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Plasma lipids, lipid peroxides and antioxidant system in osteoarthritic patients underwent spa therapy

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ABSTRACT

Objective: The molecular processes underlying degenerative cartilage disease "osteoarthritis, OA" are not fully known.. Although oxidative stress causes cell damage in various tissues, there is not enough evidence for the involvement of oxidative stress in degenerative joint diseases. On the other hand, various spa therapies such as balneotherapy, mud, mineral water, and sulfur bath have long been used for treating osteoarthritis. This study aimed to investigate the effect of spa therapy with balneotherapy on oxidant/antioxidant status and lipid levels.

Material and Methods: This prospective cross-sectional study was conducted on 28 osteoarthritis patients who had spa therapy at Bursa Military Hospital, Turkey. Osteoarthritis patients between 45-70 years who had no contraindications to spa therapy were eligible for inclusion in the study. Spa therapy included balneotherapy with acratothermal water, physical therapy modalities, and mild exercise for 15 days. Blood samples were obtained before and after the treatment cycle to determine the patients' possible changes in oxidant/antioxidant status and lipid profiles. Serum malondialdehyde (MDA), total thiol (T-SH) levels, total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx) activity, and plasma lipids were measured.

Results: We found a statistically nonsignificant decrease in MDA levels and a significant increase in GPx activity. Whereas plasma lipids, T-SH levels, TAC, and SOD activity remained unchanged.

Conclusion: We may suggest that different mechanisms may play a role in the beneficial effects of spa therapy with balneotherapy in OA besides stimulation of GPx activity.

Keywords: Balneotherapy, Lipids, Osteoarthritis, Spa therapy, Total antioxidant capacity

INTRODUCTION

Osteoarthritis (OA) is a musculoskeletal disease of synovial joints characterized by cartilage degeneration. The final steps in this disease are the progressive loss of articular cartilage and irreversible impairment of joint motion (1, 2). Although mechanical overload is thought to be involved in the degeneration of articular cartilage, molecular mechanisms and the mediators playing a role in the pathogenesis of the disease have not been elucidated yet (2).

Oxidative stress has become a popular area of research in recent years due to its importance in the pathophysiology of OA. Free radical-mediated reactions damage tissues by promoting aging, functional failure, and degenerations if produced uncontrolled (3). In addition, increased oxidative damage due to free radicals has been demonstrated to associate with knee OA (4). On the contrary, antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), were shown to be diminished in OA patients, which indicates the significance of oxidative stress in the pathophysiology of the disease (5).

Researchers are continually looking for new treatment options because there are currently no treatments available to prevent or delay OA. Osteoarthritis treatment consists of pharmacological treatments, non-pharmacological treatments, and surgical treatments. Spa therapy, including mud-pack therapy, hydrotherapy, balneotherapy with mineral and/or thermal waters, physiotherapy, and exercise, has been applied to osteoarthritic patients as the most common non-pharmacological approach (6-9). Balneotherapy has improved the quality of life by targeting pain relief and joint function (10).

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In addition to short-term analgesic, myorelaxant, and the other effects, it has long-term and preventive effects on the body from six months to a year. However, despite its long history and widespread use, the underlying mechanism regarding spa therapy's general effect and preventive aspect have not been clearly understood. Studies have suggested that therapy with hyperthermal baths, drinking cures, and peloid packs reduce oxidative stress and induce antioxidant defense mechanisms (11, 12). However, the beneficial effects of these treatments and the biological mechanisms of action are still under investigation.

There are few studies regarding the effects of spa therapy on plasma lipids and oxidant/antioxidant status in OA patients. Therefore, in the present study, we aimed to determine whether spa therapy with balneotherapy influences oxidant/antioxidant status and lipid profiles of OA patients.

MATERIAL AND METHODS

Patients

This study included 28 osteoarthritic patients (19 female, 9 male) with a mean age of 59.5 ± 7.0 years. The patients underwent spa therapy comprised of balneotherapy with acratothermal water (at 39-41 oC) for 20 min, mild exercise, and physical therapy for 15 days (with treatment on Saturdays and Sundays) at Bursa Military Hospital, Turkey. The physicochemical properties of thermal mineral water used in balneotherapy are shown in Table 1. Twelve of 28 patients took one analgesic (paracetamol, metamizole) when patients had pain. All patients were fed an identical diet. Anyone from 45-70 ages with osteoarthritis who had no contraindications to spa therapy was eligible for inclusion in the study. The exclusion criteria:The presence of any systemic disease except osteoarthritis, antioxidant and vitamin use, antihypertensive (calcium canal blockers, β blockers), antihyperlipidemic, antidiabetic drug use, alcohol use, and cigarette smoking. In addition, hematological and biochemical analyses were performed at the beginning of the study to exclude systemic disease.

The Ethics Committee approved the study of Istanbul University by following international agreements (World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended in October 2013). All subjects received prior information about the study, and written informed consent was obtained from participants.

Measurements

Fasting blood samples were obtained from patients on the study's 1st day and 16th day for measurements. Plasma lipid profiles, malondialdehyde (MDA) levels, total sulfhydryl groups (T-SH), total antioxidant capacity (TAC), glutathione peroxidase activity (GPx), and superoxide dismutase (SOD) activity were assayed.

Plasma total cholesterol, low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride levels were assayed by Roche autoanalyzer using enzymatic kits according to standard laboratory protocols.

Plasma MDA levels, a reactive aldehyde produced by lipid peroxidation of polyunsaturated fatty acids, were measured spectrophotometrically using thiobarbituric acid according to Buege & Aust (13).

Briefly, 1.0 ml plasma was mixed with the mixture of 2.0 ml trichloroacetic acid (TCA), thiobarbituric acid (TBA), hydrochloric acid (HCl), and heated in boiled water for 15 minutes. After cooling, samples were centrifuged 1000 x g for 10 minutes, and the upper phase was obtained. Absorbance was measured by spectrophotometry at 535 nm. MDA concentrations were calculated using extinction coefficient (ϵ =1.56 x 105 $M^{-1}cm^{-1}$) and presented as nmol/ml in plasma. Plasma T-SH levels were determined by the method of Hu (14), using Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid)]. TAC was evaluated by FRAP (Ferric Reducing Antioxidant Power Assay) method (15). FRAP determination was based on spectrophotometric measurement of the intense blue color change at 593 nm wavelength while Fe+3 tripyridiltriazin complex (Fe+3-TPTZ) was reducing to Fe+2 form. Change of absorbance is related to the total reducing power of electron giving antioxidant in reacting mixture. Ascorbic acid standards were used to calculate FRAP values. In addition, 1000 µM ascorbic acid used in the FRAP method equals 2000 µM FRAP value. The TAC value of samples was calculated. Plasma GPx activity was assessed using the method suggested by Paglia & Valentine (16), which measures GPx activity indirectly by measuring the rate of NADPH (nicotinamide adenine dinucleotide phosphate) oxidation to NADP+. NADPH oxidation is accompanied by a decrease in absorbance at 340 nm. Plasma SOD activity has been assayed by a spectrophotometric method based on the inhibition of superoxide-induced NADH oxidation (17). One unit of the enzyme activity is defined as the amount of SOD capable of inhibiting the rate of NADH oxidation of the control by 50 %.

Statistical Analysis

Statistical analysis in this study was made by GraphPad Prism V.8 program. The Kolmogorov-Smirnoff test was used to examine the normality of distribution. In addition to descriptive analysis, paired t-tests have been used to compare before and after treatment of the patient. Data were expressed as mean ± S.D (Standard Deviation). Results were evaluated at a $p < 0.05$ significance level, between 95 % confidence interval.

RESULTS

The study includes 28 patients with a mean age of 59.5 ± 7.0 years (age range: 45–70 years) (Table 2).

The evaluation of BMI before (27.1 ± 2.3) and after (26.9 ± 2.5) spa therapy demonstrated a minor nonsignificant reduction ($p > 0.05$) in all the population (Figure 1).

A statistically insignificant decrease was established in plasma MDA, T-SH levels, and TAC regarding the first measurement ($p > 0.05$). Also, a insignificant increase was observed in plasma SOD activity at the last measurement (Table 3).

The difference between the TAC measurements of the 1st and the 16th days was significant when patients were divided according to gender (Figure 2).

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However, we found statistically significant high plasma TAC values in male patients than in female patients (data not shown). Finally, GPx activity showed a statistically significant ($p < 0.001$) increase in patients having undergone spa therapy (Table 3).

In our study, all patients' blood lipid levels were within normal limits. Data analysis revealed a minor reduction in total cholesterol, LDL-C, and VLDL-C levels and a minor increase in patients' triglyceride levels at the end of spa therapy. However, the changes were not statistically significant. A significant decrease in HDL-C levels was found in patients after the spa therapy (Table 4). Finally, no adverse reactions were seen in participants.

Table 1. Physicochemical properties of water used in balneotherapy

Characteristic		Value
pH		7.1
Hardness (Fr^0S)		28.8
Carbon dioxide (CO_2) (mg/L)		22
Metaboric acid (HBO_2) (mg/L)		1.2
Metasilicic (H_2SiO_3) (mg/L)		40.3
Total mineral (mg/L)		602.1
Anions (mg/L)	Chloride (Cl^-)	6.1
	Bicarbonate (HCO_3^-)	341.6
	Sulfate (SO_4^-)	72.0
	Fluoride (F^-)	0.9
Cations (mg/L)	Sodium (Na^+)	31.7
	Calcium (Ca^{2+})	82.2
	Potassium (K^+)	4.7
	Magnesium (Mg^{2+})	20.1

Analysis was performed at the Balneology and Water Chemistry Laboratory of the Department of Medical Ecology and Hydroclimatology, Istanbul Faculty of Medicine

Table 2. Demographic data of patients studied at baseline

Parameters	All patients (n=28)
Sex	
Male (n, %)	9 (32)
Female (n, %)	19 (68)
Age (years)	59.5 ±7.0
Height (cm)	164.5 ± 8.7
Weight (kg)	74.1 ± 10.2
Body mass index (kg/m^2)	27.1 ±2.3
Disease duration (years)	10.2 ± 7.6

Table 3. Plasma MDA, T-SH, TAC, GPx and SOD activities changes in osteoarthritics before and after the spa therapy

	Before therapy	After therapy
MDA (nmol/ml)	6.47 ± 1.50	5.49 ± 0.80
T-SH (nmol/ml)	377 ± 62	362 ± 46
TAC	949.4 ± 226.4	896.1 ± 238.9
GPx (U/l)	376 ± 95	419 ± 78**
SOD (U/ml)	78.0 ± 21.5	78.4 ± 19.7

** $p < 0.001$ compared to before therapy, MDA malondialdehyde, T-SH total sulfhydryl groups, TAC total antioxidant capacity, GPx glutathione peroxidase, SOD superoxide dismutase

DISCUSSION

Oxidative stress in OA pathogenesis has been documented in numerous studies. Although some investigators have reported that reactive oxygen species (ROS) are involved in the degeneration of articular cartilage (2, 18, 19), there are also a few controversial studies (1). It has been suggested exogenous H2O2 has damaged cultured chondrocytes (2), and superoxide anions cause apoptotic cell death in synoviocytes (18). Mazzetti et al. (1) have found that nitric oxide (NO) rather than ROS may significantly alter chondrocyte functions in OA. DelCarlo and Loeser (20) have reported that NO, not alone but with ROS, plays an essential role in chondrocyte cell death. Although it has been suggested that the continual production of free radicals within the degenerated joint may result in the exhaustion of effective antioxidant control (3), studies related to antioxidant defenses are also conflicting. For example, Kurz et al. (2) have reported that dietary vitamins (vitamin E and C) and Se diminished osteoarthritic lesions, and it is accompanied by an increase in antioxidative enzyme activity and expression in rats. However, total antioxidant capacity in plasma and synoviocytes of osteoarthritic patients has been observed unchanged (21). Similar results have been reported by Sarban et al. (22) concerning total antioxidative capacity in plasma of OA patients. They found that GPx and CAT levels in erythrocytes were much lower, whereas SOD levels were unaffected (22).

Various therapies known to have long-lasting beneficial effects for osteoarthritis. Three weeks of spa therapy, including rest, balneotherapy with spring water, and medical care (7), two weeks of mud pack and mineral therapy (6), two weeks of balneotherapy at the Dead Sea area (8), and three weeks sulfur bath (9) are some feasible treatment options for osteoarthritis. Their mechanism of action was identified with in vitro studies. According to their results, spa therapy has been proven to have beneficial effects on the oxidant/antioxidant system. Fioravanti et al. (23) have reported that sulfated thermal waters suppress NO generation and apoptosis produced by IL-1 in OA chondrocytes. H2S has been suggested to inhibit the production of inflammatory cytokines (IL-8, IL-1, TNF-α, IL-6, and IL-10) and counteract the formation of ROS and reactive nitrogen species (RNS) by human monocytes (24). There are also in vivo studies to investigate the effects of spa therapy on the oxidation and inflammation markers and antioxidant enzymes. Jokic et al. (25) have observed reductions in serum MDA and carbonyls, as well as SOD and catalase activity (25) and also GPx activity (9) after balneotherapy with sulfurous water. This reduction in oxidative stress during sulfur therapy may be due to lower expression of antioxidant enzymes or increased production of superoxide radicals exhausting the superoxide-scavenging enzymes. Benedetti et al. (26) reported an increase in plasma thiol levels decrease in plasma MDA and carbonyl levels after balneotherapy using sulfurous water and mud in OA (26). Therapeutic baths in mineral water lowered the levels of MDA and activities of catalase, SOD, and GPx, according to Bender et al. (27).

Table 3 shows a statistically nonsignificant decrease in plasma MDA levels and TAC of osteoarthritic patients after the spa therapy. When the patients were evaluated as male and female, a statistically significant decrease in TAC value was found in females and an increase in males. All of the female patients out of two were in the postmenopausal period. Some studies have shown that the effect of estrogens, which are known as strong antioxidants, and TAC levels decrease in postmenopausal women (28). TAC decrease in women at the end of spa cure may be due to estrogens deficiency, sedentary lifestyle, and small muscle mass (28). According to this result, women may need an antioxidant supply in a spa treatment. In our study, plasma T-SH was not changed. While GPx activity was found to be elevated, SOD activity remained unchanged. This discrepancy in antioxidant enzyme activities might be due to the diversity of gene expressions. Although statistically insignificant, the decrease in MDA levels may be related with increased GPx activity.

Despite these conflicting results from the published studies, our findings are similar at some points. For instance, Bellometti and colleagues observed a significant decrease in MDA levels and a nonsignificant increase in GPx activity, transferrin, and ceruloplasmin levels after treatment with peloid packs (11, 12). Ekmekçioğlu and colleagues have found an insignificant increase in peroxide concentrations and a significant improvement in lipid levels in the patients with osteoarthritis at the end of three weeks of spa therapy (9). When the studies searching the effect of balneotherapy on oxidant and antioxidant systems were evaluated, the decrease in oxidant stress was more prominent, and the increase in antioxidant defense was less manifest. This finding may be due to the oxidant effect of hyperthermic treatment and antioxidant consumption when an organism defense against oxidative stress. Spa therapy may decrease oxidant stress by eliminating environmental oxidative factors like air pollution, mental stress and dietary regulation, regular exercise, balneotherapeutic applications like hyperthermic baths, and the chemical effects of thermomineral waters.

We also evaluated plasma lipid levels in osteoarthritics. There was a slight change in plasma lipids other than triglycerides which could be a tendency towards diminution. However, our findings did not reach statistical significance. On the other hand, the decline observed in HDL cholesterol was significant. These results differ from some published studies. For instance, a study on knee OA has shown that balneotherapy with sulfur water decreased patients' cholesterol, triglyceride, and LDL levels and unchanged their plasma total antioxidant capacity (29). However, it has been reported that decreased levels of cholesterol and triglyceride and increased HDL cholesterol levels after balneotherapy treatment (30). Our results share several similarities with Kasperczak et al. (31) findings, who found HDL cholesterol was significantly lower in male osteoarthritic patients after spa therapy. They also found a slight drop in LDL cholesterol levels and an insignificant increase in triglyceride levels after treatment (31).

We are aware that our research may have some limitations. First, we evaluated only the short-term efficacy of spa therapy in a small number of patients. Another disadvantage was the lack of a control group of OA patients who had not received treatment. However, we thought it would have been unethical to leave a patient group without any treatment. Finally, we conducted a single treatment approach. Future studies should include comparisons with other treatment techniques and long-term outcomes with participants.

CONCLUSION

Although spa therapy causes an increase in GPx activity, different mechanisms may play a role in the beneficial effects of spa therapy in OA besides stimulating of antioxidant system. However, because of the divergent results, assessing the impact of spa therapy on oxidant/antioxidant status and lipid metabolism changes will require more research in large groups of patients, preferably using different treatment protocols.

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Author Contributions: NHA, ZC, GO: Study design, Literature review, Data collection and processing, Patient therapy, Analysis **ZC:** Data collection, Writing, Revisions

Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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