PILOT STUDY

A New Treatment Strategy that Reduces Growth/ Differentiation Factor-15 in Type-2 Diabetes Mellitus: Major Autohemotherapy: A Pilot Study

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ABSTRACT

Context • Growth/differentiation factor-15 (GDF-15) is a diagnostic and prognostic marker associated with inflammation, renal damage, and cardiovascular risk in type-2 diabetes mellitus. Researchers have proposed treatment targets that reduce GDF-15 levels.

Objective • Our aim in this study is to investigate the effect of major autohemotherapy on GDF-15 levels and to evaluate it as a complementary therapy.

Design • The research team designed a laboratory study to examine the effect of major hemotherapy on GDF-15 levels in patients with type-2 diabetes mellitus and healthy volunteers. Blood was drawn from the participants in a closed system, infused with ozone gas, and reinfused into the patients.

Setting • The study was carried out from 15 August–5 October at Kırşehir Training and Research Hospital Traditional and Complementary Medicine Center, Turkey.

Participants • The study was conducted prospectively, and two groups were formed, consisting of those with type-2 diabetes mellitus (n = 21) and healthy volunteers (n = 14).

Outcome Measures • All participants received 10 sessions of major autohemotherapy at a concentration of 25–35 micrograms/milliliter twice a week. Before and after the application, GDF-15, fasting glucose, glycosylated hemoglobin, and lipid panel levels were studied and compared.

Results • Age, GDF-15, fasting glucose, glycosylated hemoglobin, and triglyceride levels were found to be higher in the type-2 diabetes mellitus group compared to the healthy group, and high-density lipoprotein cholesterol levels were found to be lower. After major autohemotherapy, GDF-15 and low-density lipoprotein cholesterol decreased significantly in the type-2 diabetes mellitus group. No change was observed in the healthy group.

Conclusions • As a new treatment strategy, major autohemotherapy reduces GDF-15 levels in type-2 diabetes mellitus and contributes to the therapeutic effects of ozone therapy. (*Altern Ther Health Med.* 2023;29(7):30-33).

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INTRODUCTION

Growth/differentiation factor-15 (GDF-15) belongs to the transforming growth factor-β (TGF-β) family and is widely expressed in many sites such as the heart, liver, kidney, placenta, vascular smooth muscle cells, endothelium, and macrophages. It is an important cytokine produced and induced by stress[1,2] and is associated with hypertension (HT), cardiovascular diseases (CVD), respiratory system diseases, malignancies, chronic renal failure, and high mortality.[3] Studies have emphasized that GDF-15 is a diagnostic and prognostic marker in diabetes. It presents higher levels in type-2 diabetes mellitus (T2DM) and positively correlates with fasting glucose, triglyceride (TRG), creatinine (CRE), and glycosylated hemoglobin (HbA_{1c}) levels, insulin resistance, and body mass index (BMI).[4-6] GDF-15 is a marker of early renal damage independent of albuminuria in diabetic kidney disease, negatively correlates with the glomerular filtration rate (GFR), and is strongly associated with major cardiovascular events.[7,8]

Ozone (O_3) gas is a dynamic and unstable mesomeric molecule consisting of three oxygen (O_2) atoms.[9] In medical ozone therapy, a mixture of 1–5% O_3 and 95–99% O_2 is used and applied by systemic or topical means.[10] The three most commonly used methods are major autohemotherapy (MAH), minor autohemotherapy, and rectal insufflation.[11] Its therapeutic efficacy in acute and chronic diseases is widely known, and it is accepted as a complementary treatment method with anti-inflammatory and anti-oxidant properties, which help reduce inflammation and oxidative stress. Medical ozone therapy is known to reduce hyperglycemia to healthy reference values, improve lipid metabolism, and reduce uric acid and lactate levels in diabetes. In addition, it contributes significantly to the control of infections due to its antimicrobial activity in the treatment of diabetic foot and reduces ischemic changes in the extremities.[12-16] Studies on diabetic rats have shown that it reduces hyperglycemia and advanced glycation products and provides a cardioprotective effect.[17,18] GDF-15 is associated with oxidative stress that occurs in ischemia, organ damage, and acute and chronic inflammation.[5] In diabetes, there is an on-going search for new treatments that reduce GDF-15 levels.

There are no studies on the effect of ozone therapy on GDF-15 levels. Therefore, our aim in this study is to investigate the effect of major autohemotherapy on GDF-15 levels and to raise awareness.

MATERIALS AND METHODS

This study was conducted at Kırşehir Training and Research Hospital Traditional and Complementary Medicine Center, Turkey, between 15 August and 5 October 2022. Two groups were formed, consisting of patients (n = 21) (patient group) with T2DM and healthy volunteers (n = 14) (control group). The age, sex, and BMI of the groups were recorded, and venous blood samples were taken from the forearm in the morning after 8–10 hours of fasting, just before receiving MAH. The blood collected in flat tubes with gel was centrifuged at 2000 g for 10 minutes after coagulation for 30 minutes. Approximately 0.5 mL of the resulting serum was transferred to microcentrifuge tubes and stored at -80°C until the GDF-15 levels were measured. The HbA1c levels were studied from blood samples collected in K2EDTA tubes. Glucose, lipid parameters [low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (T Chol), and triglycerides (TRG)] were studied in an autoanalyzer (AU5840; Beckman Coulter, CA, USA). HbA_{1c} was measured using high-performance liquid chromatography (HPLC) (Premier Hb9210; Trinity Biotech, Co Wicklow, Ireland). Serum GDF-15 concentrations were measured using the sandwich enzyme-linked immunosorbent assay (ELISA) method with commercially available (ELISA) kits (Elabscience, Beijing, China). The test was performed according to the kit instructions. Optical density was measured spectrophotometrically at 450 nm using a microplate reader (SPECTROstarNano, BMG LABTECH).

Participants with hyperthyroidism, glucose-6-phosphate-dehydrogenase deficiency, pregnancy, the presence of acute complications from diabetes, a cardiac pacemaker, changes in medical drug treatments after a follow-up, and participants who did not complete 10 sessions of MAH treatment, were excluded from the study. Three participants in the patient group (T2DM), and five participants in the control group, were excluded because they did not complete the MAH sessions, and six diabetic patients were excluded due to changes in their follow-up medical drug treatments. Therefore, the study was completed with 21 participants in the patient group and 14 participants in the control group.

Figure 1. Major Autohemotherapy Application

Major Autohemotherapy Application (MAH)

MAH is performed by taking 50–100 mL of blood in a closed system, under the influence of negative pressure, and retransfusing this blood to the donor after enriching with medical O_3 (Figure 1).[19] A total of 10 sessions of MAH, with an average concentration of 25–35 μg/mL, were applied to the participants twice per week (TURKOZONE Blue S device, Turkey). Just after the last session, venous blood samples were taken to analyze fasting glucose, HbA_{1c}, the lipid panel, and GDF-15 levels. Changes in parameters before and after MAH were examined.

Ethical Approval

Approval for the study was obtained from the ethics committee of Kayseri City Hospital Traditional and Complementary Medicine Center (Decision no/Date: 48/2022). The Helsinki Declaration was complied with. Written informed consent was obtained from all participants.

Statistical Analysis

The Shapiro–Wilk test was used to assess the normality of the data distribution. The results are expressed as mean ± standard deviation (SD) or median (25th–75th percentile) according to the data distribution. Student's *t* test was used for parametric data and the Mann–Whitney U test was used for non-parametric data to compare the two groups. The chi-squared test was used to make gender comparisons of the groups. Wilcoxon's signed-rank test was used for before and after the MAH analyses, and Fisher's exact analysis was used to compare the categorical results. All statistical analyses were performed using SPSS for Windows, version 21.0 (SPSS, Chicago, IL). $P < .05$ were considered statistically significant.

RESULTS

The demographic variables and laboratory parameters of the patient and control groups are summarized in Table 1. The mean age of the patient group (57.2 ± 10.8 years) was significantly higher than the control group (43.9 ± 8.7 years) ($P = .001$). There was no difference in terms of sex ($P = .203$). The BMI of the patient group (30.1 ± 4.3 kg/m^2) was higher when compared to the control group (25.3 ± 4.4 kg/m^2) ($P = .003$). The GDF-15 [181 pg/mL (88–271)], fasting glucose [(141 mg/dL (113–183)], HbA_{1c} [7% (6,5–8,4)] levels of the patient group were statistically higher than the healthy group GDF-15 [52 pg/mL (30–67)], fasting glucose [99 mg/dL(90–104)], and HbA_{1c} [5.5 % (5.3–5.6)] ($P < .001$). The HDL-C level of the control group (61 ± 8.8 mg/dL) was significantly higher than the patient group (53 ± 11.4 mg/dL) ($P = .042$). Also, the TRG levels of the patient group [176 mg/dL (124–240)] were significantly higher than the control group [(122 mg/dL (79–158)] ($P = .020$).

The changes in the laboratory parameters of the groups after MAH are summarized in Table 2. In the patient group, GDF-15 levels were 181 pg/mL (88–271) before MAH and 132 pg/mL (61–198) after MAH ($P = .002$). This result is important as it shows that MAH significantly reduces GDF-15 levels. Patients' LDL-C levels before MAH were 129 mg/dL (102–160) and 113 mg/dL (95–139) after MAH. This result was statistically significant, revealing that MAH reduces LDL-C levels. No significant change was observed in the control group before and after MAH.

Table 1. Demographic characteristics and laboratory parameters of the patient and control groups

Variables/Groups	Patients (T2DM) (n = 21)	Control (Healthy) (n = 14)	*P* value
Age, year	57.2 ± 10.8	43.9 ± 8.7	.001
Female, %	71.4	92.9	.203
BMI, kg/m^2	30.1 ± 4.3	25.3 ± 4.4	.003
GDF-15, pg/mL	181 (88-271)	52 (30-67)	<.001
Fasting glucose, mg/dL	141 (113-183)	99 (90-104)	<.001
HbA_{1c}, %	7 (6.5-8.4)	5.5 (5.3-5.6)	<.001
T Chol, mg/dL	215 (184-256)	203 (197-219)	.381
LDL-C, mg/dL	129 (102–160)	117(106-132)	.500
HDL-C, mg/dL	53 ± 11.4	61 ± 8.8	.042
TRG, mg/dL	176 (124-240)	122 (79-158)	.020

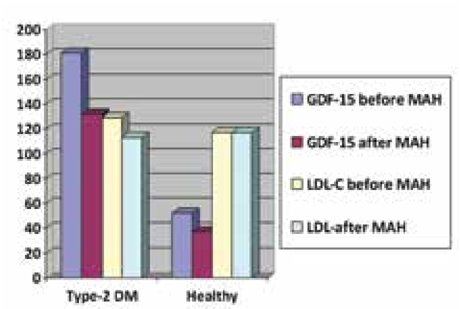
Abbreviations: DM, diabetes mellitus; BMI, body mass index; GDF-15, growth/differentiation factor-15; HbA_{1C}, glycosylated hemoglobin; T Chol, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TRG, triglyceride; mg/dL, milligram/deciliter; pg, picogram.

Table 2. Changes in the Laboratory Parameters of Groups after Major Autohemotherapy

Groups	Patient (T2DM) (n = 21)			Control (Healthy) (n = 14)		
Parameters	Before	After	*P* value	Before	After	*P* value
GDF-15, pg/mL	181 (88-271)	132 (61-198)	.002	52 (30-67)	37 (27-70)	.221
Fasting Glucose, mg/dL	141 (113-183)	149 (116-179)	.509	99 (90-104)	95 (93-97)	.010
HbA_{1c}, %	7 (6.5-8.4)	7.1 (6.3-8.0)	.109	5.5 (5.3-5.6)	5.5 (5.3-5.6)	.831
T Chol, mg/dL	215 (184-256)	196 (175-231)	.086	203 (197-219)	201 (189-248)	.975
LDL-C, mg/dL	129 (102-160)	113 (95-139)	.014	117(106-132)	117(107-146)	.925
HDL-C, mg/dL	53 ± 11.4	52 ± 11.3	.465	61 ± 8.8	60 ± 14.6	.683
TG, mg/dL	176 (124-240)	176 (119-217)	.999	122 (79-158)	126 (67-171)	.778

Abbreviations: DM, diabetes mellitus; GDF-15, Growth-differentiation factor-15; pg, picogram; HbA_{1C}, glycosylated hemoglobin; T Chol, Total cholesterol; LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; TRG, Triglyceride; mg/dL, milligram/deciliter.

Figure 2. Changes in Groups Before and After MAH



DISCUSSION

Our study is the first to show that MAH reduces GDF-15 levels in T2DM. In the patient group, GDF-15 levels decreased significantly after MAH. It tended to decrease in the healthy group, but it was not statistically significant (Figure 2). Increased GDF-15 levels are closely associated with oxidative stress, endothelial damage, ischemia, and cardiovascular risk.[20,21] In a recent study by Sendur et al., increased GDF-15 levels were shown in T2DM patients with diabetic foot ulcers. In addition, this high level of GDF-15 correlated positively with the severity of ulceration.[22]

It has been proposed that ozone bag therapy and MAH for patients with diabetic foot ulcers provide better infection control and promote wound healing, increase oxygenation and perfusion in the extremities, and significantly reduce amputation rates, morbidity, and mortality.[23,24]

Studies have shown that MAH increases oxygenation and regulates wound healing in patients with chronic extremity ischemia.[25-27] Wu et al. reported that MAH contributed to improvements in upper extremity motor functions in patients with acute cerebral infarction.[28] Sancak et al. showed that MAH improved renal ischemia and reperfusion injury in a study conducted with animal subjects.[29] Braidy et al. reported that ozone therapy improved degenerative changes in diabetes.[30]

Inflammatory markers and oxidative stress (OS) have been proposed to be implicated in the pathogenesis of T2DM.[31] Hyperglycemia leads to the reduction of anti-oxidant enzymes such as glutathione peroxidase (GPX) and glutathione reductase (GR), resulting in the formation of reactive oxygen species (ROS) such as hydroxyl radical and oxidized LDL. Moreover, the overactivation of aldose reductase, which is a nicotinamide adenine diphosphate (NADPH)-dependent enzyme, promotes sorbitol production, thereby depleting NADPH. NADPH is a co-factor of nitric oxide syntase (NOS), the enzyme responsible for NO synthesis from L-arginine; therefore, reduction in NADPH gives rise to the depletion of NO. The imbalance between NO and ROS causes oxidative stress, resulting in platelet activation, vasoconstriction, and damage to the vascular endothelium.[32,33]

Therapeutic ozone therapy increases anti-oxidant levels through the induction of antioxidant enzymes such as glutathione peroxidase (GSP), glutathione reductase (GR), glutathione transferase (GST), and superoxide dismutase (SD) and reduces the release of oxidative stress-related and inflammatory cytokines. By inhibiting the secretion of endothelin-1, which has an important vasoconstrictor effect, it inhibits smooth muscle proliferation, leading to occlusion in the vascular area. It also has a vasodilator effect by increasing the release of prostacyclin and nitric oxide (NO) from the endothelium. Along with these, it is known to improve blood circulation and oxygen delivery to ischemic tissues.[30,34]

Our study contributes to the literature on the therapeutic mechanisms of ozone therapy and evaluates it as a complementary treatment method in diabetes, because high GDF-15 is a potential prognostic marker associated with an impaired glycemic index, organ damage, and increased cardiovascular risk in diabetes.[4-8] No reported treatment strategies have been developed so far, although researchers have proposed treatments that reduce GDF-15 levels.

In addition to these, LDL-C levels decreased significantly in the patient group after MAH in our study ($P < .05$) (Table 2). No change was observed in the control group (Figure 2). In some previous studies, the therapeutic potential of MAH in severe dyslipidemia, cholesterol embolism, peripheral artery disease, and coronary artery disease has been demonstrated.[35] Zeng et al. reported that T Chol and TRG values decreased and HDL-C levels increased as a result of MAH in patients with psoriasis.[19] Sroczyński et al. also showed that intra-arterial ozone injections significantly reduced LDL-C levels in patients with T2DM.[36]

We closely followed up the patients in the T2DM group. We excluded those who had a change in their medical drug treatment because this could affect the results of GDF-15. The lack of significant changes in fasting glucose and HbA1C levels may be due to our non-intervention in medical treatments, the low number of participants, insufficient treatment sessions, and our inability to follow up for a longer period. However, GDF-15 levels were significantly reduced with MAH. GDF-15 is associated with an impaired glycemic index and has a relationship with chronic inflammation, oxidative stress, and cardiovascular risk.[20,21] MAH is likely to reduce GDF-15 levels with anti-inflammatory, antioxidant, and hemorheology effects.[30,34]

Increasing the number of sessions of MAH, combining it with other medical treatments, and continuing it as a maintenance therapy may help provide significant improvements in glycemic regulation and GDF-15 levels. Therefore, we suggest that MAH be included in diabetes treatment algorithms and that prospective studies be conducted with larger participant numbers.

CONCLUSIONS

MAH, a new treatment strategy, reduces GDF-15 levels in T2DM and contributes significantly to the mechanism of action of ozone therapy. In the management of diabetes, MAH can provide significant clinical improvements in addition to diet, physical activity, and current medical treatments and should be included in combined therapy.

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