in the world, is important. Oxidative stress and reactive oxygen species in uncontrolled diabetes can play a role in the consequences of diabetes such as neuropathy and nephropathy. The presence of minerals as bioactive compounds in the diet and their role in antioxidant enzymes can play a role in reducing the oxidative effects of diabetes. Electron beam therapy as an adjunct method can be effective in reducing free radicals and oxidative stress.

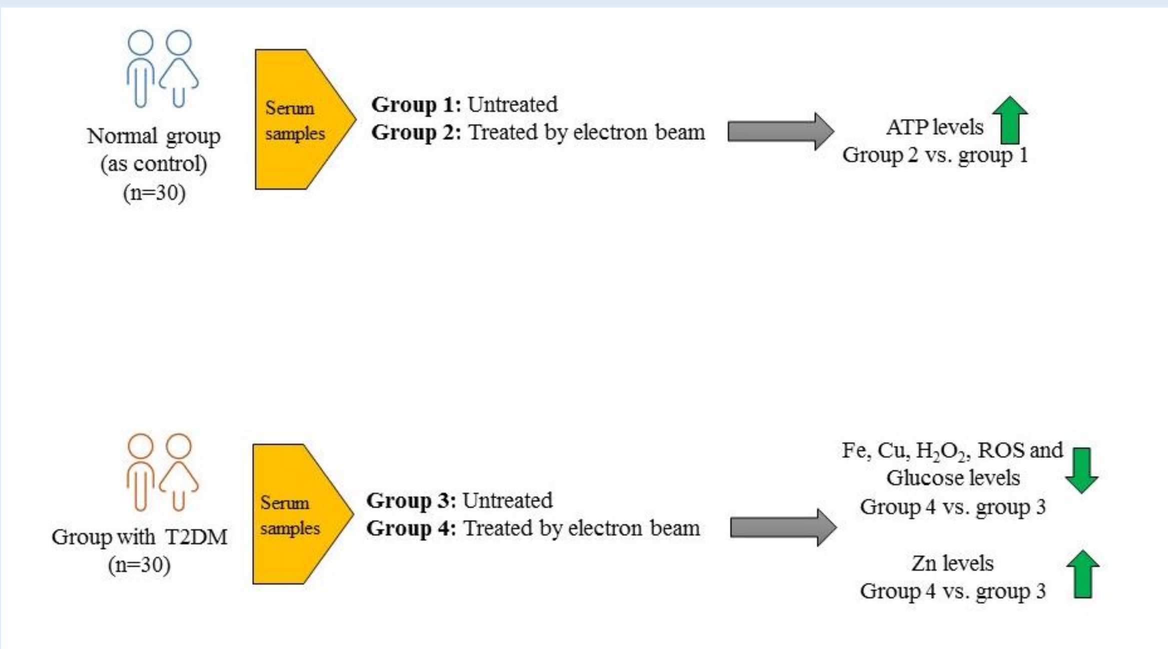
Objective: The main purpose of this study was to investigate the effect of electron beam therapy on glucose, oxidative markers and some minerals, as bioactive compound, in people with type 2 diabetes mellitus.

Methods: The study was performed on 30 volunteers with type 2 diabetes mellitus and 30 healthy volunteers as a control group. Serum samples from diabetic and control groups were assayed for glucose, hydrogen peroxide, reactive oxygen species and minerals such as iron, zinc, copper, magnesium and selenium binding protein before and after electron beam irradiation. ATP levels and NAD/NADH ratio were also evaluated. The mentioned parameters were measured by ELISA and calorimetric methods according to the relevant kit protocol. Electron beam therapy was performed using a linear accelerator. The used amount of energy was 9 MeV. The depth of treatment was 1.5 cm.

Results: The results of electron beam therapy showed that the concentrations of glucose, reactive oxygen species, hydrogen peroxide, copper and iron were significantly (P value < 0.05) reduced in diabetics. Zinc levels in this group increased significantly (P value < 0.05). In control group, ATP levels were significantly (P value < 0.05) increased by electron beam therapy.

Conclusion: According to the obtained results, electron beam therapy can be effective in reduction of oxidation indexes and thus reducing oxidative stress. Electron beam therapy can be effective in reducing the consequences of diabetes mellitus.

Keywords: Electron beam therapy, Oxidative stress, Mineral, Type 2 diabetes mellitus



INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease distinguished by inappropriately high blood glucose levels and caused by defects in insulin secretion or insulin function. Neurological, cardiovascular and renal consequences, coma, and death may be noticed in uncontrolled DM [1-3]. Type 1 and type 2 diabetes mellitus (T1DM and T2DM) are the main subtypes of DM. T2DM affects middle-aged people with chronic hyperglycemia due to poor lifestyles and is known as insulin-resistant Diabetes [4-5]. As the data obtained from investigations throughout the world, 422 million people currently are suffering from DM. Every year there are 1.6 million mortalities correlated directly to diabetes [6], Moreover, the prevalence of T2DM is much higher than T1DM. The World Health Organization (WHO) estimates that more than 250 million people worldwide will encounter type 2 DM by 2025 [7]. Various metabolic signaling pathways can be initiated by hyperglycemia that leads to inflammation, cytokines secretion, cell death, and, consequently to diabetic complications, for instance, cardiovascular disease, retinopathy, nephropathy, neuropathy [8-9]. Diacylglycerol (DAG), protein kinase C (PKC), and NADPH were culminated in reactive oxygen species (ROS) when activated by hyperglycemia. Hence, this signaling pathway is critical in controlling angiogenesis, oxidative stress with reduced ROS production, and cell death. DM patients have generated ROS through mitochondrial respiratory chain enzymes, cyclooxygenases, lipoxygenases, nitric oxide synthases, and peroxidases [10-11]. One of the most reasonable procedures in controlling diabetic intricacies is controlling PKC, NADPH-oxidase, and glucose levels, which can downregulate the pro-inflammatory cytokines and reduce ROS and cellular death [12-14]. Nowadays, many electrical methods have been introduced to affect ROS, such as electron beam therapy and electrostatic therapy [15]. Elimination of free radicals and oxidative stress can be important in preventing DM consequences. The mechanism of action of electron therapy is related to its anti-oxidative effect. Electron therapy destroys free radicals. A free radical is a molecule that has one or more unpaired electrons in its outer layer [16].

Bioactive food components (BFC) act simultaneously at identical target sites and have been revealed to have the potential to diminish the risk of cancer, cardiovascular disease, osteoporosis, inflammation, T2DM, and other chronic degenerative disorders.

Minerals predominantly include chloride, calcium, phosphorous, magnesium, sodium, potassium, and iron, whereas by triggering insulin receptor sites, certain trace elements such as cobalt, copper (Cu), sulfur, zinc (Zn), and selenium enhance insulin function [17]. These trace elements played a critical role in the pathogenesis and progression of T2DM [18]. Since vitamins and minerals elicit pharmacological effects, they can be grouped as bioactive compounds.

Polyphenols in bioactive compounds have the capability to chelate metals, and been recently considered for their reported health benefits. Polyphenols chelate a number of trace metals, including iron (Fe), Cu, and Zn [19-20]. In T2DM patients, serum ferritin concentration may impact insulin sensitivity, vascular resistance, viscosity, and oxidative damage [21]. Fe is an influential pro-oxidant and is correlated with high oxidative stress levels. Indeed, high iron levels in the body can raise the risk of developing T2DM [22].

Cu contents increased in T1DM and T2DM. Furthermore, to decrease the promotor activity of insulin genes, Cu facilitates ROS generation through regulating electron transfer, which is admiringly reactive in redox reactions [23].

Since the antioxidant properties of polyphenols contain the chelation of metals such as zinc, the investigation of the impacts of bioactive polyphenolic compounds on intestinal absorption is required [24]. The mitochondrial electron transport chain produces superoxide, and it can be converted to hydrogen peroxide (H_2O_2) by the superoxide dismutase (SOD).

(Zn)-SOD is the isoform of SOD in the cytosol and the extracellular space. Zn has an essential role in the antioxidant defense system in this context. Furthermore, it was demonstrated that zinc treatment could decline ROS and increase antioxidant activity. Zn activates key molecules involved in cell signaling, which maintain glucose homeostasis, regulate insulin receptors, and prolong the action of insulin [25-26]. Magnesium (Mg) is applied in various metabolic processes, including glucose intolerance, insulin resistance, and impaired lipid metabolism. As free radicals are often present at high concentrations in T2DM, oxidative stress and inflammation may correlate Mg deficiency with reductions in insulin sensitivity, hypertension, metabolic syndrome, and aging [27-28].

The essential trace element selenium (Se) plays a significant role in anti-oxidation, immunity, and anti-inflammation through selenium-dependent glutathione peroxidase (GPx). Se deficiency is correlated to decreased GPx activity and increased oxidative stress. Decreased insulin secretion, increase in insulin resistance, and influencing the commencement and advancement of T2DM can be mediated by oxidative stress. Thus, the progress in oxidative stress and selenium-induced insulin resistance indicates that selenium may play a protective role against DM [29-30].

The main function of mitochondrion is energy production (ATP) via oxidative phosphorylation. Two steps are involved in this process: (a) oxidizing NADH/FADH2 to receive electrons from mitochondrial electron transport chain and (b) phosphorylating Adenosine diphosphate (ADP) to yield ATP. During the electron transfer chain, free radicals leak from complex II [31]. In hyperglycemia, elevated Nicotinamide adenine dinucleotide (NADH) levels lead to excessive superoxide anion formation through the glycolytic and tricarboxylic acid pathways [32-33]. During hyperglycemia, aldose reductase is activated by increased intracellular glucose levels. By virtue of this reaction, sorbitol is converted into resilient polar sorbitol, which cannot penetrate cell membranes and brings about osmotic cell swelling, impairment of cellular structure and function, a decrease in ATPase activity, and ultimately setting in motion cellular metabolism and functional damage. Activation of protein kinase C is caused by an increase in NADH/NAD^+ ratio after sorbitol is oxidized to fructose by sorbitol dehydrogenase [34]. ROS are not generated directly in this mechanism but are caused by a redox imbalance, which drives oxidative stress to begin [35]. The aim of this study was to evaluate EBT on a number of biochemical parameters and some elements as bioactive compounds in people with T2DM.

MATERIALS AND METHODS

Materials: Zn, Cu, Fe, Mg and selenium binding protein (Sebp) assay kits were purchased from My BioSource Inc. (San Diego, CA 92195-3308, USA). Parameters were evaluated according to kit instructions. Microplate reader (Mindray, model MR-96A, Germany) and microplate spectrophotometer (model Fluostar, bmglabtech, Germany) were used for these assays. The H_2O_2 assay kit was purchased from Zell Bio (Zell Bio GmbH, Ulm, Germany). H_2O_2 was detected by the colorimetric method, quantity assay kit, and Tecan's Sunrise absorbance microplate reader (Switzerland). Human glucose (with an intra- and inter-assay CV < 8% and < 10%, respectively) was measured by enzyme-linked immunosorbent assay (ELISA) techniques according to their assay kits and ELISA reader apparatus (MR-96A, Mindary Co) (Germany). ELISA kits were purchased from My BioSource Inc. (San Diego, CA 92195-3308, USA). According to the manufacturer's instructions, the ATP assay was performed using the ATP Assay Kit (colorimetric/fluorometric) from Abcam (ab83355, Cambridge, UK). Results were then normalized by protein concentrations of each test. The NAD^+/NADH ratio [9] was measured using the NAD/NADH Assay Kit (colorimetric) (ab65348; Abcam) in accordance with the manufacturer's instructions.

Methods

Participants: In this investigation, 30 healthy control (as control group) subjects and 30 volunteers with T2DM randomly were chosen among the people referred to Vali-Asr medical laboratory in Tehran, Iran.

Figure 1. Scheme of the radiotherapy system (VARIAN, model CLINAC 2100 C/D (USA)) and samples exposed to electron beams

Among healthy subjects and subjects with T2DM, 50% were female, and 50% were male. In other words, the two groups were equal (15 males and 15 females), and the age of participants was between 63 and 88 years old. Based on the WHO criteria, individuals with glycated hemoglobin (HbA1c) ≥ 6.5% or fasting plasma glucose ≥ 126 mg/dl were considered as diabetic. Also, general characteristics of participants such as sex, age, height, weight, and body mass index (BMI) were recorded. After 12 hours of overnight fasting, blood samples were taken from all participants. Blood samples were centrifuged (250 g for 10 min), and their serum was separated, then analyzed. After 24 hours, samples were irradiated via electron beam and then examined for a second time. The sample of this investigation was divided into four groups. Group 1: Samples of healthy individuals before irradiated electron beam; Group 2: Samples of healthy individuals after irradiated electron beam; Group 3: Samples of diabetic subjects before irradiated electron beam; Group 4: Samples of diabetic subjects after irradiated electron beam. Inclusion criteria were people with T2DM and age 60 and above without a history of any other disease. People with type 1 diabetes and young age and having any other metabolic disease as well as smokers were excluded from the study. Informed consent was obtained from all volunteers.

Electron Beam Therapy (EBT): In the EBT investigation, EBT was performed using a linear accelerator, as used in our previous study [36] (Figure 1). Before exposure to electron beams, treatment was also performed in two fractions (one fraction twice in two weeks). The used amount of energy was 9 MeV (micro electron volt). The depth of treatment was 1.5 cm. In order to separate serum, the blood was centrifuged after the EBT was completed. The isolated sera were then stored at -40°C for further study. Serum in all groups was measured according to the kit's instructions.

Statistical Analysis: Statistical analyses were performed using SPSS for Microsoft Windows (version 16.0; IBM, USA). The results were thus shown as means ± standard deviation (SD). Paired sample t-tests and the mean scores of the variables between and within groups were also compared using a one-way analysis of variance (ANOVA). After that, Tukey's honestly significant difference (HSD) post-hoc test for multiple comparisons was employed. A P value ≤ 0.05 characterized the presence of a statistically significant difference.

RESULTS

General characteristics: General characteristics such as height, weight and BMI between control group and diabetics are shown in Table 1. Comparison of BMI between control and diabetic groups did not show a significant difference.

Table 1. Anthropometric parameters

Characteristics	Control group (n=30)	Diabetic group (n=30)	P value
Weight (Kg)	75.6±6.4	76.6±6.7	0.58
Height (Cm)	167.7± 6.8	166.6±6.6	0.73
BMI (Kg/m^2)	27±3.0	27.7±3.4	0.22

Data are given as mean ± SD, P value < 0.05 is significant. BMI; body mass index

Biochemical parameters: Obtained results from Tukey-HSD test with 95% family-wise confidence level for serum Zn, Cu, Fe, Mg, Sebp, glucose, H_2O_2, ATP and NAD/NADH has been shown in Table 2. According to the confidence interval resulting from Tukey's post-test, the possibility of comparing variable mean between two groups has been provided. This is a criterion to discuss significant differences in the four groups.

In the serum sample of the control and diabetic groups, some important variables were measured. These biochemical variables included blood serum Sebp, Mg, Fe, Cu, Zn, H_2O_2, NAD/NADH, ROS, ATP and glucose concentrations in the healthy and diabetic samples before and after treatment by the electron beam. A comparison of the results of parameters between the healthy people sample groups and the diabetic people sample groups is shown in Figure 2.

Data analysis, as shown in Figure 2, shows a significant difference (P value < 0.001) in the concentrations of Fe, Cu and Zn between diabetic samples before and after EBT. This significant difference (P value < 0.05) in the two mentioned groups was also observed in oxidative parameters (H_2O_2 and ROS). There was a significant difference (P value < 0.05) in the comparison of glucose concentration between groups 3 and 4. There was a significant difference (P value < 0.05) in the comparison of ATP concentrations between groups 1 and 2. No significant difference was observed in comparing ATP concentrations between groups 3 and 4.

There was no significant difference in the comparison of Sebp, Mg and NAD/NADH concentrations between groups 1 and 2 and groups 3 and 4.

Table 2. Tukey-HSD test results for biochemical parameters

Variable	Groups	Difference	95% family-wise confidence level	
			Lower bound	Upper bound
Sebp	1-2	-2.00	-191.50	187.50
	3-4	-59.13	-248.60	130.30
	2-4	1744.00	1554.00	1933.00
Glucose	1-2	1.86	12.69	16.42
	3-4	16.57	0.31	32.82
	2-4	-123.00	-137.00	-108.7
H_2O_2	1-2	1.80	13.49	17.09
	3-4	17.20	1.91	32.49
	2-4	-103.30	-118.60	-88.05
Fe	1-2	3.03	-4.82	10.89
	3-4	22.27	11.74	32.80
	2-4	-98.07	-106.6	-89.55
Cu	1-2	3.33	-3.31	9.97
	3-4	21.10	10.64	31.56
	2-4	-89.33	-97.60	-81.06
Mg	1-2	-0.16	-0.67	0.34
	3-4	-0.30	-0.91	0.31
	2-4	4.90	4.35	5.44
Zn	1-2	-1.83	-66.05	2.39
	3-4	-12.07	-16.37	-7.76
	2-4	53.43	49.77	57.10
ATP	1-2	-0.90	-1.59	-0.20
	3-4	-0.13	-0.79	0.52
	2-4	2.10	1.49	2.70
NAD/NADH	1-2	-2.86	-6.35	0.62
	3-4	-4.96	-10.87	0.93
	2-4	15.30	11.30	19.47
ROS	1-2	12.00	-164.20	188.2
	3-4	107.1	17.53	196.6
	2-4	-980.6	-1116	-845.5

Data are shown with 95% family-wise confidence level ($p < 0.001$). Group1: healthy people samples. Group 2: healthy people samples + irradiated electron beam. Group 3: Diabetic people samples and group 4: Diabetic people samples + irradiated electron beam. Sebp, selenium binding protein; Fe, Iron; Cu, Copper; Mg, Magnesium; Zn, Zinc; ROS, reactive oxygen species.

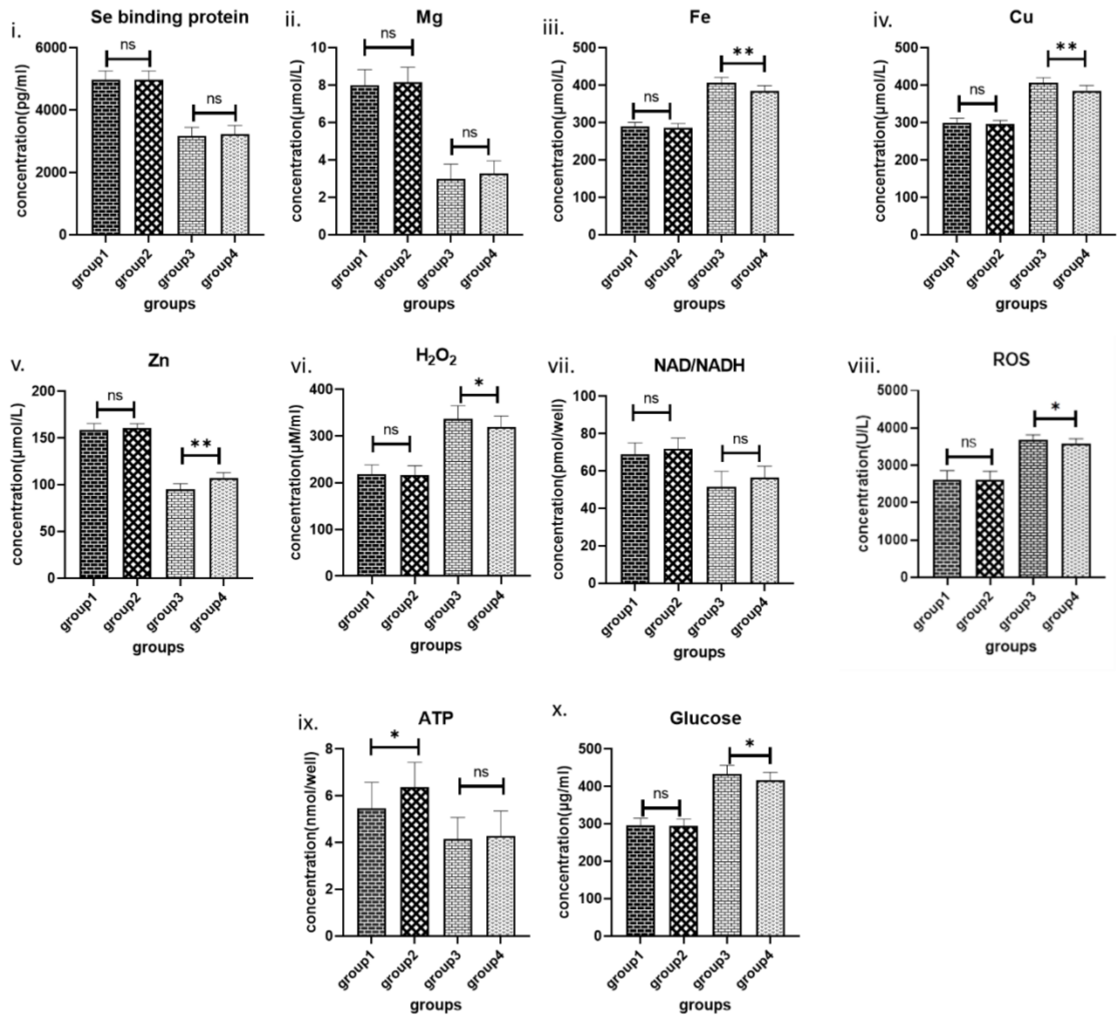


Figure 2. i. serum selenium binding protein, **ii.** magnesium, **iii.** iron, **iv.** copper, **v.** zinc, **vi.** H_2O_2, **vii.** NAD/NADH, **viii**. ROS, **ix.** ATP, and **x.** glucose concentrations in the healthy and diabetic samples before and after treatment by the electron beam. **Group1:** healthy people samples. **Group 2:** healthy people samples + irradiated electron beam. **Group 3:** Diabetic people samples, and **Group 4:** Diabetic people samples + irradiated electron beam. * Significances of data comparing groups ($p < 0.05$). ** Significances of data comparing groups ($p < 0.001$).

DISCUSSION

In this study, we investigated the effects of the EBT on some minerals, some oxidative indexes, and glucose in T2DM patients. External beam radiation is a kind of radiotherapy that works with very tiny electrically charged particles generated in a linear accelerator and directed toward the skin. The electron beam can cause severe damage to the cell DNA, leading to cell proliferation misfunction. Although this method causes serious damage to cancer cells due to their rapid cell turnover, the electron beam can affect normal cells and their characteristics. On the other side, EBT could retain some effects on blood contents. Radiation therapy and laser therapy are now being utilized to treat patients with cancer. However, no study so far has investigated the effects of EBT on patients with diabetes, although there have been investigations into laser therapy in patients with DM [37] and plasma therapy in patients with DM [38]. Also, in the previous study we examined the effect of EBT in vivo and in vitro [36]. In all types of Diabetes, hyperglycemia contributes to the formation of reactive oxygen species, and an imbalance of transition metals, such as Cu or Fe, plays a pivotal role in causing oxidative stress [39]. One valuable blood content, which plays a leading role in diabetic patients, is serum glucose [40]. Our analysis elicits that although blood under electron beam irradiation reduces the glucose content of the diabetic group, the healthy group's blood glucose concentration did not change significantly. Glucose concentration in diabetic patients is a remarkable element, which excessive glucose in the blood will damage.

Retrospective studies depicted that reducing iron levels may provide a therapeutic advantage for reducing diabetes-related morbidity and mortality [41]. By irradiation beam therapy on diabetic patients' blood, we noted that Fe serum concentration was significantly decreased due to our operation, whereas there are no significant differences in the healthy group's blood iron concentration. The copper content of the diabetic group was remarkably decreased following EBT. There were no significant changes in the healthy sample group. Another factor analyzed in this study is ATP, which is one of the critical components in states of Diabetes. Our investigation elucidates that ATP concentration in peripheral blood content in the diabetic group has no significant changes during EBT, which has no adverse effect in terms of glucose reduction. Adversely, ATP significantly decreased in the healthy group.

All forms of diabetes mellitus are associated with altered pancreatic insulin content, which is essential for the normal processing and storage of insulin [42]. Zn concentration in diabetic patients' blood increased emanating from EBT, which is in favor of T1DM and T2DM treatment. A substantial drop in serum zinc levels is observed in patients with T1DM and T2DM [43].

H_2O_2 is a factor that will increase in diabetic patient serum [44]. H_2O_2 in diabetic blood samples was decreased significantly by EBT, which favors diabetic patient treatment. Although low magnesium concentration correlated with an incident of Diabetes, there are no considerable changes applied in blood Mg through EBT. Selenium and NAD/NADH have no significant change during EBT in both diabetic and healthy blood samples. Taken together, EBT could change the blood content concentration on the side of diabetic patients based on the analysis done on their blood samples. This study concept uses EBT as a means to cure diabetic patients and lifestyles. This intervention might decrease the patient's disease-related symptoms, which will improve the health-quality life of the patients [45]. In the world, EBT has been used to control and treat skin diseases and some cancers. The use of EBT to study and change the number of free radicals as well as the study of minerals in these conditions were among the important novelties in this study.

CONCLUSION

In conclusion, the results of our study showed that EBT can be effective in reducing some oxidative indicators such as H_2O_2 and ROS as well as lowering blood glucose. EBT can also be effective in increasing and decreasing the concentration of some minerals. Some of these minerals are cofactors of antioxidant enzymes. EBT can be effective along with medication and insulin therapy as an adjunct to reduce complications of DM. Additional in vitro and in vivo studies should be performed on the role of EBT in reduction of DM consequences.

List of abbreviations: DM: diabetes mellitus, T1DM: type 1 diabetes mellitus, T2DM: type 2 diabetes mellitus, EBT: electron beam therapy, WHO: World Health Organization, HbA1c: glycated hemoglobin, ROS:

Reactive oxygen species, H_2O_2: hydrogen peroxide, BMI: body mass index, Sebp: selenium binding protein, Fe: Iron, Zn: Zinc, Mg: Magnesium, Cu: Copper, ELISA: enzyme linked immunosorbent assay.

Author's contributions: The authors' confirmed contributions to the paper are as follows: DM participated in the study design and the article editing. MSA performed treatment of samples with electron beam. FJ, MRF, FM and PF performed experimental works and data analysis. MRA and ASM participated in the writing and editing of manuscript. HM contributed to the original idea of the paper. All authors read and approved the final version before its submission.

Conflict of interest: The authors declare that there is no conflict of interest.

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