National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Assessment of biochemical markers of inflammation in subjects with knee osteoarthritis undergoing low-level laser therapy

Arun Kumar M, Venkatesh D, Savita Ravindra

Department of Physiology, M S Ramaiah Medical College, Bengaluru, Karnataka, India

Correspondence to: Venkatesh D, E-mail: venkatesh40@gmail.com

Received: December 30, 2019; **Accepted:** January 20, 2020

ABSTRACT

Background: It is a common experience that elderly population suffer from degenerative joint disease known as osteoarthritis (OA). There are several biochemical markers that are altered in this low-grade inflammation of knee joint as in OA. Low-level laser therapy (LLLT) is known to be safe and effective methods in the management of pain and inflammation in knee OA. **Aim and Objective:** This study aims to study the serum biomarkers to assess the inflammatory status in subjects with knee OA undergoing LLLT. **Materials and Methods:** The study subjects were in the age group of 45–75 years who had clinically diagnosed knee OA. The LLLT (810 nm, 10 mW) was given to the subjects for a total duration of 3 months with a periodicity of 3 times a week. Serum osteocalcin and prostaglandin E2 (PGE2) assay were done before and after therapy as well as in controls. **Results:** Mean PGE2 in the study subjects before the intervention and after laser therapy was 271.20 ± 128.30 pg/ml and 351.21 ± 98.92 pg/ml, respectively (P = 012). Mean osteocalcin (ng/ml) in the study subjects before the intervention and controls was 9.65 ± 7.90 ng/ml and 4.87 ± 3.23 ng/ml, respectively (P = 004). **Conclusion:** It can be concluded that LLLT had anti-inflammatory effects in subjects with knee osteoarthritis (KOA) as evident from significant increase in anti-inflammatory markers such as osteocalcin and PGE2.

KEY WORDS: Osteocalcin; Prostaglandin E2; Low-level Laser Therapy; Knee Osteoarthritis

INTRODUCTION

It is a common experience that elderly population suffer from degenerative joint disease known as osteoarthritis (OA). Incidence of knee OA increases with the advancement of age and the incidence is as high as 25% of population in people over 55 years.[1] Patients with knee OA have severe pain and restricted movements. Knee OA is known to occur due to wear and tear of the cartilage. This damage leads to chronic low-grade inflammation. The prolonged and dysregulated degree of inflammation leads to tissue destruction.[2] The process of healing is not effective as the joint is susceptible to wear and tear due to continuous use.

There are several proteins that are involved in the low-grade inflammation, tissue destruction, and in the healing of the cartilage in the OA of knee joint. Accordingly, these proteins may have either pro-inflammatory or anti-inflammatory properties and often work to maintain immune homeostasis.[3] These are interleukins, tumor necrosis factor-alpha, osteocalcin, prostaglandins, and other cytokines.

Osteocalcin or bone gamma-carboxyglutamic acid protein is a protein that is produced by osteoblasts.[4] Osteocalcin has been used as a serum marker for assessing the osteoblastic bone formation, various homeostases, and role of insulin on skeletal metabolism. The osteocalcin has been described as modulator of inflammatory process in several chronic

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2020.10.12396201920012020	

inflammatory diseases. A meta-analysis has reported that serum osteocalcin and C-reactive protein (inflammatory marker) exhibit with significant inverse association in the adult population.[5]

Prostaglandin E2 (PGE2) is a principal mediator of inflammation in diseases such as rheumatoid arthritis and OA. It is involved in the resolution of inflammation and also controls the mechanisms that lead to tissue repair. PGE2 has the role in expression of specific cytokines and chemokines present on immune cells, which reduces the inflammation. PGE2 directly inhibits the synthesis of interleukin-2 and also the expression of the T-cell IL-2 receptor.[6] PGE2 plays a critical role in the process of tissue repair which is considered as the final phase of the inflammatory response.[7] PGE2 causes arterial dilatation and increases microvascular permeability.[8]

Low-level laser therapy (LLLT) is a modality of treatment used in several conditions required for suppressing the pain and inflammation, stimulation of healing resulting in restoration of function. LLLT is known to be safe, effective methods in the management of pain and inflammation in knee OA.[9,10]

In this study, changes in serum biochemical markers such as osteocalcin and PGE2 were studied to assess inflammatory status in subjects with knee OA undergoing low-level laser therapy (LLLT).

MATERIALS AND METHODS

The study was done at M S Ramaiah Medical College and Teaching Hospitals. The study subjects were in the age group of 45–75 years with clinically diagnosed knee OA. Thirty symptomatic subjects satisfying radiological criteria based on Kellgren-Lawrence (KL) Grades II and III were included in the study. Subjects with KL Grades I and IV, infective arthritis, history of vascular diseases in the lower limb, photosensitivity, and patient on steroid therapy were excluded from the study. Thirty age- and gender-matched subjects without OA were included as controls. Institutional scientific and ethics committee approval was obtained. Procedure of intervention was explained to the subjects and written informed consent was obtained.

LLLT was administered using a laser device with probe giving power output of maximum 10 mW, with a wavelength of 810 nm. LLLT was given with the laser probe over 6 points around the surface of the affected knee joint. The dosage at each point was 1.5 J for the duration of 60 s. The treatment was given to the subjects for a total duration of 3 months at a frequency of 3 times a week.

Serum samples were collected from the subjects for the biochemical assay and samples were stored at −20°C. The samples were collected before the therapy and after the completion of the therapy in subjects with knee OA and also from controls. Enzyme-linked immunosorbent assay kits were used for measuring serum osteocalcin (finetest.inc) and PGE2 (Arbor Assay).

The baseline parameters of the subjects were expressed as mean±standard deviation in the study. The serum osteocalcin and PGE2 were compared before and after the therapy using paired *t*-test. The serum osteocalcin and PGE2 were compared between the study subjects before the therapy and the controls using Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

RESULTS

The average age of the subjects was 62.64 ± 7.40 years. The average body mass index (BMI) of the subjects was 27.53 ± 4.59 BMI kg/m^2. Thirty age and anthropometrically matched normal adults served as control group.

Comparison of serum biomarkers in study subjects and controls: Mean PGE2 in the study subjects before the intervention and controls was 271.20 ± 128.30 pg/ml and 246.91 ± 142.27 pg/ ml, respectively (P = 0.499). Mean osteocalcin (ng/ml) in the study subjects before the intervention and controls is 9.65 ± 7.90 ng/ml and 4.87 ± 3.23 ng/ml, respectively. There is a statistically significant difference in the mean osteocalcin level between the study subjects and controls (P = 0.004*). The results are depicted in Table 1.

Comparison of serum biomarkers assay in the study subjects before and after the laser therapy: Mean PGE2 in the study subjects before and after the laser therapy was 271.20 ± 128.30 pg/ml and 351.21 ± 98.92 pg/ml, respectively [Figure 1]. There was a statistically significant difference in the mean PGE2 in the study subjects before and after laser therapy (P = 0.012*). Mean osteocalcin (ng/ml) in the study subjects before and after the laser therapy is 9.65 ± 7.90 ng/ml and 13.87 ± 21.85 ng/ml, respectively (P = 0.352). The results are depicted in Table 2 and Figure 2.

DISCUSSION

The study considered the serum inflammatory markers in the subjects with knee OA undergoing LLLT. Inflammatory markers considered were and serum PGE2 and serum

Table 1: Comparison of serum biomarker in the study participants before the therapy and controls

Serum biomarkers (*n*=30)	Study group	Control group	*P*-value
Prostaglandin E2 (pg/ml)	271.20±128.30	246.91±142.27	0.499
Osteocalcin (ug/ml)	9.65±7.90	4.87±3.23	0.004*

*P<0.05 is considered as statistical significance

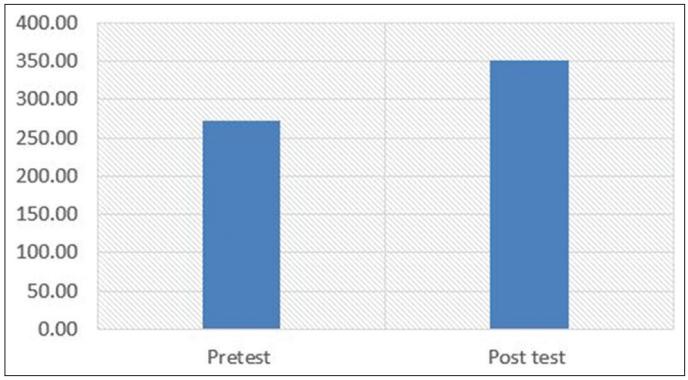

Figure 1: Comparison of prostaglandin E2 (pg/ml) before and after low-level laser therapy

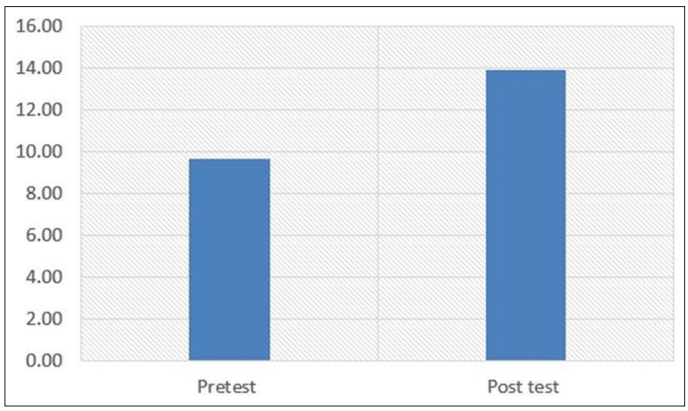

Figure 2: Comparison of osteocalcin before and after low laser therapy

Table 2: Comparison of serum biomarkers in participants before and after low-level laser therapy

Serum biomarkers	Pre-test	Post-test	*P*-value
Prostaglandin E2 (pg/inl)	271.20+128.30	351.21+98.92	0.012*
Osteocalcin (ng/ml)	9.65±7.90	13.87±21.85	0.352

*$P<0.05$ is considered as statistical significance

osteocalcin. There was a statistically significant difference in the serum PGE2 in the study subjects before and after laser therapy. The PGE2 levels were more in subjects after the LLLT in the study subjects. However, the difference in the serum levels of PGE2 was not statistically significant between the study subjects and controls. The increase in the serum levels after the therapy in the study participants signifies that there is an increase in serum PGE2 in response to LLLT.

There was an increase in the serum osteocalcin levels in subjects after they underwent LLLT. The increase in the serum osteocalcin levels was due to the response to LLLT from the osteoarthritic knee joint. There was a statistically significant difference between the serum osteocalcin levels in the study subjects and controls. This indicates that serum osteocalcin levels have decreased in the subjects with osteoarthritic knee joint and further their levels increase and/or restored following the LLLT. LLLT is known to reduce the inflammation and pain by acting at the cellular level. Low-level therapy stimulates the osteoblasts promoting the release of osteocalcin. This osteocalcin modulates in the inflammatory process in the damaged cartilage and leads to decrease in subjective pain and inflammation.

Scanzello and Loeser, 2015, described that pain and inflammation in the osteoarthritic knee have been recognized as a complex unpleasant sensory and emotional experience with actual or potential tissue damage.[11] There is a deranged inflammatory response which is subclinical and there is cartilage damage which is not able to heal completely. Hence, there is an increase in the serum biomarkers along with several symptoms in subjects with knee OA. Mahmood *et al*., 2016, reported in the study that there are altered serum markers of inflammation such as erythrocyte sedimentation rate (ESR) and total white blood cell (WBC) count along with serum boron. It was mentioned that ESR and WBC levels were high and serum boron levels were low in subjects with OA when compared to controls.[12] There are several biomarkers associated with the symptoms such as pain and inflammation in osteoarthritic knee which serve as diagnostic and prognostic indicators of disease. Moreover, serum biomarkers also have been shown to be associated with the radiological progression of the osteoarthritic disease.[13] There are alterations in the joint environment and activation of stress-induced signaling pathways due to wear and tear of joint. This leads to the production of inflammatory mediators such as cytokines, C-reactive proteins, indicating tissue injury, and cellular stress response in the joint. Prostaglandin synthesis occurs when there is an activation of arachidonic acid pathway. PGE2 secretion is increased in an endothelial cell during the inflammation and causes modulation of activities of immune cell migration, proliferation, and vascular permeability.[14,15] The enzyme cyclooxygenase-2 is known to be upregulated in tissue of osteoarthritic joint, leading to increased production of prostaglandins, PGE2.[16]

In a study reported by Liao *et al*., 2015, there is a description on the inverse association between serum osteocalcin and high sensitive C-reactive protein (hsCRP) in hyperglycemic subjects. This emphasizes the anti-inflammatory role of osteocalcin in such individuals.[17] Kanazawa *et al*., 2018, reported that osteocalcin levels were negatively associated with chronic inflammation parameters such as hsCRP, ferritin, and leukocyte subtypes in patients with diabetes mellitus type 2, thus suggesting that osteocalcin is protective against chronic inflammation.[18]

The PGE2 levels augment the tissue repairing process in the damaged cartilage in the knee OA. PGE2 can thus modulate various steps of inflammation in a and coordinate with the whole process in both pro- and anti-inflammation. This dual role of PGE2 and its receptors in modulating the inflammatory response has been observed in several disorders.[8] PGE2 also

elicits powerful immunosuppressive response that contributes to the resolution phase of acute inflammation, facilitating tissue regeneration, and the restoration of homeostasis. These multifaceted properties of PGE2 are both cell type and context specific. PGE2 has also been clearly established as a key component of anti-inflammatory processes and wound healing.[19] In a study by Attur *et al*., the PGE2 levels were known to increase in low-grade inflammation of osteoarthritic joints.[20]

The strength of the study is the biomarkers used in this study which are novel, especially serum osteocalcin and serum PGE2 in combination. In this study with LLLT or 3 months is unique and not many other previous studies have considered such long duration of therapy. Limitations of the study are that more sample size should have been considered for assay and follow-up of the patients after the therapy was not done. The follow-up may be required so as to find out when could pain reappear or worsen and what will be the pattern of biomarkers during such painful recurrence.

CONCLUSION

It can be concluded that the observations in the subjects with LLLT point toward a demonstrable improvement in the mobility, reduction of pain, and stiffness in senile OA of knee. There is an increase in the anti-inflammatory markers such as osteocalcin and PGE2. LLLT will be ideal for the patients who are motivated to undergo non-pharmacological management of OA for better compliance, long-term benefits, and little or no known adverse effects.

ACKNOWLEDGMENTS

I sincerely acknowledge the Department of Physiotherapy, M S Ramaiah Medical College and Teaching Hospitals, and Basavashree Diagnostics, Bengaluru, for the support in this study.

REFERENCES

1. Mora JC, Przkora R, Cruz-Almeida Y. Knee osteoarthritis: Pathophysiology and current treatment modalities. J Pain Res 2018;11:2189-96.
2. Heidari B. Knee osteoarthritis prevalence, risk factors, pathogenesis and features: Part I. Caspian J Intern Med 2011;2:205-12.
3. Xia B, Di Chen, Zhang J, Hu S, Jin H, Tong P. Osteoarthritis pathogenesis: A review of molecular mechanisms. Calcif Tissue Int 2014;95:495-505.
4. Zoch ML, Clemens TL, Riddle RC. New insights into the biology of osteocalcin. Bone 2016;82:42-9.
5. Fatahi S, Ghaedi E, Mousavi SM, Bawadi H, Rahmani J, Pezeshki M, *et al*. The Association between osteocalcin and C-reactive protein; a relation of bone with inflammation: A systematic review and meta-analysis. Horm Metab Res 2019;51:353-61.
6. Nakanishi M, Rosenberg DW. Multifaceted roles of PGE2 in inflammation and cancer. Semin Immunopathol 2013;35:123-37.
7. Allen JE, Wynn TA. Evolution of Th2 immunity: A rapid repair response to tissue destructive pathogens. PLoS Pathog 2011;7:e1002003.
8. Li X, Ellman M, Muddasani P, Wang JH, Cs-Szabo G, van Wijnen AJ, *et al*. Prostaglandin E2 and its cognate EP receptors control human adult articular cartilage homeostasis and are linked to the pathophysiology of osteoarthritis. Arthritis Rheum 2009;60:513-23.
9. Hegedus B, Viharos L, Gervain M, Gálfi M. The effect of low-level laser in knee osteoarthritis: A double-blind, randomized, placebo-controlled trial. Photomed Laser Surg 2009;27:577-84.
10. Enwemeka CS. Laser biostimulation of healing wounds: Specific effects and mechanisms of action. J Orthop Sports Phys Ther 1988;9:333-8.
11. Scanzello CR, Loeser RF. Editorial: Inflammatory activity in symptomatic knee osteoarthritis: Not all inflammation is local. Arthritis Rheumatol 2015;67:2797-800.
12. Sharma P, Pandey R, Deshpande SB. Protective role of prostaglandin E1 analog in indomethacin-induced deterioration in acute respiratory distress syndrome in rats. Natl J Physiol Pharm Pharmacol 2017;7:749-53.
13. Goldring MB, Otero M. Inflammation in osteoarthritis. Curr Opin Rheumatol 2011;23:471-8.
14. Mahmood NM, Barawi OR, Hussain SA. Relationship between serum concentrations of boron and inflammatory markers, disease duration, and severity of patients with knee osteoarthritis in Sulaimani city. Natl J Physiol Pharm Pharmacol 2016;6:27-31.
15. Kawahara K, Hohjoh H, Inazumi T, Tsuchiya S, Sugimoto Y. Prostaglandin E2-induced inflammation: Relevance of prostaglandin E receptors. Biochim Biophys Acta 2015;1851:414-21.
16. Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: Latest findings and interpretations. Ther Adv Musculoskelet Dis 2013;5:77-94.
17. Liao M, Huang L, Mao Y, Jiang Y, Yao Z, Lin X, *et al*. Serum osteocalcin is associated with inflammatory factors in metabolic syndrome: A population-based study in chinese males. Mediators Inflamm 2015;2015:683739.
18. Kanazawa I, Tanaka S, Sugimoto T. The association between osteocalcin and chronic inflammation in patients with Type 2 diabetes mellitus. Calcif Tissue Int 2018;103:599-605.
19. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol 2011;31:986-1000.
20. Attur M, Krasnokutsky S, Statnikov A, Samuels J, Li Z, Friese O, *et al*. Low-grade inflammation in symptomatic knee osteoarthritis: Prognostic value of inflammatory plasma lipids and peripheral blood leukocyte biomarkers. Arthritis Rheumatol 2015;67:2905-15.

How to cite this article: Kumar MA, Venkatesh D, Ravindra S. Assessment of biochemical markers of inflammation in subjects with knee osteoarthritis undergoing low-level laser therapy. Natl J Physiol Pharm Pharmacol 2020;10(04):330-333.

Source of Support: Nil, **Conflicts of Interest:** None declared.