




Introducing Matrix Metalloproteinases as Crucial Targets for Intra-articular Laser Therapy in Patients with Synovial Fluid of Knee Osteoarthritis

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Abstract

Background: Many elder people have knee osteoarthritis (OA). The patients are faced with pain and disability in movement. Given the challenging lifestyle of the patients, finding an efficient therapy approach is necessary. Since low-level laser therapy applies to the treatment of many diseases, it seems it can be a suitable option for the treatment of knee OA. The present study aimed to evaluate the molecular mechanism of laser therapy on knee OA via a protein expression change study.

Methods: The present study examines the gene expression profile of patients with OA in the knee using bioinformatics. The protein expression change profile of synovial fluid of knee OA patients is extracted from the literature and is analyzed based on the rate of expression and interactions between the differentially expressed proteins (DEPs). The results are compared with the DEPs of similar tissue of the treated knee OA patients (from published documents) after laser therapy.

Results: Apolipoprotein B (APOB) and matrix metalloproteinase 2 (MMP2) were determined as the hub bottlenecks of the protein-protein interaction (PPI) network of synovial fluid of knee OA patients. MMP2, complement 5, transthyretin, and apolipoprotein A-1 from laser-treated patients were related to the PPI network of knee OA patients. The reduction of Interleukin-6 activity was highlighted as a critical event as a function of laser on the human body.

Conclusion: In conclusion, it was noted that the main phenomenon associated with laser therapy-induced improvement in the condition of knee OA patients is the downregulation of MMP2.

Keywords: Low-Level Laser Therapy, Knee Osteoarthritis, Proteomics, MMP2, Network Analysis

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Introduction

Older people are facing some diseases such as osteoarthritis (OA), a degenerative joint disease that is accompanied by pain and disability. Synovial thickening, destruction of the articular cartilage, and alterations of the subchondral bone cruciate ligament, and characteristic changes of the meniscus are highlighted as a stamp of this joint

disease (1-3). It is reported that 37% of 60-year-old people or older have knee OA. This disease is more common in women relative to men (4). Light-to-moderate physical activity as nonpharmacological management of knee OA is recommended (5). The efficacy of low-level laser therapy to treat knee OA in patients has been investigated by

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↑What is “already known” in this topic:

Many older people have knee osteoarthritis (OA). These patients are faced with pain and disability in movement. The patients' demanding lifestyle implies trying to find an effective therapeutic method.

→What this article adds:

The present study aims to evaluate the molecular mechanism of laser therapy on knee OA via a protein expression change study. It was highlighted that one of the main methods by which laser therapy improves the condition of knee OA patients is through the downregulation of MMP2.

several researchers (6, 7).

Different types of diseases are targeted via laser therapy and the findings have satisfied the experts. However, laser therapy's effectiveness on some human diseases is clear, and the molecular mechanism perspective needs more investigation (8-10). Proteomics, as a useful tool, is applied to detect various aspects of low-level laser therapy (11). OA is one of the numerous diseases that is assessed via proteomics. Liao et al published a document about proteomic analysis of synovial fluid to detect OA biomarkers (12). In such studies, many differentially expressed proteins that discriminate patients from healthy people are introduced via 1 experiment (13).

Network analysis is an excellent method to screen proteomic data to introduce the critical differentially expressed proteins (DEPs) linked to the researched disease since many DEPs related to the studied disease are identified using proteomics and bioinformatics (14). In such analysis, the critical proteins that control central biological processes and molecular functions are highlighted as biomarkers. The protein-protein interaction (PPI) network analysis is a method that is applied widely to analyze such complex experiments (15). Network analysis is applied to detect laser therapy efficacy for several diseases (16). Several actions such as activation, inhibition, and coexpression relationships between some proteins can be identified via PPI network analysis (17).

Chen et al published a document about intra-articular laser therapy as a possible method to treat knee OA in elderly patients. As it is reported in this investigation, 19 patients with moderate degree of knee OA received low-level laser therapy (red laser followed by infrared irradiation) under ultrasound guidance via intra-articularly mode. They found that after laser therapy, the concentrations of matrilin, transthyretin (TTR), complement 5, osteoprotegerin (OPG), and immunoglobulin kappa chain were changed 2-fold (FC >2) as upregulated proteins and apolipoprotein A-1 (APOA1), cartilage acidic protein 1, immunoglobulin kappa chain, and matrix metalloproteinase (MMP) as downregulated proteins, respectively (1). According to Naserin et al, 7 patients—four men and 4 women—had early-stage OA synovial fluid proteome profiling compared to 13 controls (5 men and 8 women). A total of 156 DEPs (95% CI, the range of alteration that the mean value was significant) were introduced as distinct people between patients and controls, comprising 114 upregulated and 42 downregulated proteins (18). For the upregulated and downregulated DEPs, respectively, a fold change of >1.78 and <0.56 were taken into account. In the present study, the proteomics profile of knee OA synovial fluid was extracted from the literature and analyzed via the PPI network analysis to find the critical proteins that differentiate the patients from healthy people. These critical proteins are compared with the DEPs that are appeared in the treated patients via low-level laser therapy. Finally, the key proteins that are targeted via laser therapy in the patients with knee OA disease are introduced.

Methods

In this study, the gene expression profile of patients with OA was analyzed using bioinformatics. The set of proteins that were dysregulated after laser treatment in the study by Chen et al (1) are called “laser related proteins” (LRPs) and were pointed out for analysis. The OA related proteins were selected from Naserin et al report (18). This set of proteins are called “OA related proteins” (OAPs). Comparison of LRPs and the significant DEPs of OAPs revealed that the only common protein between LRPs and OAPs is the MMP protein. The top10 upregulated and downregulated proteins of OAPs were selected for more analysis.

In the present investigation, the 156 DEPs of proteomics profiling of synovial OA are investigated via the STRING database by Cytoscape software Version 3.7.2 to construct a PPI network. Because of the weak interactions between the queried DEPs, the optimum number of first neighbors from the STRING database were added to the DEPs and the network was renewed. The top 10% of the queried DEPs based on the degree value and the top 5% of DEPs considering betweenness centrality were identified as the hub and bottleneck nodes, respectively. The common hubs and bottlenecks were identified as hub bottlenecks.

Using the Cytoscape program CluePedia Version 1.5.7, an action map was made in order to determine potential connections between LRPs and the newly added initial neighbors. An action map was created for LRPs and OAPs in the comparable performance.

Results

Since the rate of fold change is an important criterion, the 10 extremely upregulated and downregulated proteins of OAPs were selected for more investigation (Figure 1). As it is depicted in Figure 1, GPX3 and PGS2 are the 2 proteins that are characterized with maximum and minimum upregulation and downregulation, respectively. Among the 156 queried DEPs, 95 individuals were identified via the STRING database. The network—including

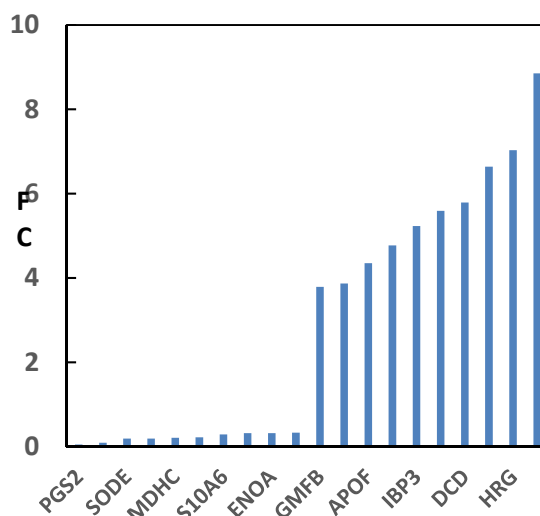


Figure 1. Top 10 extremely upregulated- and downregulated proteins among 156 significant DEPs among OAPs. $P < 0.01$.

Table 1. Two hub bottlenecks of OAPs PPI network. The nodes are selected among the queried DEPs. FC > 1 was considered as significant fold change.

Display name	Degree	Betweenness centrality	FC
APOB	67	0.019	2.15
MMP2	40	0.016	1.91

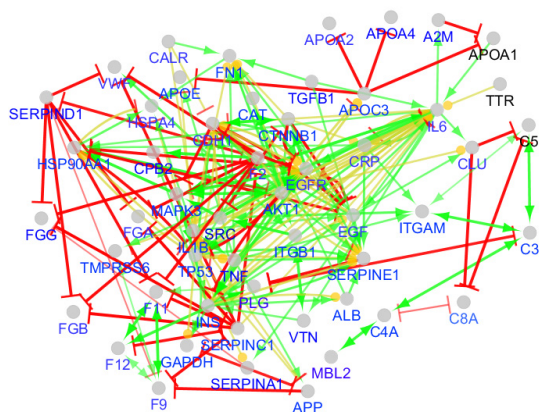


Figure 2. The main connected component of the action map of the LRPs plus the 60 added first neighbors. The LRPs (important nodes) are labeled with black color. Red, yellow, and green links refer to inhibition, expression, and activation actions respectively.

24 isolated proteins, 2 paired DEPs, and a main connected component of 69 nodes which were connected via 225 edges—was formed. Analyses revealed that adding 60 first neighbors to the queried DEPs minimized the number of the isolated DEPs. The renewed network—including 10 isolated DEPs and a main connected component of 145 nodes (85 queried DEPs plus 60 added first neighbors) which were connected by 2613 edges—was constructed. Among the queried DEPs, APOB and MMP2 were determined as hub bottlenecks (Table 1).

To screen the “laser related proteins,” the 8 proteins of LRPs plus 60 added first neighbors were comprised in a regulatory network. Three LRPs plus 48 added first neighbors were included in the main connected component (Figure 2). As it is shown in Figure 2, three proteins of LRPs are presented in the action map. Because of the unclear details of connections, these 3 proteins and their first neighbors are presented in the Figure 3 for more assessment. In the next step, an action map—including 8 LRPs proteins plus 156 OARs proteins—was constructed. The interacted proteins are presented in the Figure 4.

Discussion

Chen et al investigation led to the introduction of intra-articular laser therapy as a possible useful therapeutic method to treat knee OA. Proteomics as a valuable technique is performed in the Chen et al assessment to confirm the validity of knee laser therapy (1). In the present examination, 3 sets of data—including ORPs, LRPs, PPI network finding (the results related to the queried DEPs and the added first neighbors)—can be integrated to find a clear prospective from molecular events in patients after laser therapy. As it is presented in the Figure 1, PGS2, CXCL7, SODE, MYOC, MDHC, TPIS, S10A6, CATB,

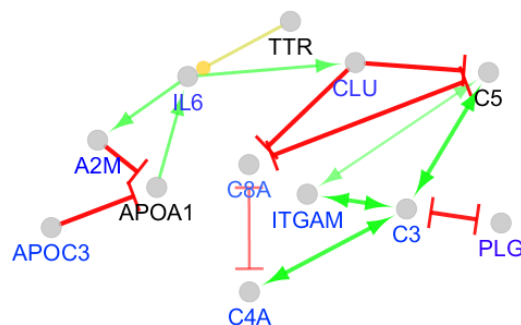


Figure 3. A subnetwork of LRPs plus the 60 added first neighbors action map, including TTR, C5, and APOA1 and their first neighbors. Red, yellow, and green refer to inhibition, expression, and activation actions respectively.

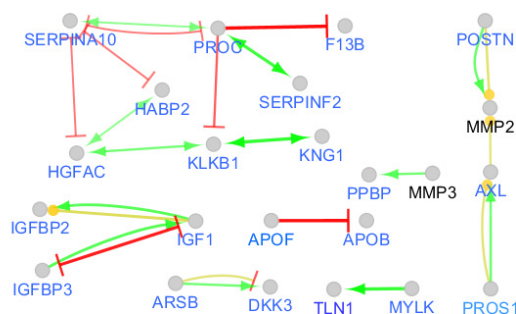


Figure 4. Action map of the LRPs plus the ORPs. The isolated individuals are removed. Red, yellow, and green refer to inhibition, expression, and activation actions respectively.

ENOA, and CILP1 are the extremely downregulated proteins in knee synovial fluid of the patients. GMFB, FA5, APOF, MYLK, IBP3, ZHX3, DCD, POSTN, HRG, and GPX3 are appeared as the top upregulated genes. There is no common protein between this set of proteins and the introduced LRPs. It can be concluded that the introduced LRPs are not important in knee OA based on expression change criterion. According to earlier analyses, the rate of expression change can be a valuable tool for understanding molecular mechanisms, but it is insufficient for comprehending entire molecular events (19).

PPI network assessment showed APOB and MMP2 are 2 hub bottleneck nodes among ORPs. Since the hub bottlenecks are used as key nodes to interpret the studied networks, it seems that APOB and MMP2 are 2 critical players in knee OA (20, 21). As it is tabulated in Table 1, these proteins are upregulated about 2-fold. APOB and MMP2 are ranked as 70th and 95th upregulated proteins, respectively. Comparison between LRPs and the hub bottlenecks of ORPs indicates that MMP2 is common between the compared proteins. Based on the study by Chen et al, MMP2 is downregulated after laser therapy (1). The results provide a clear understanding of the involvement of MMP2 in knee OA; patients have overexpressed MMP2, which is downregulated following laser therapy

and appears to be a crucial protein in the progression of knee OA.

We added 60 first neighbors to the queried DEPs of ORPs to form the PPI network. Results indicates APOA1 and TTR are common between the added first neighbors and LRPs. TTR and APOA1 are upregulated and down-regulated proteins among LRPs, respectively. In **Figures 2 and 3**, interactions of TTR, APOA1, and C5 protein with the members of added first neighbors is presented. As it is depicted in these figures, APOA1 activates IL6. It is reported that IL6 is a crucial player in OA (22). It can be concluded that downregulation of APOA1 is accompanied with decrement of IL6 activity. The inverse role of APOA1 and TTR has a coexpression change with IL6; thus, its upregulation increases level of IL6. The beneficial effects of laser therapy in treating knee OA are correlated with favorable clinical outcomes (1).

Another role of MMP2 in the progression of knee OA is illustrated in the **Figure 4**. There is a reciprocal activation relationship between POSTN and MMP2. As it is shown in the **Figure 1**, POSTN is the third ranked upregulated proteins in ORPs. **Figure 4** illustrates how the addition of POSTN to activate MMP2 boosts its expression. Consequently, one important step in slowing the advancement of knee OA is the downregulation of MMP2 achieved using laser therapy. Previous investigations have shown the significance of the fall in MMP2 serum level in the prevention of knee OA (23). Our study offers fresh insights into how laser therapy can manage OA. Combining 2 independent studies with the use of network analysis resulted in the introduction of crucial laser targets that are accountable for the advancement of OA in the knee.

Conclusion

In conclusion, intra-articular laser therapy regulates knee OA in a multifaceted manner. The MMP family of proteins emerged as the primary target for intra-articular laser therapy of knee OA. In patients with OA, downregulation of MMP2 is associated with a significant change in molecular function. In order to determine the most efficient therapy approach, it is advised that the effects of low-level lasers be investigated in the presence of MMP2 inhibitors.

Ethical Considerations

The study protocol was approved by the Research Ethics Committee of Laser Application in Medical Sciences Research Center (IR.SBMU.LASER.REC.1401.007).

Authors Contribution

R.M.R.: project management
Z.R.: research & analysis
B.A.: conceptualization & investigation
M.R.T.: writing-original draft & research
F.SH.: writing, review, editing

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Conflict of Interests

The authors declare that they have no competing interests.

References

- Chen JL, Hsu CC, Chen WC, Peng YN, Chen CP, Suputtitada A. Intra-Articular Laser Therapy May Be a Feasible Option in Treating Knee Osteoarthritis in Elderly Patients. *Biomed Res Int.* 2022;2022.
- Heinegård D, Saxne T. The role of the cartilage matrix in osteoarthritis. *Nat Rev Rheumatol.* 2011;7(1):50-6.
- Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum.* 2012;64(6):1697-1707
- Sharma L. Osteoarthritis of the knee. *N Engl J Med.* 2021;384(1):51-9.
- Mora JC, Przkora R, Cruz-Almeida Y. Knee osteoarthritis: pathophysiology and current treatment modalities. *J Pain Res.* 2018;2:189-96.
- Tascioglu F, Armagan O, Tabak Y, Corapci I, Oner C. Low power laser treatment in patients with knee osteoarthritis. *Swiss Med Wkly.* 2004;134(1718):254-8.
- Wyszyńska J, Bal-Bocheńska M. Efficacy of high-intensity laser therapy in treating knee osteoarthritis: a first systematic review. *Photomed Laser Surg.* 2018;36(7):343-53.
- dos Santos Santinoni C, Oliveira HFF, de Souza Batista VE, Lemos CAA, Verri FR. Influence of low-level laser therapy on the healing of human bone maxillofacial defects: A systematic review. *J Photochem Photobiol B Biol.* 2017;169:83-9.
- De Marchi T, Leal Junior ECP, Bortoli C, Tomazoni SS, Lopes-Martins RÁB, Salvador M. Low-level laser therapy (LLLT) in human progressive-intensity running: effects on exercise performance, skeletal muscle status, and oxidative stress. *Lasers Med Sci.* 2012;27:231-6.
- Berni M, Brancato AM, Torriani C, Bina V, Annunziata S, Cornella E, et al. The Role of Low-Level Laser Therapy in Bone Healing: Systematic Review. *Int J Mol Sci.* 2023;24(8):7094.
- Shahrokhs S, Razzaghi Z, Mansouri V, Ahmadi N. The impact of Proteomic investigations on the development and improvement of skin laser therapy: A Review Article. *J Lasers Med Sci.* 2019;10(Suppl 1):S90.
- Liao W, Li Z, Zhang H, Li J, Wang K, Yang Y. Proteomic analysis of synovial fluid as an analytical tool to detect candidate biomarkers for knee osteoarthritis. *Int J Clin Exp Pathol.* 2015;8(9):9975.
- Taha IN, Naba A. Exploring the extracellular matrix in health and disease using proteomics. *Essays in biochemistry.* 2019;63(3):417-32.
- Abbaszadeh HA, Peyvandi AA, Sadeghi Y, Safaei A, Zamanian-Azodi M, Khoramgah MS, et al. Er: YAG laser and cyclosporin A effect on cell cycle regulation of human gingival fibroblast cells. *J Lasers Med Sci.* 2017;8(3):143.
- Rezaei-Tavirani M, Rezaei-Tavirani S, Mansouri V, Rostami-Nejad M, Rezaei-Tavirani M. Protein-protein interaction network analysis for a biomarker panel related to human esophageal adenocarcinoma. *Asian Pac J Cancer Prev.* 2017;18(12):3357.
- Wada Y, Suzuki A, Ishiguro H, Murakashi E, Numabe Y. Chronological gene expression of human gingival fibroblasts with low reactive level laser (LLL) irradiation. *J Clin Med.* 2021;10(9):1952.
- Berger SI, Iyengar R. Network analyses in systems pharmacology. *Bioinformatics.* 2009;25(19):2466-72.
- Ali N, Turkiewicz A, Folkesson E, Tjörnstand J, Neuman P, Önerfjord P, et al. Proteomics profiling of human synovial fluid suggests increased protein interplay in early-osteoarthritis (OA) that is lost in late-stage OA. *Mol Cell Proteomics.* 2022;21(3).
- Mansouri V, Arjmand B, Hamzeloo-Moghadam M, Razzaghi Z, Khodadoost M, Tavirani MR, et al. Introducing BDNF and SNAI1 as the Crucial Targeted Genes by UV Radiation. *J Lasers Med Sci.* 2022;13.
- Dehghan Z, Mirmotalebisohi SA, Sameni M, Bazgiri M, Zali H. A motif-based network analysis of regulatory patterns in Doxorubicin effects on treating breast cancer, a systems biology study. *Avicenna J Med Biotechnol.* 2022;14(2):137-153.
- Belenahalli Shekarappa S, Kandagalla S, Hanumanthappa M. A network pharmacology approach to investigate the pharmacological effect of curcumin and capsaicin targets in cancer angiogenesis by module-based PPI network analysis. *J Protein Proteomics.* 2019;10:109-20.

22. Akeson G, Malesud CJ. A role for soluble IL-6 receptor in osteoarthritis. *J Funct Morphol Kinesiol.* 2017;2(3):27.
23. Yuan PW, Yu HC, Zhou HZ, Chao Z, Qiang Q, Liu DY. Preventive Administration of Juanbi Capsules () for Knee Osteoarthritis: Effects on Serum MMP-2 and MMP-9 levels and Cartilage Repair. *J Tradit Chin Med.* 2011;31(4):334-7.