Effect of Mud-Bath Therapy on Serum Biomarkers in Patients with Knee Osteoarthritis: Results from a Randomized Controlled Trial

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ABSTRACT: **Background:** Balneotherapy is one of the most commonly used non-pharmacological approaches for osteoarthritis (OA). Recent data indicate that some biomarkers could be useful to predict OA progression and to assess therapeutic response.
Objectives: To evaluate the effects of mud-bath therapy on serum biomarkers in patients with knee OA.
Methods: The study group comprised 103 patients with primary symptomatic bilateral knee OA who were randomly assigned to receive a cycle of mud-bath therapy over a period of 2 weeks or to continue their standard therapy alone. Clinical and biochemical parameters were assessed at baseline and after 2 weeks. Clinical assessments included global pain score on a visual analogue scale (VAS) and the Western Ontario and McMaster Universities Index (WOMAC) subscores for knee OA. Cartilage oligomeric matrix protein (COMP), C-terminal cross-linked telopeptide type II collagen (CTX-II), myeloperoxidase (MPO) and high sensitivity C-reactive protein (hsCRP) serum levels were assessed by ELISA.
Results: At the end of mud-bath therapy we observed a statistically significant improvement in VAS and WOMAC subscores. Serum levels of COMP, MPO and hsCRP did not show any significant modification in either group, while a significant increase ($P < 0.001$) in CTX-II serum levels was observed in the mud-bath group after the treatment.
Conclusions: A cycle of mud-bath therapy added to the usual treatment had a beneficial effect on pain and function in patients with knee OA. The evaluation of serum biomarkers showed a significant increase of CTX-II only, perhaps due to an increase of cartilage turnover induced by thermal stress.

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KEY WORDS: balneotherapy, mud-bath therapy, osteoarthritis (OA), serum biomarkers, C-terminal cross-linked telopeptide type II collagen (CTX-II)

Although various systematic reviews, meta-analyses and clinical trials on balneotherapy for rheumatic diseases have been published, its role in modern medicine is still a subject of debate [2]. Furthermore, the mechanism by which mud-packs and/or balneotherapy improves the symptoms of rheumatic diseases is still not fully understood [3]. Numerous studies have identified potential biochemical markers in serum, urine or synovial fluid samples as a means to evaluate local inflammation and cartilage degradation in OA [4].

Articular cartilage is primarily composed of type II collagen and proteoglycans such as aggrecan. Cartilage oligomeric matrix protein (COMP) is an important component of hyaline cartilage; serum level of COMP is a potential indicator of radiological progression in knee OA [5]. Fragments of C-terminal cross-linked telopeptide type II collagen (CTX-II), a breakdown product of collagen type II, are detectable in urine, serum and synovial fluid and have been shown to be predictive of joint degeneration in patients wth OA [6].

Myeloperoxidase (MPO) is the main protein present in primary granules of circulating polymorphonuclear cells and the major enzymatic source of leukocyte-generated oxidants, released by activated neutrophils. MPO is used as a marker of leukocyte recruitment and function and subsequent inflammation [7]. Moreover, serum MPO concentration was found to be elevated in patients with knee and hip OA [7]. C-reactive protein (CRP) is a marker of low grade systemic inflammation; high serum levels of high sensitivity C-reactive protein (hsCRP) are present in early OA and are associated with reduced cartilage volume [8]. The hsCRP level was also reported to be a predictor of knee pain [9].

The objective of this study was to assess whether a cycle of mud-bath therapy influences the serum levels of COMP, CTX-II, MPO and hsCRP in patients with knee OA.

PATIENTS AND METHODS

This was part of a prospective randomized single-blind controlled trial (RCT) aimed at evaluating the efficacy of mud-

Balneotherapy is one of the most commonly used non-pharmacological approaches for patients suffering from diseases of the musculoskeletal system, such as osteoarthritis (OA) [1].

bath therapy in patients with knee OA [10]. The study protocol was approved by the Ethics Committee of Siena University Hospital and was registered on http://www.clinicaltrials.gov (NCT01538043). For more details about the methodology of the trial, see Fioravanti et al. [10].

The study population included 103 outpatients of both sexes with primary bilateral knee OA who fulfilled the American College of Rheumatology (ACR) criteria [11]. The patients were screened at the Rheumatology Unit of Siena Hospital to verify the inclusion and exclusion criteria. The physician who performed the screening visit (G.M.G.) did not participate in the randomization process. We selected all patients with pain in both knees during the previous 3 months or longer and rated ≥ 30 mm on a visual analogue scale (VAS, 0–100 mm) and with the Kellgren-Lawrence radiological score of I–III [12]. The exclusion criteria were severe co-morbidity contraindicating mud-bath therapy, a body mass index (BMI) ≥ 32 kg/m^2, other rheumatic diseases with palpable fluid in the knee or any previous knee injury, pregnancy or breastfeeding. Patients who had received intra-articular corticosteroids during the past 3 months, treated with symptomatic slow-acting drugs for OA (SYSADOA) or received intra-articular hyaluronic acid less than 3 months before the study were excluded. Also excluded were patients who had had a spa treatment in the 3 months previous to enrolment.

At the screening visit, blood samples were processed for erythrocyte sedimentation rate (ESR), electrolytes, creatinine, aspartate and alanine aminotransferases, and complete blood count analysis. Urinalysis was also performed to check the absence of exclusion criteria. All patients provided an informed consent form.

TREATMENT

Patients fulfilling the inclusion criteria were randomized 1:1 in two groups: the mud-bath therapy group (n=53) was treated with a combination of daily local mud-packs and baths in the water of the Chianciano Spa Resort (Siena, Italy), in addition to the usual treatment. The control group (n=50) continued their regular care routine alone (exercise, symptomatic drugs, SYSADOA, intra-articular hyaluronic acid). Both groups of patients were advised to continue their established pharmacologic and non-pharmacologic treatments, but were allowed to change them if needed. Washout of symptomatic drugs was required for an entire week before randomization and 24 hours before every assessment.

OUTCOME PARAMETERS

Clinical assessments of the patients were performed 7 days before enrolment (screening visit), at the time of enrolment (basetime) and after 2 weeks. Clinical examination included measurement of global pain score and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) sub-scores for knee OA. Global pain experienced during the last 24 hours prior to assessment was assessed on a 0–100 mm VAS (0 = no pain, 100 = the worst pain possible) [13]. The WOMAC subscores were used to characterize knee joint pain (W-TPS), joint stiffness (W-TSS), and physical function (W-TPFS). For WOMAC, we used a 5-point Likert scale; the Likert scale may be more favorable than the VAS version.

Blood samples (6 ml) were collected from an antecubital vein with the patient in the supine position in the morning after an overnight fast. The blood was immediately centrifuged and the serum was stored at -80°C until analyzed.

COMP levels were measured in serum using the Quantikine human COMP ELISA kit as described by the manufacturer (R&D Systems Inc., Minneapolis, MN, USA). The reported mean intra- and inter-assay coefficients of variation of the assay were 3.8% and 4.8%, respectively, and the minimum detectable dose was reported to be 0.010 ng/ml.

Serum CTX-II levels were detected by enzyme-linked immunosorbent assay (ELISA) as described in the data sheet (Uscn Life Science Inc., Wuhan, Hubei, China). The detection range was 123.5–10,000 pg/ml and sensitivity 44.5 pg/ml.

To measure the level of MPO in the serum, we used a Human MPO ELISA (BioVendor GmbH Heidelberg, Germany) and followed the manufacturer's instructions. The assay was performed with a sensitivity of 0.4 ng/ml.

Human hsCRP in the serum was obtained by using CircuLex high sensitivity CRP ELISA (CycLex Co. Ltd., Nagano, Japan) according to the user's manual. The measurement ranged from 59.4 to 3800 μg/ml and the limit of detection was 28.6 pg/ml of samples.

STATISTICAL ANALYSIS

The Shapiro-Wilk test was used to test the data distribution: for the four markers a parametric (normal) distribution was observed. Comparisons between each parameter for the mud-bath therapy group and the control group were done using Student's t-test for independent samples (data are expressed as mean ± standard deviation). A correlation study on the effect of clinical parameters on the four markers was performed using the non-parametric Spearman's correlation coefficient (Rho). Holm's correction was used to avoid high Type I error due to multiple comparisons.

In order to evaluate the effect of mud-bath therapy on the variation in COMP, CTX-II, MPO and hsCRP values, two-way ANOVA with repeated measures were calculated. Each subject was considered as the reference unit, Time as the within-subunit repeated measure, while Group was treated as the between-individuals factor. The Time:Group interaction was also tested (in order to infer if different treatments cause a significant change in the specific marker over time). All the statistical analyses were performed using the R statistical software [14].

Table 1. Baseline and final results of clinical parameters

Parameters	Control group (n=50)		Mud-bath group (n=53)	
	Baseline	15 days	Baseline	15 days
VAS for pain (0–100 mm)	57.1 ± 25.32	50.9 ± 22.70***	51.54 ± 21.58	31.66 ± 20,63ᵒᵒᵒ
W-TPS (0–20)	7.04 ± 4.4	5.82 ± 3.56***	5.41 ± 2.75**	2.66 ± 1.95ᵒᵒᵒ
W-TSS (0–8)	3.00 ± 2.07	2.42 ± 1.72***	2.47 ± 1.45	0.94 ± 1.05 ᵒᵒᵒ
W-TPFS (0–68)	23.60 ± 14.54	21.36 ± 13.49***	18.73 ± 10.07	8.79 ± 6.89ᵒᵒᵒ

Values are expressed as mean ± SD
Significance within groups ᵒᵒᵒ $P < 0.001$
Significance between groups ** $P < 0.01$, *** $P < 0.001$
VAS = visual analogue scale, W-TPS = WOMAC total pain score, W-TSS = WOMAC total stiffness score, W-TPFS = WOMAC total physical function score

Table 2. Results of the two-way ANOVA (Time = within factor; Group = between factor)

COMP (ng/ml)					CTX-II (ng/ml)				
Factor	SS	Df	F test	P value	Factor	SS	Df	F test	P value
Group	10825	1	1.3296	0.2502	Group	0.812	1	1.5668	0.212
Time	5306	1	0.6517	0.4204	Time	2.136	1	4.1205	0.043*
Group:Time	49	1	0.006	0.9381	Group:Time	4.974	1	9.5967	0.002**
Residuals	1,644,597	202			Residuals	1,644,597	202		

MPO (ng/ml)					hsCRP (mg/L)				
Factor	SS	Df	F test	P value	Factor	SS	Df	F test	P value
Group	1539	1	0.7574	0.3852	Group	43	1	1.4058	0.2371
Time	2057	1	1.0122	0.3156	Time	0.7	1	0.0215	0.8835
Group:Time	2677	1	1.3169	0.2525	Group:Time	0.9	1	0.0281	0.867
Residuals	410,579	202			Residuals	6183.4	202		

COMP = cartilage oligomeric matrix protein, CTX-II = fragments of C-terminal cross-linked telopeptide type II collagen, MPO = myeloperoxidase, hsCRP = high sensitivity C-reactive protein

RESULTS

At baseline, the mean age of patients in the therapy group was 68.49 ± 9.01 years and 69.66 ± 11.1 years in the control group. Twenty-three patients (43%) were male and 30 (57%) were female in the mud-bath group, while in the control group 6 (12%) were male and 44 (88%) were female. Duration of disease (years) was 7.10 ± 5.90 and 7.01 ± 6.53 in the mud-bath and control groups, respectively. Thirteen patients (24%) were graded as Kellgren-Lawrence score I, 23 (44%) as score II, and 17 (32%) as score III in the mud-bath group; 10 patients (20%) were graded as Kellgren-Lawrence score I, 13 (26%) as score II, and 27 (54%) as score III in the control group.

Comparison of the two groups showed no statistically significant differences in demographic and clinical characteristics except for gender ($P < 0.001$). At baseline we observed some co-morbidities. In the mud-bath group, 8 (15.09%) presented arterial hypertension, 4 (7.5%) had non-insulin-dependent diabetes, 2 (3.77%) had gastric ulcer, and 2 (3.77%) had prostatic hypertrophy. In the control group, 7 patients (14%) presented arterial hypertension, 4 (8%) had type 2 diabetes and 3 (6%) had hypothyroidism. Routine laboratory tests did not show any significant abnormalities in the two groups.

At the end of the treatment (2 weeks), a statistically significant reduction ($P < 0.001$) in spontaneous pain and in all WOMAC subscores was observed in the mud-bath group. In the control group, there were no significant differences after 2 weeks. The differences between the two groups were significant at day 15 ($P < 0.001$) [Table 1].

After mud-bath therapy, mean serum levels of COMP, MPO and hsCRP did not show any significant modification in comparison to baseline values, for both the mud-bath group and the control group [Table 2 and Figure 1A, C, D]. Conversely, a significant increase ($P < 0.001$) in CTX-II serum levels was observed in the mud-bath group after treatment but not in the control group (significant interaction $P = 0.002$ in ANOVA [Table 2 and Figure 1B].

Furthermore, we tested the relationships between demographic and clinical parameters and serum levels of the four investigated markers, measured in the mud-bath group at baseline and at the end of the study. As seen in Table 3, COMP resulted in a positive correlation with age of the patient ($P = 0.002$ before therapy, and $P = 0.01$ after therapy); CTX-II showed a significant ($P = 0.03$) negative relationship with the W-TPFS (only after therapy); hsCRP was positively correlated (baseline $P = 0.006$, and $P = 0.014$ after therapy) with BMI, with no effect of the mud-bath therapy on the correlation values. Finally, MPO was negatively correlated with the duration of disease (baseline $P = 0.04$, and $P = 0.03$ after therapy), and after the therapy was provided resulted in a negative correlation with age ($P = 0.063$) and radiological score ($P = 0.043$).

DISCUSSION

Most recent data indicate that some markers could be of value to predict OA progression and assess therapeutic response, although they still have limitations. Indeed, none of them can be considered a valid tool for the diagnosis and prognosis of OA in routine clinical practice. As recently reviewed, developing specific and sensitive biochemical markers for use as surrogate end-points is challenging [4].

Balneotherapy is one of the most common non-pharmacological approaches for OA in many European countries and Israel. It uses thermal mineral water from natural springs and peloids (muds) for medical treatment [1]. The mechanism by which mud-packs and/or balneotherapy improves the symptoms of rheumatic diseases is still not fully understood [3].

Recent OARSI guidelines for the non-surgical management of knee OA [15] suggested balneotherapy as appropriate non-pharmacological intervention in individuals with multiple-joint OA and relevant co-morbidities. In this study

Figure 1. Values of serum levels of **[A]** COMP, **[B]** CTX-II, **[C]** MPO, and **[D]** hsCRP in the control group and the mud-bath treatment group at basetime and at the end of the study (15 days). Data are expressed as mean ± SD. Significance within group ^{ooo} P ‹ 0.001, significance between groups $^{**}P$ ‹ 0.01
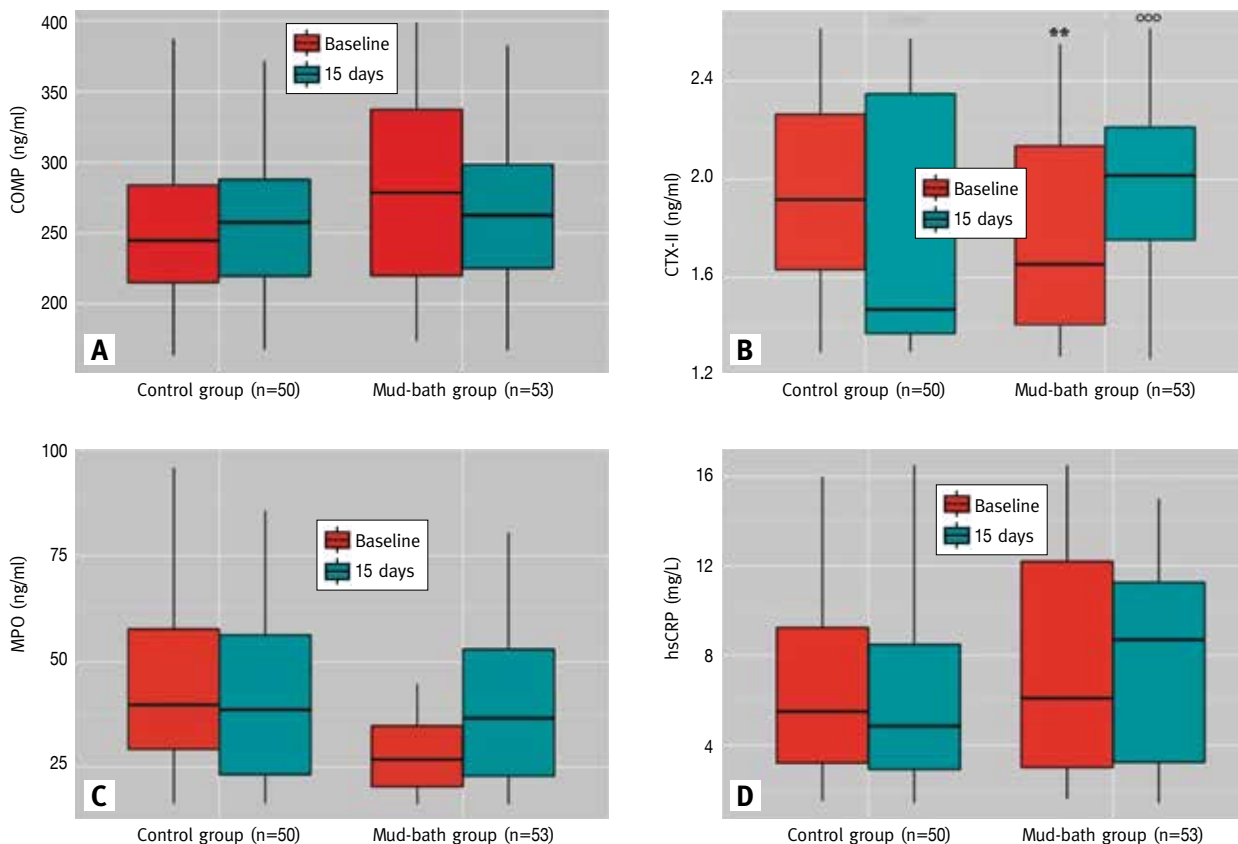
Table 3. Correlations of COMP, CTX-II, MPO and hs-CRP markers with clinical, functional and radiological parameters, measured in the mud-bath group at baseline and at study end (15 days)

Marker	Time	Age (years)	BMI (kg/m²)	Disease duration (years)	RX score	VAS for pain (0–100 mm)	W-TPS (0–20)	W-TSS (0–8)	W-TPFS (0–68)
COMP (ng/ml)	Baseline	0.292*	-0.019	0.198	0.026	0.028	0.001	0.016	-0.068
	15 days	0.244*	0.115	0.015	0.052	-0.029	-0.111	-0.064	-0.091
CTX-II (ng/ml)	Baseline	-0.150	-0.160	0.053	0.017	0.161	0.145	0.076	0.187
	15 days	-0.009	0.119	-0.094	0.018	-0.151	-0.165	-0.154	-0.202*
MPO (ng/ml)	Baseline	0.018	0.064	-0.200*	-0.136	-0.029	-0.054	-0.041	-0.003
	15 days	-0.267*	0.122	-0.213*	-0.200*	-0.018	-0.085	-0.116	-0.045
hsCRP (mg/L)	Baseline	-0.082	0.354**	0.064	0.069	0.100	0.070	-0.001	0.077
	15 days	-0.009	0.335**	0.153	0.050	0.168	0.175	0.114	0.190

Displayed values represent Spearman's Rho correlation coefficients. Holm's method was applied to calculate adjusted P values.
*P ‹ 0.05
**P ‹ 0.01

COMP = cartilage oligomeric matrix protein, CTX-II = fragments of C-terminal cross-linked telopeptide type II collagen, MPO = myeloperoxidase, hsCRP = high sensitivity C-reactive protein, BMI = body mass index, VAS = visual analogue scale, W-TPS = WOMAC total pain score, W-TSS = WOMAC total stiffness score, W-TPFS = WOMAC total physical function score

we evaluated whether a cycle of mud-bath therapy has an effect on serum levels of COMP, CTX-II, MPO and hsCRP in patients with knee OA. At the end of mud-bath therapy, neither group exhibited any significant modification of COMP, MPO and hsCRP levels in comparison to baseline values. Our data on hsCRP are in agreement with those of Gungen et al. [16] who did not observe any significant change in hsCRP levels in 44 patients with knee OA during the follow-up period (treatment). Conversely, Oláh et al. [17] highlighted a decrease in hsCRP levels in 22 obese and 20 hypertensive patients who underwent balneotherapy with mineral thermal water at 38°C temperature, in 15 sessions of 30 minutes, compared to matched controls. The same authors, in 2009 [18], showed how CRP levels followed a decreasing tendency, which still persisted 3 months after balneotherapy in 42 patients with musculoskeletal degenerative diseases.

Increased synovial infiltration is reported to correlate with elevated hsCRP levels in OA patients. None of the patients in our study had active joint inflammation either at the beginning of the treatment or during follow-up. This might explain our observation.

As for MPO and COMP levels, our results did not differ from those reported by Ceccarelli et al. [19], who did not demonstrate any change in 53 patients with symptomatic knee OA after mud-pack applications for 12 consecutive days. Conversely, other studies demonstrated different results: Bellometti et al. [20] showed a significant decrease of MPO levels in 37 OA patients after mud-bath therapy, and Benedetti et al. [21] pointed out a similar significant decrease of COMP values in 30 OA patients compared to baseline values.

In our results, the only significant data concerned CTX-II levels, which increased slightly after mud-bath therapy. Despite the limitation of not having tested the urinary CTX-II, our data could reflect thermal stress induced by mud-bath treatment, resulting in an increased turnover of cartilage. Similarly, Duclos et al. [22], in an animal model of OA, highlighted an early rise in serum CTX-II after surgical OA induction, suggesting that stressing factors (i.e., mud-baths or surgery) could influence CTX-II values. Conversely, in the most recent published paper, Gungen et al. [23] did not show any difference in urinary CTX-II between patients with knee OA treated, or not, with mud compress therapy. Nevertheless, our study and other currently available data do not allow for identification of any specific mechanism of action able to modify CTX-II serum levels after mud-bath therapy.

The tested correlations between demographic and clinical parameters and the four investigated serum markers underline a positive correlation between hsCRP and BMI, according to the current trend of considering overweight and obesity a low grade inflammatory disease [24].

COMP correlated positively with age, both at baseline and after mud-bath therapy. When considering age as a strong risk factor for OA progression and COMP as a marker of cartilage degradation, the positive correlation is clear [5].

The pathologic changes seen in OA joints include not only the degradation of articular cartilage, but also subchondral bone, edema and synovial inflammation. In particular, synovitis plays an important role in OA pathogenesis and may be a precursor rather than simply a consequence of joint failure [25]. In our study we observed a negative correlation between MPO and age, radiological score and disease duration; this could reflect the trend of OA which presents more inflammatory features in the early than the late stages, when the mechanical/degenerative aspects become prevalent [7].

The present study has some limitations which warrant mention. First, we did not include a control group of healthy subjects. Second, we did not evaluate the possible long-term effects of mud-bath therapy on serum concentrations of these markers. The third limitation is represented by the fact that we did not test urinary CTX-II.

CONCLUSIONS

A cycle of mud-bath therapy added to the regular treatment has a beneficial effect on pain and function in patients with knee OA. However, the evaluation of biomarkers showed only a significant increase of CTX-II, perhaps due to an increase in cartilage turnover induced by thermal stress. Further studies on a larger number of patients and a longer follow-up would provide verification of these findings and enhance our understanding of the potential of biomarkers as outcome measures following spa treatment.

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