Rheumatol Int (2012) 32:1235–1244
DOI 10.1007/s00296-010-1727-4

ORIGINAL ARTICLE

The effect of mud pack therapy on serum YKL-40 and hsCRP levels in patients with knee osteoarthritis

Gonca Güngen · Fusun Ardic · Gülin Fındıkoğlu · Simin Rota

Received: 23 June 2010 / Accepted: 30 December 2010 / Published online: 22 January 2011

Abstract The aim of this study was to evaluate the efficacy of treatment with mud pack in knee osteoarthritis (OA) and to determine whether mud pack effects serum levels of YKL-40 and high-sensitivity C-reactive protein (hsCRP) which are reported to be biological markers for articular damage or inflammation in patients with OA. Forty-four patients with the diagnosis of knee OA assigned into two groups were treated with local natural mineral-rich mud pack or hot pack. Treatments were applied for 6 days a week for 2 weeks as a total of 12 sessions. Patients were assessed at baseline, post-treatment, and 3 months after the treatment. VAS, range of motion, 15-m walking time, WOMAC index, Nottingham Health Profile, serum YKL-40, and hsCRP levels were the outcome measures. Pain intensity and joint stiffness decreased in both groups at all follow-ups. Physical activity status was found to persist for 3 months after treatment only in mud pack group. Serum mean YKL-40 and hsCRP levels of the patients were higher compared to healthy control group. Serum YKL-40 level increased significantly only in hot pack group 3 months after the treatment ($P < 0.017$). No significant change was observed in hsCRP levels in both groups during the whole follow-up periods ($P > 0.05$). Mud pack and hot pack therapy were both demonstrated to be effective in symptomatic treatment of knee OA until the end of the 2-week treatment period, whereas only mud pack therapy was shown to be effective in functional status over time. In the hot pack group, increased serum YKL-40 level 3 months after the treatment might indicate persistence of cartilage degradation. Maintenance of YKL-40 level in mud pack therapy seems to slow down the progression of knee OA.

Keywords Mud pack · Knee osteoarthritis · YKL-40 · hsCRP

Introduction

Osteoarthritis (OA) is a chronic disease characterized by progressive destruction of articular cartilage and subchondral bone and synovial reaction. Abnormal and degraded cartilage, inflamed and/or thickened synovial tissue, and altered bone structure being the major presentation of OA results in pain, mobility impairment, and disability [1].

Biochemical markers of arthritis are molecules detectable in synovial fluid (SF), serum, or urine that may reflect the underlying degenerative or inflammatory joint disease [2]. Such biochemical markers might be useful for the early identification of patients with OA or patients at high risk for progression and for assessing therapeutic response in OA, because of their greater sensitivity compared to radiographs [3, 4].

YKL-40 of which name comes from the one-letter code for its first three N-terminal amino acids and from its molecular weight of 40 kDa is also called human cartilage glycoprotein-39 (HC gp-39) or chondrex [5, 6]. While the function of YKL-40 is not clearly identified, some propose that YKL-40 functions as a glycosidic bond hydrolase involved in the tissue-remodeling process [6, 7]. YKL-40 in SF of an arthritic joint may originate from articular

The preliminary report of this paper was presented in the 5th World Congress of the International Society of Physical and Rehabilitation Medicine (ISPRM), June 13–17, 2009, Istanbul.

G. Güngen · F. Ardic (✉) · G. Fındıkoğlu · S. Rota
Pamukkale University, Denizli, Turkey
e-mail: fardic@pau.edu.tr

cartilage (chondrocytes), synovial membrane (synovial cells, macrophages), or polymorphonuclear leukocytes [6, 8]. Increased serum and SF YKL-40 levels are found in patients with active rheumatoid arthritis (RA) or severe knee OA compared to normal subjects [7, 9–11]. In OA, YKL-40 expression is almost proportional to the severity of the cartilage fibrillation [8]. Connor et al. [12] reported enhanced expression of YKL-40 in osteoarthritic cartilage and osteophytes which may reflect increased osteogenesis as well as cartilage degeneration. Therefore, YKL-40 may be a useful marker for joint damage, joint inflammation, and disease activity in patients with OA and RA [7, 9–11, 13].

Multiple studies have demonstrated that hsCRP is modestly elevated in the plasma of patients with OA compared to age-matched controls although hsCRP is a non-specific marker of inflammation and has a limited use as a diagnostic marker of OA [2, 14]. It is suggested that both hip and knee OA are associated with elevated hsCRP level and hsCRP level increases for each level of Kellgren–Lawrence scoring system [14–16]. In addition, a relationship among elevated plasma levels of hsCRP, elevated SF levels of IL-6 and the presence of chronic synovial inflammation in patients with idiopathic OA has been demonstrated [17]. Higher CRP level is also found to be associated with both prevalent and incident OA of knee joint [15].

The optimal management of knee OA requires a combination of pharmacological treatment and non-pharmacological modalities [18]. Balneotherapy is one of the recommended non-pharmacological interventions for its efficacy in OA by the European League Against Rheumatism (EULAR) [19]. Mud is a therapeutic substance, which consists of various amounts of organic and inorganic materials and provides heat transfer by conduction. The beneficial effects of mud in pain relief and functional status improvement has been reported in patients with OA [20, 21], RA [22, 23], psoriatic arthritis [24], and fibromyalgia syndrome [25]. The beneficial effects of mud pack are often attributed to its thermal effect; however, a specific mode of action due to its influence on bio-humeral mediators of the inflammatory response may also be considered. To our knowledge, this is the first study evaluating serum YKL-40 and hsCRP levels in monitoring the efficacy of mud pack therapy so far.

Denizli where the study was carried on is one of the thermal spa centers in Turkey. The aim of this study is to determine the effect of mud pack treatment obtained from Sarayköy-Denizli on serum levels of YKL-40 and hsCRP, biological markers for local inflammation and cartilage degradation in patients with knee OA with respect to the improvement in the functional status.

Materials and methods

Patients

Fifty patients attending to Physical Medicine and Rehabilitation Clinic of Pamukkale University between January and June 2008 ages between 45 and 75 years (18 men and 32 women) presenting knee pain and diagnosed as bilateral knee OA according to American College of Rheumatology Classification Criteria and with a radiographic evidence of OA (Kellgren Lawrence score III or IV) were enrolled for this study. Four and two patients dropped from the hot pack–treated and mud pack–treated groups, respectively (Fig. 1). The study was completed with 44 patients (21 and 23 patients in hot pack–treated and mud pack–treated groups, respectively).

Patients with a previous history of operation for OA or with intra-articular injection or secondary inflammatory symptoms or having physical therapy/balneotherapy in the last 6 months were not included to the study. Additionally, patients with a dermatological lesion at the site of mud pack application, diabetes mellitus, coronary artery disease, liver dysfunction/cirrhosis, thyroid dysfunction, hypercholesterolemia, cancer, peripheral vascular disease, infection, cooperation difficulty, or on anti-inflammatory drugs were not considered for this study. All women patients were post-menopausal, and none of the patients were receiving treatment that might interfere with bone metabolism.

Serum analysis results for YKL-40 and hsCRP of 26 healthy people ages between 44 and 65 years (6 men and 20 women) who were eligible in terms of inclusion and exclusion criteria and who were previously enrolled for a thesis study accepted as the control group for comparison of serum biochemical analysis [26]. These participants were also not given any medical and/or physical therapy.

The research protocol was approved by the Ethics committee of Pamukkale University. Each participant was informed about the study verbally and signed informed consent.

Treatment protocol

All participants were screened by regular physical and musculoskeletal examination as well as bilateral anterio-posterior radiographs of knee, biochemical, and hematological analyses including blood count, erythrocyte sedimentation rate, rheumatoid factor, liver function tests, glucose, and lipid profile.

The study was planned as a prospective randomized controlled clinical study. All knee OA patients were assigned into groups using random number table. Both modalities were applied on right and left knees for 20 min, 6 days a week for 2 weeks, as a total of 12 sessions. All

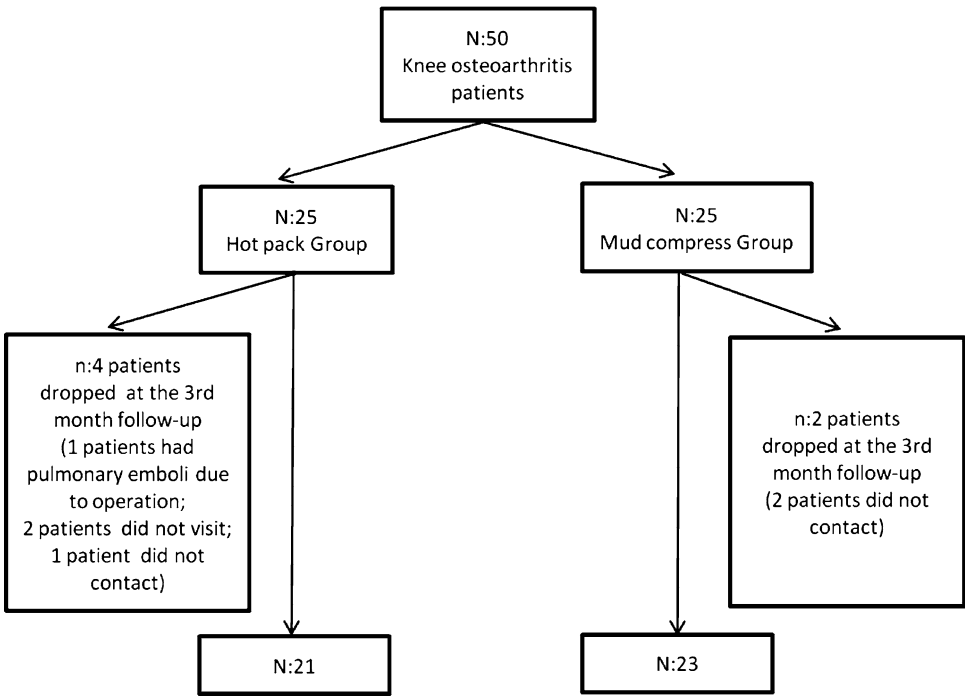

Fig. 1 Flow diagram for the number of patients dropped out of our study

patients were informed to take paracetamol in case of intensive pain and were given a timetable to follow their paracetamol consumption. All patients were evaluated at baseline (pre-treatment), at the end of treatment (post-treatment), and 3 months after the treatment.

Mud pack application

Mud pack treatment was applied to group 1 patients ($n = 23$) at Umut Thermal & Spa Resort, Sarayköy-Denizli, Turkey. It has been classified as turf based on its physical, chemical, and geological properties. It was rich in organic substances; its lignin and humin content was 80.99 g/l. Heat retention and water retention capacities (84%) were also high (Table 1). Approximately 4-cm-thick mud pack at 45°C was laid on the skin from 10 cm distal to the inferior border of patella to 10 cm proximal to the superior border of patella on both knees by pleiodo therapists.

Hot pack application

Hot pack was applied to group 2 patients ($n = 21$) in Physical Medicine and Rehabilitation Clinic at the Pamukkale University in Denizli city center by the GG on both knees. Hydrocollator hot packs (Chattonoga®) with dimentions of 25 × 50 cm at 42–45°C were used.

Biochemical analysis

Venous blood samples were drawn from the patients 12 h after fasting in the morning hours between 8:30 and 10:30. Samples were collected in serum separator tubes, allowed to clot for 20 min, centrifuged at room temperature for 15 min at 3,000 *g*. The samples were stored at −20°C until analyzed.

Serum YKL-40 levels were measured by commercial "METRA YKL-40 EIA® (Quidel Corporation,CA, USA)" kit, a sandwich enzyme immunoassay in a microtiter stripwell format, according to the manifacturer's instructions. The Fab fragment of a monoclonal anti-YKL-40 conjugated to biotin binds to streptavidin and captures YKL-40 in the sample. A polyclonal anti-YKL-40 antibody conjugated to alkaline phosphatase binds to the captured YKL-40. Bound enzyme activity is detected with p-nitrophenly phosphate as substrate.

Serum hsCRP levels were measured by commercial "High sensitivity C-reactive Protein Enzyme Immunoassay® (BioMERICA Inc. CA, USA)" kit, a solid-phase enzyme immunoassay in a microtiter stripwell format according to the manufacturer's instructions. In the monoclonal anti-CRP antibody–coated wells, the second anti-CRP antibody in the enzyme conjugate binds to the CRP in serum. Bound enzyme activity is detected with tetramethylbenzidine as substrate.

Outcome measures

Pain Patients were questioned about the intensity of pain at rest, at night, and during activity by visual analogue scale (0–10).

Range of motion Degrees of flexion and extension of knee were measured with a goniometer by same person,

Table 1 Physical and chemical properties of mud used for therapy

Physical properties	Findings
Color	Dark gray
Odor	Smells like petroleum
Thickness	Fine
Homogeneity	Fine
Sediments	None
Coarse particles	None
Other	Plant root segments in small amounts
Ph	6.76
Water retention capacity (at 105°C)	83.60
Chemical properties	**Results of analysis**
Water content (%) (at 105°C)	51.51
Mineral content (%) (dry weight at 800°C)	70.47
Humic acid (g/l)	9.98
Butiminous substances(g/l)	3.22
Hemicellulose, cellulose(g/l)	38.83
Lignin, humin (g/l)	80.99
Pectin and carbohydrates (g/l)	9.49
Hydrogen sulphate (g/l)	0.28
Iodine(g/l)	2.766
Total of inorganic substances (g/l)	593.55
Total of organic substances (g/l)	142.51

GG. While the patient is in supine position, the hip joint was stabilized at the neutral position, and the arms of the goniometer were placed parallel to the long axis of femur and tibia.

15-m Walking time Patients were asked to walk for three times on a flat smooth surface by the same commands. Time to walk 15 m was measured by the same chronometer. Mean of three trials was calculated and used for the study.

Western Ontario and McMaster Universities multifunctional (WOMAC) index, A disease-specific index, was used to assess three dimensions; pain, stiffness, and physical functional disability using a 5-point Likert scale (0 = no pain, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe pain) as a grading system [27]. A total score was calculated by summation of WOMAC total pain score (W-TPS), WOMAC total stiffness score (W-TSS), and WOMAC total physical function score (W-TPFS). Reliability and validity of WOMAC in Turkish were shown by Tüzün et al. [28].

Nottingham Health Profile (NHP), assessing perceived physical, social, and emotional health with 38 items, was answered as "yes" or "no" [29]. NHP includes physical mobility, pain, emotional reaction, energy level, sleep, and social isolation evaluation. Pain score (NHP-PS), physical activity score (NHP-PAS), tiredness score (NHP-TS), sleep score (NHP-SS), emotional reaction score (NHP-ERS), and social isolation score (NHP-SIS) were calculated by weighted score of the related question. The overall score was obtained from sum of the scores in each six subscales. The adaptation study of the Turkish version was also done, and the questionnaire was reported to be valid and reliable [30].

Statistical analysis

As the data did not show normal distribution characteristics, Mann–Whitney *U* test was used to find the effectiveness of therapy compared to control group. Friedman test was used to determine the differences before and after treatment in each therapy group. P values < 0.05 were accepted as significant. Comparison of groups (baseline and follow-up measurements) was performed by using Wilcoxon test with Bonferroni correction for each group, $P < 0.017$ was considered as significant. Spearman's correlation test was used for correlation. SPSS 10.0 program was used for statistical calculations.

Results

Mean age of patients was 61.87 ± 6.73 years (50–74 years) in the hot pack group and 65.04 ± 7.11 years (54–74 years) in the mud pack group. Twelve (%57) of patients were female, and 9 (%42) were male in the hot pack group; 13 (%56) of patients were female and 10 (%43) were male in the mud pack group. The mean body mass indices were 27.60 ± 2.42 and 27.95 ± 2.83 in the hot pack and mud pack groups, respectively. Ten (%47.8) of patients were graded as Kellgren Lawrence (KL) stage III and 11 (%52.2) of patients as stage IV in hot pack group. There were 12 (%52.1) KL stage III patients and 11(%47.8) stage IV patients in mud pack group (Table 2). Both groups did not show any difference in terms of any of the measures considered for this study (YKL-40, hsCRP, pain, range of motion for knee joint, 15-m walking time, WOMAC, Nottingham Health Profile) in addition to age and body mass index at baseline ($P > 0.05$) (Tables 3, 4).

A significant difference in serum YKL-40 (131.35 ± 90.91 vs. 114.16 ± 43.73 ng/ml) and hsCRP (4.71 ± 3.65 vs. 1.85 ± 1.57 mg/l) values was found between patients with OA in both treatment groups compared to the values of the age-matched healthy control (Table 5) ($P < 0.05$). Serum YKL-40 level was not found to be correlated with

age, sex, and BMI ($P > 0.05$). Pre-treatment serum hsCRP level correlated with BMI (r: 0.345, $P < 0.05$) and pain at night (r: 0.298, $P < 0.05$) but not with age ($P > 0.05$).

Table 2 Demographic characteristics of patients

	Hot pack group	Mud pack group
No of patients	21	23
Age, years (mean ± SD)	61.87 ± 6.73	65.04 ± 7.11
Gender, F/M	12/9	13/10
BMI (kg/m^2) (mean ± SD)	27.60 ± 2.42	27.95 ± 2.83
Radiographic OA severity (Kellgren–Lawrence Score)		
III	10 (47.8%)	12 (52.1%)
IV	11 (52.2%)	11 (47.8%)

The results and statistical comparisons for biochemical and functional parameters of the pre-treatment, post-treatment, and 3 months after the treatment in hot pack and mud pack groups were shown in Table 3.

Although serum YKL-40 did not differ between the two therapy groups at all follow-ups ($P > 0.05$), in the hot pack group 3 months after the treatment compared to pre-treatment values, a significant increase was demonstrated ($P < 0.017$). On the other hand, the YKL-40 values of mud pack group did not differ in the follow-up periods with respect to pre-treatment values.

No significant changes were seen in hsCRP levels in both groups during the whole follow-up period ($P > 0.05$).

Pain intensity values during activity, at rest, and at night measured by VAS were significantly lower at the end of the treatment and 3 months after the treatment compared to

Table 3 Comparison of biochemical and functional parameters within each group and between hot pack and mud pack groups

Measure	Hot pack group ($n = 21$) (mean ± SD)	Mud pack group ($n = 23$) (mean ± SD)	Mann–Whitney U test (P)
YL-40 (ng/ml)			
Pre-treatment	130.69 ± 92.93	132 ± 87.1	0.934
Post-treatment	128.74 ± 70.46	154.4 ± 102.9	0.482
3 months after treatment	189.95 ± 110.9*	161.1 ± 82.3	0.528
hsCRP (mg/l)			
Pre-treatment	5.4 ± 4	3.8 ± 3.4	0.252
Post-treatment	5.9 ± 3.6	3.7 ± 3.4	0.065
3 months after treatment	5 ± 3.5	3.6 ± 3.4	0.182
VAS in activity			
Pre-treatment	6.3 ± 1.9	6 ± 2.1	0.673
Post-treatment	4.6 ± 2.4**	3.8 ± 2.4**	0.273
3 months after treatment	5.2 ± 2.4*	4.5 ± 2.8**	0.387
VAS at rest			
Pre-treatment	4 ± 2.3	3.7 ± 1.7	0.673
Post-treatment	2.8 ± 1.9**	2.3 ± 1.9**	0.339
3 months after treatment	2.9 ± 2.5*	2.6 ± 2.2**	0.625
VAS at night			
Pre-treatment	4.5 ± 2.7	3.9 ± 2.4	0.255
Post-treatment	3.2 ± 2.7**	2.1 ± 2**	0.357
3 months after treatment	3.5 ± 2.7*	2.7 ± 2.9**	0.180
15-m Walking time			
Pre-treatment	15.4 ± 3.3	15.4 ± 2.8	0.884
Post-treatment	14.2 ± 2.7*	13.7 ± 2.2**	0.497
3 months after treatment	14.2 ± 3.2	14 ± 2.6**	0.991
Degree of knee flexion			
Pre-treatment	135.4 ± 11.6	132.2 ± 7.8	0.529
Post-treatment	136 ± 10.8	132.2 ± 7.8	0.532
3 months after treatment	135.4 ± 11.4	132.3 ± 7.9	0.526
Degree of knee extension			
Pre-treatment	4 ± 4.2	3.5 ± 4.0	0.730
Post-treatment	3.6 ± 3.8	3.5 ± 4.0	0.992
3 months after treatment	3.8 ± 4.2	3.7 ± 4.0	0.135

Comparison of evaluations with respect to pre-treatment

* $P < 0.017$

** $P < 0.001$ (Wilcoxon rank sum test with Bonferroni correction)

Comparison of evaluations between hot pack and mud pack groups

#$P < 0.05$ (Mann–Whitney U test)

Table 4 Comparison of quality of life parameters within each group and between hot pack and mud pack groups

Measure	Hot pack group ($n = 21$) (mean ± SD)	Mud pack group ($n = 21$) (mean ± SD)	Mann–Whitney U test (P)
WOMAC TPS			
Pre-treatment	15.9 ± 4.3	14.5 ± 3.9	0.284
Post-treatment	12.6 ± 3.4**	10.9 ± 3.9**	0.136
3 months after treatment	13.6 ± 3.7**	12.3 ± 4.5**	0.215
WOMAC TSS			
Pre-treatment	6.6 ± 1.8	6 ± 2.3	0.293
Post-treatment	5.2 ± 1.8**	4.1 ± 1.8**	**0.048**$^{\#}$
3 months after treatment	5.5 ± 1.9**	4.2 ± 2.1**	**0.024**$^{\#}$
WOMAC TPFS			
Pre-treatment	54.4 ± 13.1	47.1 ± 14.7	0.107
Post-treatment	49.1 ± 13.8**	41.4 ± 14.3**	**0.04**$^{\#}$
3 months after treatment	52.5 ± 14.6	42.3 ± 14.2*	**0.049**$^{\#}$
NHP-PS			
Pre-treatment	78.5 ± 70.2	75.4 ± 26.3	0.897
Post-treatment	54 ± 25.7**	43.5 ± 33.7**	0.197
3 months after treatment	67.3 ± 28.8*	51.9 ± 33**	0.132
NHP-PAS			
Pre-treatment	45.3 ± 19.3	43.5 ± 17.8	0.827
Post-treatment	38.8 ± 19.4*	36 ± 19.5*	0.92
3 months after treatment	39 ± 18.9	36.5 ± 18.6*	0.743
NHP-SS			
Pre-treatment	33.6 ± 24.3	45.6 ± 29.2	0.108
Post-treatment	24.8 ± 18.5	24.8 ± 25.3**	0.562
3 months after treatment	31.4 ± 20.6	33.04 ± 27.4*	0.901
NHP-SIS			
Pre-treatment	16.8 ± 30	25.6 ± 26.8	0.11
Post-treatment	11.2 ± 24.5	7.2 ± 16.2*	0.688
3 months after treatment	14.3 ± 30.4	18.3 ± 20.8	0.118
NHP-ERS			
Pre-treatment	39.3 ± 35	44 ± 38.1	0.617
Post-treatment	28.8 ± 31.6**	17.3 ± 24**	0.311
3 months after treatment	34.4 ± 35.8	34.3 ± 35.4*	0.867
NHP-ELS			
Pre-treatment	64 ± 31.8	60 ± 39.7	0.617
Post-treatment	56 ± 34.3	43.5 ± 37.2*	0.311
3 months after treatment	58.7 ± 33.2	50.7 ± 37.4*	0.867

Statistically significant values are given in bold

WOMAC Western Ontario and McMaster Universities multifunctional index, *W-TPS* WOMAC total pain score, *W-TSS* WOMAC total stiffness score, *W-TPFS* WOMAC total physical function score, *NHP* Nottingham Health Profile, *NHP-PS* pain score, *NHP-PAS* physical activity score, *NHP-SS* sleep score, *NHP-ERS* emotional reaction score, *NHP-SIS* social isolation score, *NHP-ELS* energy level score

Comparison of evaluations with respect to pre-treatment. * $P < 0.017$; ** $P < 0.001$ (Wilcoxon rank sum test with Bonferroni correction)

Comparison of evaluations between hot pack and mud pack groups. # $P < 0.05$ (Mann–Whitney$U$ test)

pre-treatment values in both hot pack and mud pack groups ($P < 0.017$) and were not different among both groups ($P > 0.05$).

Although 15-m walking time did not differ between the two groups, it was significantly shortened after the treatment compared to pre-treatment scores in both hot pack and mud pack groups. This difference persisted only in the mud pack group 3 months after the treatment.

Degree of knee flexion and extension did not change significantly in both groups compared to pre-treatment levels ($P > 0.05$).

The results and statistical comparisons of the pre-treatment, post-treatment, and 3 months after the treatment evaluations for quality of life parameters in hot pack and mud pack groups were shown in Table 4. Significantly lower scores were attained in WOMAC TSS and TPFS in mud pack group compared to hot pack group. WOMAC TPS, TSS, and TPFS decreased significantly at the end and 3 months after the treatment compared to pre-treatment values in mud pack group. Except for lack of the persistence of beneficial effects in WOMAC TPFS 3 months after treatment, hot pack–treated group demonstrated

Table 5 Comparison of serum YKL-40 and hsCRP values between patients with knee OA in both treatment groups and healthy control group

	Hot pack and mud pack groups ($n = 50$)	Control group ($n = 20$)	P
YKL-40 ng/ml	131.35 ± 90.91	114.16 ± 43.73	0.045*
hsCRP mg/l	4.71 ± 3.65	1.85 ± 1.57	0.028*

Mann–Whitney U test was used for comparison

* $P < 0.05$

significant changes in other dimensions of WOMAC in all evaluations.

All dimensions of NPH improved 2 weeks after the treatment, and this improvement persisted for 3 months after the treatment except for the NHP-SIS in mud pack group. In the hot pack group, while NHP-PS persisted to be significant for 3 months after the treatment, NHP-PAS and NHP-ERS improved significantly only till the end of treatment but no change was detected in NHP-SS, NHP-SIS, NHP-ELS. All subscales of NHP were not significantly different in both groups.

Discussion

The present randomized, controlled clinical study indicates that pain intensity and joint stiffness decreased in both groups at all follow-ups. Physical activity status was found to persist for 3 months after treatment only in mud pack group contrary to hot pack group in which this change continued until the end of the treatment.

Previous studies have shown that mud pack treatment alone or in combination with other balneologic treatments is effective in OA [21, 24, 31, 32]. The beneficial effect of mud pack treatment has been ascribed mainly to heat especially for a relatively prolonged time [33]. Pain, and muscle spasm diminish due to the increase in the extensibility of collagen-rich tissues with thermal stimulation [34]. It was hypothesized that human skin can release opioid peptides that may affect the threshold of pain under different stimuli, such as heat or UV radiation [35]. These mechanisms that relate to the high temperature of the mud therapy could explain the short-term clinical efficacy. Because superficial heat was given in hot pack and mud pack applications; heating might be responsible for the analgesic effect which was demonstrated by VAS and pain scales of both WOMAC and NHP for both groups in our study.

Only mud pack therapy was able to influence NHP-ELS scores and NHP-SS scores at all follow-ups compared to pre-treatment. In the mud pack group, as the patients were transferred to spa center all together, NHP-SIS was significantly lower during the therapy, and on the other hand, this parameter did not change for hot pack group for whom the application was made individually. Degree of knee flexion and extension did not change in both groups compared to pre-treatment levels. This may be due to the fact that patients did not do regular therapeutic exercise.

While a significant improvement was found in physical activity status determined by 15-m walking time, WOMAC TPFS, NHP-PAS, and NHP-ERS of the hot pack group 2 weeks after the treatment compared to pre-treatment levels; improvement was found to persist only in mud pack group as long as 3 months after the treatment. The results of our trial confirm those reported previously in the literature for knee OA [20, 31]. Effects of mud pack on the functional status over time might be related to its chemical effect, a distinct property from hot pack.

Chemical effects of mud pack can be explained by organic substances or minerals that are absorbed through the skin. Mud used in our study which is similar to the one used in Odabaşı and colleagues' study was rich in organic substances with high lignin and humin content [31]. Fulmic, ulmic, and humic acids have definite effects on spontaneous contractile activity of smooth muscle tissue acting on the α2 adreno and D2 dopamine receptors [36, 37]. An anti-inflammatory compound of mud, sulphoglycolipid, produced by colonized microorganisms during maturation process, might contribute to the therapeutic activity [33]. It was assumed that galic, vanilic, and procatechic acid derivatives may have a role in chemical effects of mud. Human skin possesses a selective permeability for organic acid derivatives. However, it is not clear which elements or organic substrates are essential and what is the ideal concentration of these elements in order to attain an optimal response to treatment [38].

It was demonstrated that mudpack treatment in OA could reduce serum IL-1 and prostaglandin E2 (PGE2) and leukotriene (LTB4) levels which are potent inflammatory compounds [39, 40]. Bellometti et al. reported that mud pack treatment decreases serum levels of matrix metalloproteinase-3 and tumor necrosis factor α (TNF-α) receptors consequently reducing cartilage inflammation and damage [41, 42]. Insulin-like growth factor-1, known to have cartilage protective properties, was found to be elevated after mud pack therapy in patients with OA [43, 44]. Mud pack treatment may have effects on cartilage homeostasis and inflammatory reactions, influencing NO and decreasing serum myeloperoxidase levels [45].

It was previously reported that direct mud pack application is superior to nylon covered mud pack application in

knee OA patients [38]. This finding underlines the contribution of chemical properties of the mud pack treatment. Similarly in our study, patients in both hot pack and mud pack groups were exposed to thermal effects but only patients with mud pack treatment maintained low levels of YKL-40 which might be related with the anti-inflammatory properties of mud.

Increased serum and SF YKL-40 levels are found in patients with knee OA compared to normal subjects [7, 9, 11]. Higher CRP level is also found to be associated with both prevalent and incident OA of knee joint [15]. In our study, serum mean YKL-40 and hsCRP levels of the patients were higher compared to healthy control group. Although serum levels of YKL-40 did not differ between the therapy groups, it was increased significantly only in hot pack group 3 months after the treatment. In our study, although serum YKL-40 level was not found to be correlated with hsCRP level, in a report, it was suggested YKL-40 may be a marker for joint inflammation in OA because of correlation between YKL-40 and CRP levels [10]. The production of CRP by hepatocytes, the main source of this acute-phase reactant [9], appears to be regulated primarily by the proinflammatory cytokines interleukin-6 (IL-6) [46] and interleukin-1 (IL-1) [17]. In OA, increased hsCRP levels have been demonstrated to be associated with disease progression as well as with clinical severity [14]. Higher levels of IL-1, IL-6, and TNF-α in the SF of persons with knee OA have been reported to be related with joint destruction [47–49].

In our study, pain at night was found to be correlated with hsCRP levels before treatment. Increased synovial infiltration is reported to correlate with elevated hsCRP levels in OA patients. None of the patients included to this study had active joint inflammation neither at the beginning of the treatment nor during follow-ups. No significant change was seen in hsCRP levels in both groups during the whole follow-up period. This might be the explanation of why temporary elevation was not observed in hsCRP level.

BMI that was found correlated with hsCRP level in our study might be responsible for the persistence of elevated levels [50]. As a limitation of our study, hsCRP level was not corrected for BMI or regression analysis was not applied.

YKL-40-positive cells were found in synovial membrane lining and stromal cells (macrophages) of OA patients. A correlation among the number of YKL-40-positive cells and the severity of the synovitis was reported [9, 13]. This finding was also confirmed by the correlation of SF YKL-40 level with the synovial membrane cells and the joint effusion volume determined by magnetic resonance imaging [9]. It was shown that whereas there was no YKL-40 mRNA expression in normal human cartilage chondrocytes of the hip joint, a high number of YKL-40-positive chondrocytes were detected in articular cartilage from the hip joint of patients with OA [8]. YKL-40-positive chondrocytes were noticed to locate particularly in the superficial and middle layer of the cartilage and especially in areas of the joint with a considerable biomechanical load [8]. Johansen et al. [5] suggested that YKL-40 in SF and serum might reflect human articular cartilage degradation and synovial inflammation in severe knee OA and RA. Connor et al. [12] reported that YKL-40 is expressed in osteoarthritic human cartilage and osteophytes.

SF YKL-40 level was found to be higher than serum YKL-40, and a relationship exists among the both levels [5, 9], suggesting that in patients with both OA and RA most of the serum YKL-40 originate from the joint tissues [5]. High serum YKL-40 levels in OA patients in our study were considered to reflect degenerative changes in the knee joint cartilage.

Serum level of YKL-40 was found to reflect local articular changes in response to medical treatment. For instance, intra-articular glucocorticoid injection results with a decrease in clinical signs of synovitis and a decrease in serum YKL-40 [9]. Decreased YKL-40 values indicated the clinical improvement in patients treated with disease-modifying antirheumatic drug therapy [5]. Similarly, serum YKL-40 level was not elevated in knee OA patients treated with mud pack 3 months after therapy in our study which might indicate that mud pack retarded the cartilage degeneration or synovial inflammation in the joint.

Functionally improved status together with low levels of YKL-40 for the long term might indicate beneficial effects of mud pack distinct from hot pack treatment in patients with knee OA.

Mud pack and hot pack therapy were both demonstrated to be effective in symptomatic treatment of knee OA until the end of the treatment period. Whereas only mud pack therapy was shown to be effective in functional status over time. This is the first study using YKL-40 and hsCRP in monitoring efficacy of mud pack therapy. Increased serum levels of YKL-40 3 months after the treatment might indicate persistence of cartilage degradation in the hot pack group. Maintenance of YKL-40 level in mud pack therapy seems to slow down the progression of knee OA in long-term follow-up.

As a conclusion, our study demonstrated the superiority of mud pack therapy compared to hot pack therapy in the treatment of knee OA, probably due to the specific effects of the minerals found in this mud. Treatment with mud pack may relieve pain and improve the function; moreover, it may decrease the ongoing cartilage loss in patients with knee OA as evidenced by YKL-40 levels.

Acknowledgments We would like to thank Umut Thermal & Spa Resort for their contribution for sponsoring mud and transportation.

"Pamukkale University Scientific Research Projects Management Unit" provided funding for the study (Project number: 2002MHF001).

References

1. Garnero P, Delmas PD (2003) Biomarkers in osteoarthritis. Curr Opin Rheumatol 15:641–646
2. Abe M, Takahashi M, Naitou K, Ohmura K, Nagano A (2003) Investigation of generalized osteoarthritis by combining X-ray grading of the knee, spine and hand using biochemical markers for arthritis in patients with knee osteoarthritis. Clin Rheumatol 22:425–431
3. Garnero P, Ayral X, Rousseau JC, Christgau S, Sandell LJ, Dougados M, Delmas PD (2002) Uncoupling of type II collagen synthesis and degradation predicts progression of joint damage in patients with knee osteoarthritis. Arthritis Rheum 46:2613–2624
4. Vignon E, Garnero P, Delmas P et al (2001) Recommendations for the registration of drugs used in the treatment of osteoarthritis: an update on biochemical markers. Osteoarthr Cartil 4:289–293
5. Harvey S, Weisman M, O'Dell J, Scott T, Krusemeier M, Visor J, Swindlehurst C (1998) Chondrex: new marker of joint disease. Clin Chem 44:509–516
6. Hakala BE, White C, Recklies AD (1993) Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. J Biol Chem 268:25803–25810
7. Johansen JS, Hvolris J, Hansen M, Backer V, Lorenzen I, Price PA (1996) Serum YKL-40 levels on healthy children and adults. Comparison with serum and synovial fluid levels of YKL-40 in patients with osteoarthritis or trauma of the knee joint. Br J Rheumatol 35:553–559
8. Volck B, Ostergaard K, Jhansen JS, Garbarsch C, Price PA (1999) The distribution of YKL-40 in osteoarthritic and normal human cartilage. Scand J Rheumatol 28:171–179
9. Volck B, Johansen JS, Stoltenberg M et al (2001) Studies on YKL-40 in knee joints of patients with rheumatoid arthritis and osteoarthritis. Involvement of YKL-40 in the joint pathology. Osteoarthr Cartil 9:203–214
10. Conrozier T, Carlier MC, Mathieu P, Colson F, Debard AL, Richard S, Favret H, Bienvenu J, Vignon E (2000) Serum levels of YKL-40 and C reactive protein in patients with hip osteoarthritis and healthy subjects: a cross sectional study. Ann Rheum Dis 59:828–831
11. Garnero P, Piperno M, Gineyts E, Christgau S, Delmas PD, Vignon E (2001) Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage. Ann Rheum Dis 60:619–626
12. Connor JR, Dodds RA, Emery JG, Kirkpatrick RB, Rosenberg M, Gowen M (2000) Human cartliage glycoprotein 39 (HC-gp 39) mRNA expression in adult and fetal chondrocytes, osteoblasts and osteocytes by in situ hybridization. Osteoarthr Cartil 8:87–95
13. Kawasaki M, Hasegawa Y, Kondo S, Iwata H (2001) Concentration and localization of YKL-40 in hip joint diseases. J Rheumatol 28:341–345
14. Wolfe F (1997) The C-reactive protein but not erythrocyte sedimentation rate is associated with clinical severity in patients with osteoarthritis of the knee or hip. J Rheumatol 8:1486–1488
15. Sowers M, Jannausch M, Stein E, Jamadar D, Hochberg M, Lachance L (2002) C-reactive protein as a biomarker of emergent osteoarthritis. Osteoarthr Cartil 10:595–601
16. Sturmer T, Brenner H, Koenig W, Gunther KP (2004) Severity and extent of osteoarthritis and low grade systemic inflammation as assessed by high sensitivity C reactive protein. Ann Rheum Dis 63:200–205
17. Pearle AD, Scanzello CR, George S, Mandl LA, DiCarlo EF, Peterson M, Sculco TP, Crow MK (2007) Elevated high-sensitivity C-reactive protein levels are associated with local inflammatory findings in patients with osteoarthritis. Osteoarthr Cartil 15:516–523
18. Lange U, Müller-Lander U, Schmidt KL (2006) Balneotheraphy in rheumatic diseases: an overview of novel and known aspect. Rheumatol Int 26:497–499
19. Zhang W, Doherty M, Arden N et al (2005) EULAR evidence based recommendations for the management of hip osteoarthritis: report of a task force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). Ann Rheum Dis 64:669–681
20. Flusser D, Abu-Shakra M, Friger M, Codish S, Sukenik S (2002) Therapy with mud compresses for knee osteoarthritis: comparison of natural mud preparations with mineral-depleted mud. J Clin Rheumatol 8:197–203
21. Evcik D, Kavuncu V, Yeter A, Yigit I (2007) The efficacy of balneotherapy and mud-pack therapy in patients with knee osteoarthritis. Joint Bone Spine 74:60–65
22. Codish S, Abu-Shakra M, Flusser D, Friger M, Sukenik S (2005) Mud compress therapy for the hands of patients with rheumatoid arthritis. Rheumatol Int 25:49–54
23. Sukenik S, Buskila D, Neumann L, Kleiner-Baumgarten A, Zimlichman S, Horowitz J (1990) Sulphur bath and mud pack treatment for rheumatoid arthritis at the Dead Sea area. Ann Rheum Dis 49:99–102
24. Elkayam O, Ophir J, Brener S, Paran D, Wigler I, Efron D, Even-Paz Z, Politi Y, Yaron M (2000) Immediate and delayed effects of treatment at the Dead Sea in patients with psoriatic arthritis. Rheumatol Int 19:77–82
25. Fioravanti A, Perpignano G, Tirri G, Cardinale G, Gianniti C, Lanza CE, Loi A, Tirri E, Sfriso P, Cozzi F (2007) Effects of mud-bath treatment on fibromyalgia patients: a randomized clinical trial. Rheumatol Int 27:1157–1161
26. Sert F (2008) Osteoartrit hastalarında YKL-40 ve yüksek-duyarlıklı CRP (hsCRP) düzeylerinin değerlendirilmesi. Dissertation, University of Pamukkale
27. Bellamy N, Buchanan WW, Goldsmith CH, Campbell SLW (1988) Validation of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol 15:1833–1840
28. Tüzün EH, Eker L, Aytar A (2005) Acceptability, reliability, validity and responsiveness of the Turkish version of WOMAC osteoarthritis index. Osteoarthr Cartil 13:28–33
29. Martini CJM, Waugh IM (1978) A method for self-assessment of disability before and after hip replacement operation. BMJ 2:258–259
30. Kucukdeveci AA, McKenna SP, Kutlay S, Gursel Y, Whalley D, Arasil T (2000) The development and psychometric assessment of the Turkish version of the Nottingham health profile. Int J Rehabil Res 23(1):31–38
31. Odabaşı E, Turan M, Erdem H, Pay S, Güleç M, Karagülle MZ (2009) The effect of Mud pack treatment in knee osteoarthritis. Turk J Rheumatol 24:72–76
32. Fioravanti A, Iacoponi F, Bellisai B, Cantarini L, Galeazzi M (2010) Short- and long-term effects of spa therapy in knee osteoarthritis. Am J Phys Med Rehabil 89(2):125–132
33. Tolomio C, Ceschi-Berrini C, Moschin E, Galzigna L (1999) Colonization by diatoms and antirheumatic activity of thermal mud. Cell Biochem Funct 17:169–175
34. Sulenik S, Abu-Shakra M, Flusser D (1997) Balneotheraphy in autoimmun disease. Isr J Med Sci 33:258–261

35. Ghersetich I, Freedman D, Lotti T (2000) Balneology today. J Eur Acad Dermatol Venereol 14:346–348
36. Beer AM, Junginger HE, Lukanov J, Sagorchev P (2003) Evaluation of the permeation of peat substances through human skin in vitro. Int J Pharm 253:169–175
37. Beer AM, Lukanov J, Sagorchev P (2000) Mechanism of the effect of aqueous peat extract on spontaneous contractile activity of smooth muscles. Forsch Komplementärmed Klass Naturheilkd 7:237–241
38. Odabasi E, Turan M, Erdem H, Tekbas F (2008) Does mud pack treatment have any effect? A randomized controlled clinical study. J Altern Complement Med 14:559–565
39. Cechettin M, Bellometti S, Lalli A, Galzigna L (1995) Serum interleukin-1 changes in arthrosis patients after mud-pack treatment. Phys Rehab Kur Med 5:92–93
40. Bellometti S, Galzigna L (1998) Serum levels of a prostaglandin and a leukotriene after thermal mud pack therapy. J Investig Med 46:140–145
41. Bellometti S, Richelmi P, Tassoni T et al (2005) Production of matrix metalloproteinases and their inhibitors in osteoarthritic patients undergoing mud-bath therapy. Int J Clin Pharmacol Res 25:77–94
42. Bellometti S, Galzigna L, Richelmi P et al (2002) Both serum receptors of tumor necrosis factor are influenced by mud-pack treatment in osteoarthritic patients. Int J Tissue React 24:57–64
43. Bellometti S, Giannini S, Sartori L, Crepaldi G (1997) Cytokine levels in osteoarthrosis patients undergoing mud bath therapy. Int J Clin Pharmacol Res 17:149–153
44. Bellometti S, Cecchettin M, Galzigna L (1997) Mud pack therapy in osteoarthrosis: changes in serum levels of chondrocyte markers. Clin Chim Acta 268:101–106
45. Bellometti S, Poletto M, Gregotti C, Richelmi P, Berte F (2000) Mud bath therapy influences nitric oxide, myeloperoxidase and glutathione peroxidase serum levels in arthritic patients. Int J Clin Pharmacol Res 20(3–4):69–80
46. Swaak AJ, van Rooyen A, Nieuwenhuis E, Aarden LA (1988) Interleukin-6 (IL-6) in synovial fluid and serum of patients with rheumatic diseases. Scand J Rheumatol 17:469–474
47. Abramson SB, Attur M, Amin AR, Clancy R (2001) Nitric oxide and inflammatory mediators in the perpetuation of osteoarthritis. Curr Rheumatol Rep 3:535–541
48. Westacott CI, Whicher JT, Barnes IC, Thompson D, Swan AJ, Dieppe PA (1990) Synovial fluid concentration of five different cytokines in rheumatic diseases. Ann Rheum Dis 49:676–681
49. Guerne PA, Zuraw BL, Vaughan JH, Carson DA, Lotz M (1989) Synovium as a source of interleukin 6 in vitro. Contribution to local and systemic manifestations of arthritis. J Clin Invest 83:585–592
50. Ridker PM (2001) High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation 103:1813–1818