ADIPONECTIN CORRELATION WITH BIOCLINICAL BENEFITS OF USING NATURAL THERAPEUTIC FACTORS IN KNEE OSTEOARTHRITIS

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Abstract

Context and objective. The new insights in the pathogenesis of osteoarthritis (OA) reveal the implications of adipocytokines. This study aims to analyze the correlations between the serum value of adiponectin and the clinical rehabilitation effects in patients diagnosed with knee OA, admitted and treated in the complex balneal resort of Techirghiol lake.

Subjects and methods. The prospective randomized clinical study included 23 patients in the study group, diagnosed with knee OA according to ACR criteria, and a matching control group of 23 subjects. Serum level of adiponectin (using ELISA technique), uric acid, triglycerides, cholesterol, HDL-cholesterol and clinical response using a visual analog scale (VAS) were evaluated in all patients on their admission day and after 10 days of balneal treatment. Control group benefited from the same procedures except for cold mud therapy and mineral water baths.

Results. Plasma adiponectin levels (23.73 ± 6.44 ng/dL) were statistically higher ($p < 0.05$) in the study group compared to the control group (18.15 ± 6.49 ng/dL). The mean VAS in both groups was decreased ($p < 0.005$) compared to the initial moment.

Conclusions. Cold peloidotherapy combined with physical therapy and balneal factors induces serum adiponectin elevation and improves knee pain in OA. Therapeutic properties of Techirghiol mud still need further research.

Key words: adiponectin, osteoarthritis, mud, knee, VAS, Techirghiol.

INTRODUCTION

In the past years, in medical literature, emphasis has been placed on implication of adipocytokines in inflammatory processes of the osteoarticular system as well as in osteoporosis (1). Both white and brown adipose tissue is producing interleukin (IL)-1 β ,

tumor necrosis factor (TNF)- α , IL-6, IL-8, IL-17, basic fibroblast growth factor, vascular endothelial growth factor (VEGF), leptin, resistin, adiponectin and probably many others.

Infrapatellar fat pad (IPFP), fat tissue situated intracapsular and extrasynovial in the knee joints, rich in vessels, nerve fibers, immune cells, produces all the above mentioned adipocytokines which can play a role in the initiation and progression of knee osteoarthritis (2, 3).

OA is a multifactorial degenerative joint disease characterised by damaged articular cartilage, modified subchondral bone architecture, formation of bone exostosis and inflammation of the synovia.

Even if OA is the most common so-called “non inflammatory” rheumatic disease, according to new research, inflammation plays an important role in its etiology, along with obesity, age, previous injuries etc. (4, 5).

In the last years, balneological research was directed towards the study of specific inflammatory cytokines involvement in musculo-skeletal disorders. Correlations between peloid therapy and inflammatory pathways, at molecular level, are established and new insights in the world of chondrocytes and osteoblasts are brought to light (6).

In general, adiponectin is considered a benefic adipokine because of its systemic anti-inflammatory effect based on reduction of T cell activation, of the seric levels of IL 6 and TNF- α , of its antiatherosclerotic properties etc. (7).

Medical data is contradictory regarding the role of adiponectin in osteoarticular disorders. There is proof that adiponectin decreases both in the serum and in synovial fluid along with the aggravation of OA (8) and also with the radiologic deterioration of hand OA. (9). Contrary to all that, there are other opinions

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that adiponectin is involved in the pathogenesis of OA with a proinflammatory effect at chondrocytes level and also in the degeneration of interstitial cartilaginous matrix (10-12). In chondrocytes, *in vitro*, this adipokine induces pro-inflammatory mediators such as nitric oxide, IL-6 and matrix metalloproteinase with catabolic effects (13, 14). Furthermore, one study revealed a higher level of adiponectin in the IPFP of the patients with knee OA, especially in advanced stages, than in the subcutaneous tissue (15).

Mud, a balneotherapeutic agent used for centuries, preserves its mysteries regarding its implications in the pathophysiology of osteoarticular system. The complex biochemical composition of Techirghiol peloid, with its rich microbial flora, represents a valuable therapeutic option in musculoskeletal disorders.

In an already published paper we obtained proof of the influence of warm mud applications over the serum levels of adiponectin in patients with knee OA (16).

This study aims to evaluate the serum level of adiponectin and the rehabilitation benefits using a visual analogous scale for pain (VAS) under the effect of natural therapeutic factors after 10 procedures of cold mud applications (CMA) in knee OA. We also aim to find out if there are any correlations between adiponectin and uric acid, considered a pro-inflammatory articular factor, and if balnear treatment affects adiponectin's cardiovascular protective role, regarding lipid profile.

PATIENTS, MATERIAL AND METHOD

In our prospective randomized clinical study, we included 2 groups: the study group consisting in 23 patients, between 30 and 70 years old, diagnosed with knee OA, according to ACR criteria (17) and a matching control group of 23 patients. The patients were hospitalized in the Balnear and Rehabilitation Sanatorium of Techirghiol between 01.06.2016-01.09.2016.

All subjects signed a special informed consent with the agreement to participate in this study and approval of the sanatorium ethical committee was obtained.

Patients from the study group (cold mud applications group-CMAG) performed 10 complex physical and balnear therapy procedures, in Techirghiol specific area, consisting in hydrotherapy (general cold mud baths and salt bath in the lake Techirghiol),

heliotherapy, electrotherapy (current of low, medium, high frequency, laser therapy, ultrasonotherapy, phototherapy), massage therapy, kinesiology; while the patients in control group (CG) performed the same therapy except for specific hydrotherapy (mud baths or salt baths in Techirghiol lake).

Serum adiponectin, uric acid, cholesterol, HDL-cholesterol, triglycerides and clinical response after natural treatment with sapropelic mud were evaluated in all patients as follows. All patients were evaluated on their admission day and after 10 days of balnear treatment. Blood samples were collected at admission, before treatment and at discharge, both determinations respecting an overnight 12 h digestive rest. Serum adiponectin was determined at admission and after 10 days of treatment. Specific reagents were used for the determination of human adiponectin using ELISA technique produced by "DRG Instruments GmbH", Germany, with normal reference values of adiponectin according to BMI in Table 1 (however, the producer considered that every laboratory should establish their own values).

Table 1. Adiponectin reference values according to BMI

Gender	BMI (kg/m ²)	Mean (µg/mL)	SD (µg/mL)
Men	< 25	15.9	4.0
	25-30	13.8	4.0
	> 30	13.3	2.8
	Total	14.5	3.9
Women	< 25	18.6	5.4
	25-30	18.9	8.6
	> 30	16.4	3.8
	Total	18.2	6.1

In parallel, the groups were analyzed in terms of age, gender, average age of the disease, body mass index and severity of pain in the knee joints assessed by visual analogue scale (VAS).

Exclusion criteria were: patients with erythrocyte sedimentation rate (ESR) > 40 mm/h; patients with skin disorders (chronic or acute), including skin lesions; with serious conditions such as: cardiovascular, respiratory, digestive, renal, neurological; cancer; presence of connective tissue disorders, pregnancy. Previous treatments were restricted as follows: Nonsteroidal Antiinflammatory Drugs (NSAIDs) and steroids at least 4 weeks before study entry; surgery (arthroplasty, arthroscopy, abrasion, etc.) performed at least 3 months before the study; administration of corticosteroid intra/periarticular at least 6 weeks before starting the trial or hyaluronic acid with at least 6 months before the study; treatment with opioids for at least 4 weeks before study

entry; physiotherapy, acupuncture, transcutaneous electrical nerve stimulation (TENS), massage and physical therapy at least 4 weeks before study entry; administration of sedatives, hypnotics, anticonvulsant and muscle relaxant at least 2 weeks before study entry.

Statistical interpretation of the results was performed by processing data using IBM SPSS 18.0. The results were analysed with mathematical methods in order to obtain an average of the values, standard deviation and also the standard error of the media (descriptive statistics in order to characterise discrete and defined continuous variables); parametric statistical tests (t-Test to compare media of two independent samples and t-Test to compare media of paired samples).

Normal distribution was confirmed for all studied variables by Shapiro-Wilk test ($p > \alpha = 0.05$). By assessing Skewness and Kurtosis coefficients resulted that distributions are symmetrical and they do not present an abnormal vaulting.

Correlations were considered according to the values of the r correlation coefficient (adapted after D.E. Hinkle, W. Wiersma si G.S. Jurs, 1988, p.118).

The values of adiponectin, VAS, cholesterol, HDL-cholesterol, triglycerides, uric acid measured in the first day of treatment were registered as “Adiponectin 1”, “VAS 1” etc. At the end of treatment, after 10 days of balneotherapy, the values were registered as “Adiponectin 10”, “VAS 10” etc.

Statistical measurements were presented in Table 1.

RESULTS

Presentation of the study groups

The two groups contained 23 patients each and were considered statistically similar in terms of demographic features and of associated comorbidities, such as metabolic syndrome and diabetes which might have had an impact over adiponectin values (18).

- sex (60.9% females in CG, 65.2% females in CMAG, $p > 0.05$),

- age (medium value in CG=56.17±12.2 years vs. 55.17±8.28 years in CMAG, $p = 0.747 > 0.05$),

- medium value of BMI (29±4.64 in CG vs. 27.69±4.33 in CMAG; $p = 0.328 > 0.05$),

- medium value of VAS scale at admission (5.43±2.52 vs. 5.45±1.87, $p = 0.974 > 0.05$),

- values of adiponectin 1 (17.90 ±8.04 ng/dL in CG vs. 20.24±9.90 ng/dL in CMAG, $p = 0.198 > 0.05$). Adiponectin values were correlated

with BMI in both groups ($p < 0.001$, $r = -0.690$, negative correlation in CG and $p = 0.002$, $r = -0.609$, negative correlation on CMAG),

- values of glycemia in day 1 (CG: medium value 111.60 ±19.21 mg/dL vs. 118.52±34.20 mg/dL, $p = 0.40 > 0.05$,

- proportion of diabetic patients (considered both from anamnesis and after a cut-off value of glycemia of 126 mg/dL, twice registered) was 21.7% in CG vs. 26.1% in CMAG, ($\lambda^2 = 0.00$, $p > 0.05$),

- length of disease (in CG: 8.7% of the patients had a duration of 1-6 months, 17.4% had a duration of 6-12 months, 43.5% had a duration of 1-5 years, 13% had a duration of 5-10 years and 17.4% of more than 10 years, in the CMAG the percentages are: 4.3%, 13%, 52.2%, 17.4% and 13 %. All proportions compared revealed a $p > 0.05$),

- regarding the presence of metabolic syndrome the control group was statistically similar with the study group ($\lambda^2 = 0.392$; $p > 0.05$),

- both cholesterol and HDL –cholesterol and uric acid values did not differ significantly between groups.

We divided time of illness in the following time intervals: 1 – 6 months, 6 months – 1 year, 1 to 5 years, 5 to 10 years and more than 10 years. Occurrence of pain was considered the time of OA onset (the main feature of knee OA). We found that the patients suffering from pain for 1 up to 5 years were in a bigger number than in the other time intervals.

In CMAG, adiponectin 10 (23.73±6.44 ng/dL) is significantly higher than in day 1 (20.24±9.90 ng/mL) ($t = -2.919$; $df = 22$; $M_{diff} = -3.482$; $p = 0.008$).

The difference between adiponectin 10 in CMAG and the one in CG was significant, also, in favor

Figure 1. Values of Adiponectin 1 and 10 in both groups.

of the CMAG ($t = -2.922$; $df = 44$; $M_{diff} = -5.573$; $p = 0.005$), see Figure 1.

We found no statistical difference between the values of adiponectin 1 and adiponectin 10 in CG (17.58 ± 7.02 ng/mL vs. 18.15 ± 6.49 ng/dL; $t = -0.461$; $df = 22$; $M_{diff} = -0.573$; $p = 0.649 > 0.05$).

Adiponectin significant negative correlation with BMI persists in the 10th day of treatment.

VAS, a tool for measuring subjective pain that cannot be measured directly, was performed both in the research project baseline, day 1, and after 10 days of treatment.

VAS 10 in CG is 3.93 ± 2.30 while VAS 10 in CMAG is 3.76 ± 2.10 .

Even if the difference between VAS 1 and VAS 10 is significant in each group ($t = 4.657$; $df = 22$; $M_{diff} = 1.5$; $p < 0.001$ in CG, $t = 6.28$; $df = 22$; $M_{diff} = 1.69$; $p < 0.001$ in CMAG), there is no difference between VAS 10 in CG and the one in CMAG ($t = -0.267$; $df = 44$; $M_{diff} = 0.17$; $p = 0.791$).

In our study, VAS 10 had a significant negative correlation with adiponectin 10 value only in the CMAG ($p = 0.036 < \alpha = 0.05$, $r = -0.44$, considered a mild correlation).

DISCUSSION

The variety of the natural therapeutic agents used in balneotherapy, the influence of seasons and of the climate - different from the one of the patients' residence place, and of the changes in diet and daily activity, make strong scientific studies difficult in the field of balneology. Because of all that, debate exists in medical literature regarding the benefits of mud therapy.

In our study most of the patients were suffering from knee pain for 1 up to 5 years. It seems that patients with symptoms for less than 1 year are underestimating the knee OA evolution and prognosis, while those with more than 5 years of suffering are somehow "adapted and consoled" with the situation.

VAS is a psychometric response scale that can be used in questionnaires. It is a tool for quantifying subjective characteristics or attitudes that cannot be measured directly. When responding to a VAS item, respondents specify their level of agreement to a statement that indicates a position along a continuous line between 2 end points (pain with intensity from 1 up to 10, for example). In our study VAS was significantly lower on the 10th day of treatment in both groups. This is, once more, the proof of balneal treatment efficiency in knee osteoarthritis.

Our study brings proof not only of mud therapy efficiency but also of its possible correlations with adiponectin.

Adiponectin, an adipocytokine discovered in 1995, produced by adipocytes synthesis in many forms: low-molecular weight trimers, medium and high-molecular weight trimers, plays an important role in metabolic regulation and in inflammatory/anti-inflammatory processes. Among these, adiponectin enhances 5' AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor (PPAR α) pathway in the striate muscle and in the liver, prevents insulin resistance by increasing fatty acids oxidation, offers cardio-vascular protection by augmenting the endothelial nitrous oxide production and reducing platelet aggregation, while its reduction seems to increase the risk of coronary heart disease, steatohepatitis, insulin resistance (19) as well as of biliary sludge and lithiasis (with similar contribution as other risk factors: pregnancy, parity, dyslipidemia etc) (20, 21).

If up to 5-6 years ago, medical data sustained the pro-inflammatory effect of adiponectin, with a deleterious effect over the cartilage (22-24) and just a few considering that adiponectin may have a protective role in the progression of OA, proving that both the serum and the synovial level of adiponectin decrease significantly with the severity of osteoarthritis (25), or that adiponectin modulates cartilage destruction in chondrocytes (up-regulating TIMP-2 and down-regulating IL-1 β - induced MMP-13) (26), new studies (27-29) are sustaining the beneficial effect of adiponectin over joint cartilage. Under the same light, the study of Zheng S *et al.* published in 2016 in Scandinavian Journal of Rheumatology sustains that serum adiponectin is significantly and negatively associated with radiologic severity of knee osteoarthritis (30).

Looking at our results, we found a statistically significant variation of adiponectin after 10 days of complex balneal therapy in the study group compared to control group. Cold mud bath induced a significant increase in serum adiponectin values both related to value of adiponectin 1 in CMAG and to the adiponectin 10 of the control group. Based on our previous study, in which we found that hot peloid bath induced significant decrease in serum levels of adiponectin value after 10 days (16), we suppose that thermoregulation interferes with therapeutic results of peloid baths, along with other similar results from medical literature (31, 32). This is why we will extend our study with repeated

dosage of serum levels of adiponectin at 3 months/ 6 months after balneal contrastant treatment, because we have a new work hypothesis.

By excluding the possibility of a pregnancy, in fertile patients, we, first, reduced the necessary investigations, some of them being rather specific for our region (33). We have, also, took care of the possible adverse effect of anticonvulsant therapy – even before the 4 weeks prior the beginning of the study (34). A possible effect of the cortisol, due to high estrogen levels (35) was also avoided.

We did not find a significant correlation between adiponectin values and the levels of uric acid, HDL-cholesterol, triglycerides in either group.

The results of this study, that adiponectin values are higher in case of cold mud applications than in the control group, are in contrast with our previous results (16), in which hot mud baths reduced the value of adiponectin. At the same time the significant negative correlation between VAS scale and adiponectin level is pleading for a protective role of adiponectin over knee osteoarthritis. So, we consider that thermoregulation is involved, and further research is needed to establish the relation between adiponectin, reduction of pain and cold peloid treatment with Techirghiol mud.

In conclusion, although the study included a small number of cases, it allowed the issuance of plausible assumptions on understanding the beneficial effects of Techirghiol peloid on osteo-articular degenerative disorders.

In our study we find a significant elevation of adiponectin level after 10 cold sapropelic mud applications in the study group as related to the control group.

Even if prevention of obesity related to pro-inflammatory cytokines' production is still relying on physical activity, reduction of fat body mass and healthy diet, new data about control of adipokines and their effect over osteoarthritis may represent the base of new pharmacological approaches in this disease.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contribution

Ionescu Elena-Valentina and Tica Irina equally contributed to this article.

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