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1. Programme (PR-1) Border-lines of Health

**Investigating the effects of the thermal mineral water of Szigetvár in a randomized
controlled double-blind clinical trial**

Doctoral (Ph.D) thesis

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Introduction

Previous studies have shown the beneficial effects of balneotherapy in musculoskeletal (chronic low back pain, rheumatoid arthritis, etc), gynaecological, dermatological conditions (psoriasis in particular), peripheral vascular disease, fibromyalgia and many other disorders. Thanks to its special geothermal conditions and great natural power, Hungary is one of the leading countries worldwide using thermal water for medical purposes. Hungary is rich in thermal-mineral waters as it has more than 1300 thermal wells. From the 18th century chemical analyses focused mainly on the inorganic substances of natural thermal-mineral waters, and therefore these waters were categorized according to their inorganic compositions. Mineral waters are defined as waters containing more than 1000 mg of total dissolved solids (Ministry of Health Decree no. 74/1999 (XII. 25.)), and thermal waters have to reach the temperature of more than 30 °C (General Directorate of Water Management). In the second half of the 20th century several analytical methods were developed to analyse the organic compounds of medicinal waters. Still, the presence of organic components was almost completely ignored both in the categorization of thermal-mineral waters and their mechanism of action. We could not find any publications investigating the effects of the organic compounds of medicinal waters on diseases.

Osteoarthritis (OA), the most common musculoskeletal condition of aged population, is a chronic degenerative disease involving large weight bearing joints resulting in stiffness, pain and impaired movement. The 2010 Global Burden of Disease Study reports that the burden of musculoskeletal disorders accounts for 6.8% of DALYs worldwide. OA can affect any joints, but most commonly develops in the hips, knees and hands. Its prevalence is 18.3% in Hungary, higher than the worldwide estimates (9.6% of men and 18.0% of women aged over 60 years) (WHO Department of Chronic Diseases and Health Promotion) and it is increasing due to ageing and related factors such as obesity. OA has a significant negative impact on patients' quality of life. The goals of OA treatment include alleviation of pain and improvement of functional status. Non-pharmacological therapies are very important in OA together with pharmacological treatments. These treatments should be individually devised and should undergo rigorous randomized controlled trials in a similar manner to pharmacological studies. One of the widely used non- pharmacological treatment options is balneotherapy. The beneficial effects of the thermal mineral waters and/or medicinal mud on OA were

investigated by several randomized controlled trials. However, these studies were focusing on the inorganic compounds of the thermal mineral waters. (Branco et al. 2016; Fioravanti et al 2014; Fioravanti et al. 2015; Kovacs et al 2012; Kulisch et al 2014).

In our previous clinical trial (Hanzel et al. 2018) we have already proven the positive health effects of Szigetvár thermal-mineral water on patients suffering from osteoarthritis of the hips and the knees. Several other studies did the same with other thermal waters, spa therapies (Pittler et al. 2006; Franke et al. 2007; Kulisch et al. 2009; Horvath et al. 2012). In a recent systematic review Morer et al. described these health effects as well and emphasized that double-blind randomized clinical trials are needed to study the role of mineral elements and other chemical compounds appropriately (Morero C et al. 2017).

Fulfilling this requirement the aim of the present study was to assess the therapeutic effect of the organic fraction of Szigetvár thermal-mineral water in a double-blind, randomized, controlled clinical trial on patients suffering from OA of the hips and the knees. This is a completely new approach providing insight into the mechanism of action of thermal-mineral waters.

We hypothesized the followings:

- We hypothesized that we obtained significantly better results in terms of pain, activity, and total scores in the medicinal water and concentrate groups in long term compared to the tap water group.
- According to our hypothesis, based on VAS, the feeling of pain is significantly reduced in the groups, that were treated long-term with medicinal water and concentrate, however, this is not assumed in the tap water group.
- According to our hypothesis, immediately after the treatment and at the 3-month follow-up we get a significant improvement in the quality of life in the group of medicinal water and organic matter concentrates compared to the group of tap water
- We hypothesized that the range of motion changes would be significantly better in the medicinal water and organic matter concentrate group compared to the tap water group, using the same treatments in the long run
- According to our hypothesis, the effect can be related to organic matter

Patients and methods

We performed the study at Szigetvár Spa between September 2014 and December 2017. The Regional Ethics Committee of University of Pécs, Pécs, Hungary approved the study protocol (Permission No. 5351), in accordance with the 2008 Helsinki declaration.

Inclusion criteria were: osteoarthritis of the hip and/or the knee, age between 50 and 75 years, willingness to take part in our 3-week-long outpatient rehabilitation treatment, Kellgren-Lawrence radiological stages I–III in the joint investigated, at least mild (1 point on the Likert scale) hip or knee pain for a minimum of 5 days a week for at least 3 months, characteristic complaints of knee joint OA for at least 3 months, no limitations in the range of joint motion and no severe disability.

Exclusion criteria were: any kind of physiotherapy with the exception of home-exercise in the last 2 months, any previous knee joint operation, any hip joint or spine operation within 1 year before the study, any knee or hip joint trauma within 1 year before the study, knee joint arthroscopy within 3 months before the study, intraarticular hyaluronic injection within 2 months before the study, the onset of lumbar radiculopathy, the onset of palpable Baker cyst, sedimentation rate higher than 30 mm in females and higher than 20 mm in males and non-compliant patients. Further exclusion criteria were: lumbago, sciatica, any previous fracture in the hip joint, subluxation, luxation, algodystrophy, fibromyalgia, gout, balneotherapy within the past 6 months, intra-articular corticosteroid treatment of the affected hip joint within 3 months or any other joint within 1 month, systemic corticosteroid treatment within 1 month prior to the study, and initiation of symptomatic slow-acting drugs for osteoarthritis (SYSADOA) within 3 months prior to the study.

We paid special attention to include only those patients who had never received underwater jet massage either with tap water, or with medical water.

Recruitment of patients

To minimize dropout, only patients living maximum 15 km far from the spa were involved. Patient information sheet was distributed among patients. The patients signed the consent form before the treatment period. Patient selection was based on age, sex and disease parameters. Among 135 patients, suffering from OA of the hips and the knees, enrolled into the study 61 were excluded and 74 (24 males, 50 females, mean age: 67.3 ± 4.48 years) were

randomized and treated. After randomization patients were divided into three groups according to the water type used for treatment: tap water group (n=24), mineral water group (n=26) and organic fraction group (n=24).

Preparation of organic fraction

Organic concentrate from medicinal water was prepared according to Varga et al. (Varga et al. 1991). Samples of thermal-mineral water concentrates were isolated by adsorption-elution cycles on macroreticular adsorbent resins. A stainless steel pilot chromatography column (size: 103.5 mm x 1000 mm, own product) was filled with a mixture of two adsorbent resin types (XAD-4 (Serva); XAD-1180 (Rohm&Haas) in a rate of 1:1 in a volume of 5 liters. 50 m³ of thermal-mineral water was passed over the column at an average flow rate of 1 bed volume per minute. Inert gas was used to eliminate the residual water from the column. Adsorbed organic substances were eluted with 96% ethanol of 1 bed volume to produce a 5,000-fold concentration of the original thermal-mineral water. The concentrate contained the vast majority of organic substances of the water, but not inorganic components. During the jet-massage treatment in bath tub the organic concentrate was diluted to the original concentration and temperature with tap water.

Interventions

The patients received tub and underwater jet massage treatment (15 minutes per occasion). The enrolled patients were randomly divided into three groups. One group received jet treatment in tap water, another in thermal mineral water and the third in tap water containing the re-dissolved organic concentrate. To minimize the difference between the three water types, all were colored by commercially available water coloring tablets. Moreover, the treatments were performed in the same room; therefore, all patients smelled the odor of the thermal water. The pH of the water was adjusted as well. The sensation of water containing high concentration of minerals can be different from tap water. The enrolment of patients who were unfamiliar with the underwater jet massage treatment and the general properties of the mineral water reduced this factor. All patients were treated in individual tubs with 34 C° warm water. The water type filled into the tub was only known by the study assistant who provided tap water, thermal mineral water or tap water with organic fraction for the patients

according to the randomization. The treatment included 30-min sessions 5 days of the week for 3 weeks, thus a there were a total of 15 treatments. The examining physician was responsible for the detailed medical history and patient selection.

Outcome parameters

Patients' condition was measured with both objective and subjective methods. To evaluate disease severity, the range of movement (ROM) of the involved joints and Western Ontario and McMaster University Osteoarthritis Index (WOMAC) were measured. Visual analog scale (VAS) was applied to measure current pain severity. Quality of life was assessed by filling in the Short Form 36 questionnaire (SF-36). ROM score, WOMAC index and VAS were determined before the first treatment (baseline), after the last treatment (short-term) and 3 months after the last treatment (long-term). SF-36 questionnaire was filled in before the first treatment and after the last treatment. To rule out interobserver error, the same physiotherapist assessed ROM score and WOMAC index, and the same researcher evaluated VAS score. The articular joint movement was measured by a protractor (Goniometer). To minimize scaling errors, the mean of three measurements was recorded. Goniometry plays an essential role in the clinical measurement of range of motion. It is apparent from several papers that traditional plastic goniometer evaluates hip and knee joint angles efficiently and reliably, however, use of goniometer requires careful attention in evaluation.

Randomization

Random number generator was used to allocate the patients into groups upon arrival ([Suresh K 2011](#)).

Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics for Windows Version 22 (IBM Corp., New York, NY, USA). Significance of the results was analyzed by Kruskal–Wallis, Mann-Whitney, Wilcoxon signed-rank test and chi square tests. $P < 0.05$ was considered the criterion of significance.

Results

Among 135 patients, suffering from OA of the hips and the knees, enrolled into the study 61 were excluded and 74 (24 males, 50 females, mean age: 67.3 ± 4.48 years) were randomized and treated. After randomization patients were divided into three groups according to the water type used for treatment: tap water group (n=24), mineral water group (n=26) and organic fraction group (n=24). Table 1 shows the clinical characteristics of the patient groups. Comparing the three groups, there were no significant differences between age, sex distribution, body mass index and number of hypertensive patients.

Table 1. Clinical characteristics of the patients. BMI, body mass index.

	Tap water (n=24)	Mineral water (n=26)	Organic fraction (n=24)	p value
Mean age, years	67.43 ± 4.95	66.22 ± 4.68	68.46 ± 3.58	0.206
Male, n (%)	8 (33.3)	9 (34.6)	7 (29.2)	0.912
BMI, kg/m ²	27.08 ± 3.42	26.66 ± 3.05	26.33 ± 3.19	0.725
Diabetes type 2, n (%)	12 (50)	13 (50)	11 (45.8)	0.908
Hypertension, n (%)	18 (75)	20 (76.9)	17 (70.8)	0.850

Note: Comparing the three groups, no significant differences were found between age, sex distribution, body mass index and number of hypertensive patients. Mean age, years and BMI estimates are from Kruskal–Wallis test; and Male, Diabetes, Hypertension estimates are from chi-square test.

ROM score

Tap water – Mineral water comparison

Significant changes of ROM score were found in the tap water group; hip flexion ($p=0.017$), hip abduction ($p=0.039$), hip extension ($p=0.034$), knee flexion ($p=0.003$) and knee extension ($p=0.016$) showed significant improvement when baseline values were compared to short-term values. No significant changes of ROM were found in the tap water group, when comparing the baseline values to the long-term values. On the other hand, significant changes of ROM score were found in the thermal water group; hip flexion ($p=0.005$), hip extension ($p=0.011$) hip abduction ($p=0.011$), hip adduction ($p=0.005$), knee flexion $p<0.001$, and knee

extension ($p=0.014$) showed significant improvement when baseline values were compared to short-term values. Furthermore, when baseline values were compared to long-term values, hip extension ($p=0.023$) and flexion ($p=0.007$), hip abduction ($p=0.007$), hip adduction ($p=0.005$), knee flexion ($p<0.001$) and knee extension ($p=0.014$) improved significantly, as well. There was significant change in parameters when comparing the ROM of Mineral water group patients to the ROM of Tap water group patients after the treatment, significant improvements were detected in these parameters (hip flexion $p=0.023$, hip adduction $p=0.001$, knee flexion $p=0.003$ and extension $p=0.006$)

When comparing the ROM measured in the Mineral water group to the ROM measured in the Tap water group after long term, significant improvements were detected in all parameters.

Tap water – Mineral water – Organic fraction comparison

When comparing the ROM measured in the Organic fraction group to the ROM measured in the Tap water group after 15 treatments, significant improvements were detected in all parameters, except changes in hip extension. We performed the same comparison between the Organic fraction and the Mineral water group. There was no significant change in parameters when comparing the ROM of Organic fraction group patients to the ROM of Tap water group patients after 3 months, significant improvements were detected in all parameters (hip flexion $p=0.028$, hip abduction $p=0.004$, hip adduction $p=0.002$, knee flexion and extension $p<0.001$), except changes in hip extension ($p=0.059$).

WOMAC score

Tap water – Mineral water comparison

Regarding the WOMAC index, when baseline scores were compared to short-term scores significant improvement was found among pain ($p=0.036$), stiffness ($p=0.009$), activity ($p=0.007$) and total ($p=0.004$) scores in the tap water group. However, comparing the baseline scores to the long-term scores, no significant differences were found in the tap water group.

Comparing the baseline scores to the short-term scores, the WOMAC activity ($p<0.001$), stiffness ($p=0.005$), pain ($p=0.002$) and total ($p<0.001$) scores improved significantly in the mineral water group. Comparing the baseline scores to the long-term scores ,significant differences were found in activity score ($p<0.001$) and total score ($p<0.001$) in the mineral water group.

Patients in the Organic fraction group presented significant improvement in the dimensions of the WOMAC score during the short-term period, and in the long-term period, as well, except stiffness parameter.

Tap water – Mineral water – Organic fraction comparison

Comparing the WOMAC scores determined in the Organic fraction group to the Tap water group no significant differences were detected in the short-term. However, 3 months after the treatment we found significant improvements in the Organic fraction group concerning pain ($p=0.004$), activity ($p<0.001$) and the WOMAC total score ($p<0.001$).

Comparing the Organic fraction group to the Mineral water group in the short-term period, significant difference was not found in pain, stiffness, activity and total WOMAC scores. In the long-term period no significant differences were found.

SF 36 questionnaire

Tap water – Mineral water comparison

Evaluating the dimensions of the SF 36 questionnaire, we compared the short- and long-term results, and have found a significant improvement in both groups. However, in the dimension of social function we got a significant change only in the medicinal water group, not in the tap water group.

Tap water – Mineral water – Organic fraction comparison

We found improvement in all dimensions of the SF 36 questionnaire, except in the dimension of “General health perceptions”, both short- and long-term in organic fraction group. The “Emotional role functioning” dimension showed positive change only in the long-term period. 3 months after the treatment there were significant improvements only in the dimension of “Physical functioning” when comparing the results of the SF 36 questionnaire of Organic fraction group to the Tap water group ($p=0.020$).

VAS

Tap water – Mineral water comparison

Regarding the results of VAS, tap water treatment significantly reduced the pain in the short-term ($p=0.006$), however, no beneficial long-term effects were demonstrable. On the other hand, both short-term ($p<0.001$) and long-term ($p=0.008$) reduction of VAS scaled pain were demonstrated in the mineral water group.

Tap water – Mineral water – Organic fraction comparison

Regarding the results of VAS, re-dissolved organic fraction treatment significantly reduced the pain both in short-term ($p < 0.001$) and long-term ($p = 0.025$).

There were no significant differences when comparing Organic fraction group to Tap water group or Mineral water group, either in short-term ($p = 0.107$; $p = 0.670$) or in long-term ($p = 0.525$; $p = 0.564$).

Discussion

This double-blind, randomized, controlled clinical trial proved the therapeutic effect of the re-dissolved organic fraction of the Szigetvár mineral water according to international standards in patients suffering from OA of the knee and hip. To the best of our knowledge, previous researches were only limited to the inorganic components of the thermal-mineral water e.g. sulfur Rn^{222} Dead Sea salt solution, CO_2 in humans. No researcher has examined the possible healing effects of the organic fractions of mineral waters.

Along our initial hypothesis, the re-dissolved organic substance concentrate could partially provide the therapeutic effect of the original medicinal water. Our finding is the first direct evidence for the so-called "Varga's organic hypothesis", namely that the biological effects of thermal waters are caused by bioactive organic molecules more likely than by the inorganic salt content. Inorganic ion absorption through the skin is either unproven or insignificant due to higher ion concentrations in the human body. (This does not apply to the high-ionic mineral water consumption as course of treatment.) However, for example hormone-like molecules with high biological activity can be found among the organic substances. These molecules that are absorbed can be highly active at very low concentrations. Furthermore, Bender et al. (2013) in their meta-analysis concluded that the beneficial effects of Hungarian thermal-mineral waters were independent of their inorganic salt content.

Interpreting ROM results, when only the effect of the re-dissolved organic fraction was examined short- and long-term, it caused significant improvements in ROM score, just like the original mineral water. So in this case the positive effect of the concentrate is very similar. When the three groups were compared, changes in ROM scores were higher in the Organic fraction group than in the Tap water group, both in short- and long-term. Results refer again to the same favorable effect of the re-dissolved organic fraction as the original mineral water, both short- and long-term. Our hypothesis was confirmed, and at 3-month

follow-up, a significant improvement in the range of motion of the mineral water and concentrate groups was also observed.

WOMAC scores also indicated significant positive effects in the Organic fraction group, short- and long-term, too. Comparing the three types of water-treated group Tap water group showed significantly worse WOMAC scores after 3 months of follow-up than the Organic fraction group. WOMAC scores indicated similar beneficial or no effects of the mineral water compared to the organic fraction, both short- and long-term. Based on our results, our hypothesis was confirmed.

Based on SF 36, our hypothesis was not confirmed, although we observed a significant improvement in several dimensions after treatment compared to the pre-treatment condition, however, comparing the groups, there was only a significant difference in the physical function dimension of the concentrate group compared to the tap water group. Thus, of the eight dimensions, only one confirmed this. In several dimensions of the SF 36 questionnaire the organic fraction induced positive changes both short- and long-term.

Regarding the VAS results the organic fraction decreased the pain severity significantly also short- and long-term. Based on our results, our hypothesis was correct.

These novel findings emphasize the role and positive effects of the organic fraction of Szigetvár thermal-mineral water.

Conclusion

On the field of balneology science, this unique idea is to suggest the beneficial healing effects of the organic substances in Szigetvár thermal-mineral water. However, this phenomenon also needs to be proven in other medicinal waters. Once it is confirmed in numerous other investigations, our theory can lead to paradigm shift in the science of balneology. The line of further research is set for our team, as several medicinal waters in Hungary contain considerable amount of organic matter according to previous gas chromatography studies. The presented concentration method also provides the opportunity to produce medicinal water-based products with therapeutic effects, which can be used both bedside and homeside.

Novel findings and practical applications

- Our analyses presented in the dissertation have provided several new results. To the best of our knowledge, this is the first study to use water-coloring tablets to eliminate the color difference between tap water, medicinal water, and concentrated water.
- Treatment with Szigetvár medicinal water and concentrated water significantly improved the range of motion in patients with knee and hip osteoarthritis, as well as WOMAC scores and SF-36 value.
- Our results are the first direct proof of the so-called “organic hypothesis”, according to which the biological effects of medicinal waters are more related to bioactive organic molecules than to inorganic salinity.
- Our experiments can lead to a paradigm shift if we prove the phenomenon in several medicinal waters.
- We have found little national or international literature on this topic, which includes both objective and subjective forms of measurement, so we would like to develop a unified method for the classification of medicinal waters.
- To verify our results, we perform a counter-test (by diluting the inorganic salts)

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