Journal of Lasers in Medical Sciences

J Lasers Med Sci 2023;14:e27

Original Article

http://journals.sbmu.ac.ir/jlms

doi 10.34172/jlms.2023.27

Long and Short-terms Effects of Ablative Fractional Laser Therapy on Human Skin: A Network Analysis

Zahra Razzaghi[1], Babak Arjmand[2,3], Mostafa Rezaei Tavirani[4*], Maryam Hamzeloo-Moghadam[5], Mohammad Rostami Nejad[6], Reza Mohamoud Robati[7]

[1]Laser Application in Medical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
[3]Iranian Cancer Control Center (MACSA), Tehran, Iran
[4]Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5]Traditional Medicine and Materia Medica Research Center, School of Traditional Medicine Shahid, Beheshti University of Medical Sciences, Tehran, Iran
[6]Celiac Disease and Gluten Related Disorders Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[7]Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

***Correspondence to**
Mostafa Rezaei Tavirani,
Email: tavirany@yahoo.com

Received: January 3, 2023
Accepted: June 17, 2023
Published online August 27, 2023

Abstract

Introduction: Time-dependent effects of laser radiation have been investigated by researchers. An understanding of the molecular mechanism of the time course effect of the laser needs molecular assessment and function evaluation of the related genes. In the present study, the importance of repetition of treatment after 4 weeks and gene expression alteration after 7 days of laser radiation versus one day on the human skin was evaluated via protein-protein interaction (PPI) network analysis and gene ontology enrichment.

Methods: The differentially expressed genes (DEGs) were extracted from Gene Expression Omnibus (GEO) and assessed via PPI network analysis. The critical DEGs were enriched via gene ontology. The related biological processes and biochemical pathways were retrieved from "GO-Biological process" and "Kyoto Encyclopedia of Genes and Genomes" (KEGG) respectively.

Results: The repetition of laser therapy after 4 weeks of the first treatment did not have a significant effect on treatment efficacy. Sixty-three significant DEGs and six classes of biological terms discriminated the samples seven days after the treatment from individuals one day after the treatment. The studied DEGs were organized into two clusters with certain functions.

Conclusion: Based on the findings after laser therapy, several days are required to complete the critical processes such as DNA biosynthesis and skin cornification.

Keywords: Human; Laser radiation; Time of radiation; Gene expression change; Gene ontology.

Introduction

The time-dependent effect of laser radiation in medicine is an attractive subject for investigators. There are many documents about the effect of time which alters the efficacy of the treatment of patients with low-level laser therapy. The in vivo and ex vivo investigations revealed the important role of time in the effectiveness of laser therapy.[1,2] Time-dependent events are reported for radiotherapy and other fields of radiation. Nagtegaal et al reported an evidence about dose-time dependent way in radiation.[3] Sherrill et al reported a document about the considerable alteration of the gene expression of treated patients with a fractional laser one day after radiation, and these variations remained after one month.[4]

The important point related to the application of therapeutic methods is the molecular mechanism of treatment. Many researchers have administrated various projects to explore molecular events during the treatment of diseases. Gene expression changes are a suitable criterion to detect the details of the molecular mechanism of the applied therapeutic methods. In such research studies, a large number of genes that are up- or down-regulated have been identified and assessed via suitable methods.[5,6] Arjmand et al have published an article about the post-radiation time effect on the gene expression of the treated sample with a UV Laser. They compared the

Please cite this article as follows: Razzaghi Z, Arjmand B, Rezaei Tavirani M, Hamzeloo-Moghadam M, Rostami Nejad M, Robati RM. Long and short-terms effects of ablative fractional laser therapy on human skin: a network analysis. *J Lasers Med Sci.* 2023;14:e27. doi:10.34172/jlms.2023.27.

biological effects of 15 and 30 minutes post laser radiation times.[7]

Bioinformatics is a common method for analyzing gene expression changes. Hossain et al have discussed a model to determine ovarian cancer gene expression patterns that are associated with mortality and progression of disease via bioinformatics and machine learning.[8] Xiao et al have studied the gene expression changes of the treated sample after laser therapy by using bioinformatics.[9] Protein-protein interaction (PPI) network analysis as a bioinformatics trend is used to assess the laser effect on the body. Rezaei-Tavirani et al have studied the effect of erbium: yttrium–aluminum–garnet laser irradiation on the skin via PPI network analysis. The main targeted genes by laser radiation are reported in this investigation.[10] Tian et al have studied molecular response in early glaucoma via PPI network analysis. The hub genes are introduced and discussed in this report.[11] In the present study, the gene expression profiles of the treated patients with laser radiation one day and seven days after the treatment were extracted from Gene Expression Omnibus (GEO) and analyzed via PPI network analysis and gene ontology to find the main targeted genes, biochemical pathways, and biological processes.

Methods

Due to the effect of repetition on the efficacy of treatment, the gene expression profiles of the back skins of 14 women, which were treated with an ablative fractional laser, were extracted from GSE168760, which is recorded in GEO. The samples that received one session laser therapy and were prepared four weeks later are nominated as 4-weeks group. The other group (8-weeks group), received two sessions of laser with two four weeks intervals. In the first step, the gene expression profiles of the 8-weeks and 4-weeks samples were compared by GEO2R. The gene expression alterations were assessed via volcano plot analysis.

In the second step of the assessment, the gene expression profiles of 14 samples seven days after receiving one ablative fractional laser (7-day samples) were compared with 14 gene expression profiles of the samples provided one day after receiving one treatment (one-day samples). The feasibility of analysis was assessed via box plot evaluation and the investigated profiles were compared by the volcano plot.

250 top differentially expressed genes (DEGs) that differentiate "seven-day samples" from "one-day samples" were extracted by using GEO2R. To find the significant DEGs, the queried DEGs were screened, considering P value ≤ 0.05 and (Fold change) ≥ 1.5. To construct a PPI network, the significant DEGs were imported in the "Protein query" of the STRING database by Cytoscape software v 3.7.2. The network was created by the recognized significant DEGs and was analyzed by the "Network analyzer" application of Cytoscape. The main connected component of the network was determined and based on degree value visualized. More details of the organization of the main connected component elements were assessed via cluster analysis. DEGs of cluster-1 and cluster-2 were enriched via gene ontology via ClueGO v 2.5.7. The biological terms were extracted from "Kyoto Encyclopedia of Genes and Genomes" (KEGG) and GO-Biological process. The biological terms were grouped based on the kappa score.

Results

Volcano plot analysis of the gene expression profiles of "eight-week samples" versus "four-week samples" is shown in Figure 1. As it is presented, there are no significant differences between the compared samples. The box plot related to the "seven-day samples" versus "one-day samples" is presented in Figure 2. All gene expression profiles are median center and comparable. The gene expression pattern of the evaluated profiles is

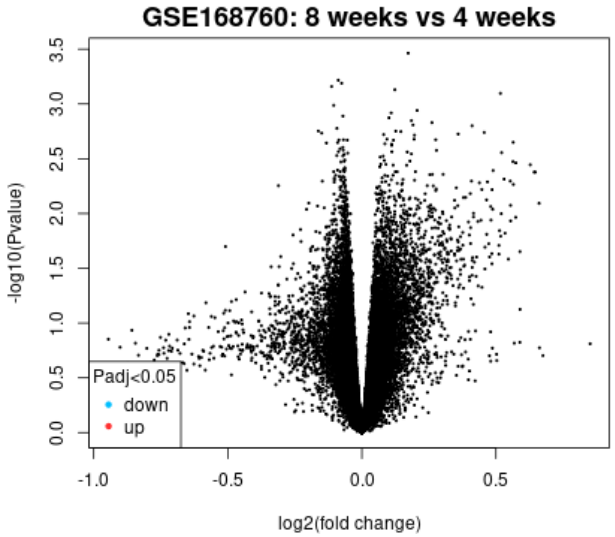

Figure 1. Volcano Plot of "8-Week Samples" Versus "4-Week Samples"

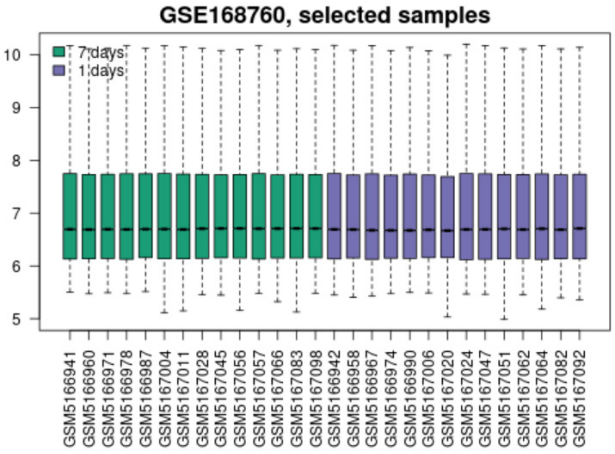

Figure 2. Box Plot of "7-Day Samples" Versus "1-Day Samples" Analysis

depicted in Figure 3. As it is shown, there are numerous up- and down-regulated genes which discriminate the "seven-day samples" from "one-day samples".

Among 250 top DEGs, 89 individuals were determined as the significant DEGs. 86 genes were recognized by the STRING database and were included in the PPI network. A network including a main connected component containing 63 nodes, a pentad sub-network, two-paired genes, and 16 isolated DEGs was created. As it is depicted in Figure 4, the main connected component is formed from two clusters: cluster-1 including 42 nodes and cluster-2 containing 21 DEGs. Due to the presence of a limited family of genes in cluster-2, the organization of nodes in this cluster is shown in Figure 5. The elements of both clusters are presented in Tables 1 and 2.

The result of gene ontology enrichment for nodes of cluster-1 is shown in Figure 6. Five groups of biological terms including "nucleoside metabolic process", "positive regulation of telomerase RNA localization to Cajal body", "NADH regeneration", "disulfide oxidoreductase activity", and "ribosomal large subunit biogenesis" are related to the elements of cluster-1. The three groups of biological terms ("cornification", "antimicrobial humoral response", and "intermediate filament cytoskeletal organization") that are related to the nodes of cluster-2 are represented in Figure 7.

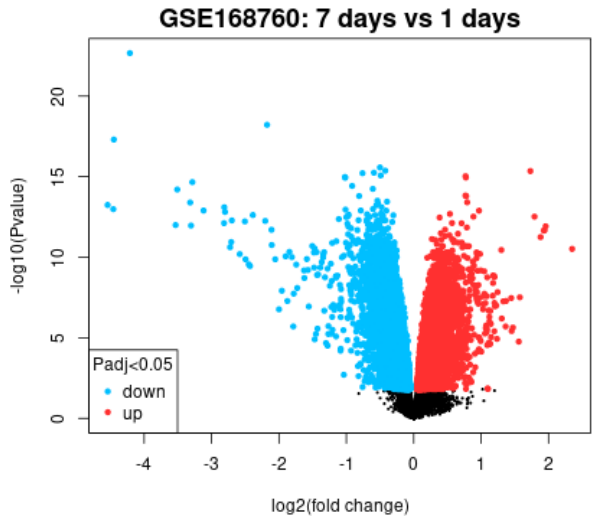

Figure 3. Volcano Plot of "7-Day Samples" Versus "1-Day Samples" Analysis

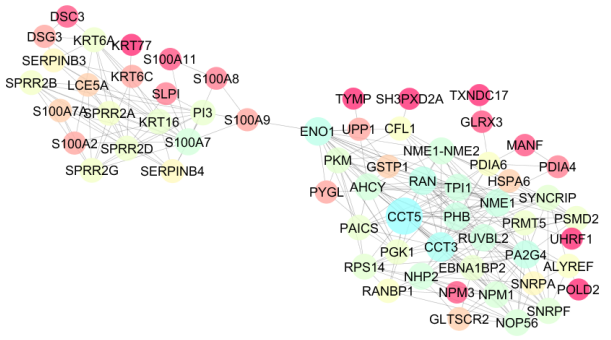

Figure 4. Main Connected Component of the PPI Network Related to "7-Day Sample" Versus "1-Day Sample" Analysis. The nodes are laid out based on degree value; blue to red and the increase in node size refer to the rise of degree value. The confidence score was considered to be 0.4

Discussion

The efficacy evaluation of laser radiation in medicine is an attractive activity. Manuskiatti et al evaluated the long-term efficacy of the safety of laser radiation in 26 Fitzpatrick skin types III and IV patients that received a 1064-nm picosecond laser in combination with the MLA handpiece for an average of three passes, for 6 monthly sessions.[12] In the present study, the effect of laser treatment on patients who received two repetitions at 28-day intervals was compared with the patients 28 days after one-time laser radiation. As it is depicted in Figure 1, repetition does not have a significant effect on the gene expression profiles of the studied patients. Based on Drnovšek-Olup and colleagues' report, the application of repetitive Er:YAG laser irradiation in human skin deeply affects the denaturation and remodeling of skin collagen. This finding indicates that repetitive radiation is more effective and is associated with less epidermal damage compared to standard Er:YAG laser skin resurfacing.[13] It can be concluded that two possible mechanisms have occurred in the present analysis. The first event refers to the similar patterns of effects of the single and repeated doses. The second mechanism is consisted with a moderate effect of the second laser radiation.

Time dependency of the laser effect was studied in the irradiated samples after one day and seven days of radiation. Based on box plot analysis (Figure 2), the gene expression profiles of the irradiated samples are comparable. Differences between the gene expression profiles of samples seven days and one day after radiation

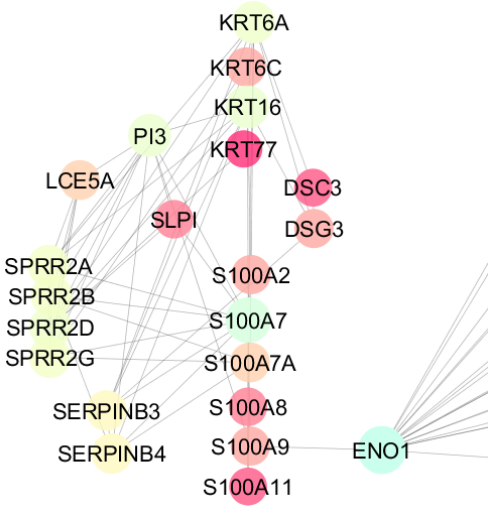

Figure 5. Organization of the Main Connected Component Elements of Cluster-2. ENO1 is the bridge between cluster-2 and cluster-1. The confidence score was considered to be 0.4.

Table 1. Nodes of Cluster-1

R	Display name	Degree	BtwC	Cc	Stress	FC
1	CCT5	24	0.12	0.43	2332	
2	CCT3	21	0.07	0.42	1622	
3	ENO1	18	0.49	0.47	7812	
4	RAN	18	0.05	0.40	1086	
5	PA2G4	17	0.02	0.35	338	
6	NME1	16	0.04	0.40	812	
7	PHB	16	0.08	0.41	1574	
8	RUVBL2	15	0.01	0.35	316	
9	TPI1	15	0.03	0.40	728	
1	AHCY	14	0.03	0.39	648	
2	NHP2	14	0.02	0.34	356	
3	NME1-NME2	14	0.02	0.39	524	
4	NOP56	13	0.01	0.33	160	
5	NPM1	13	0.06	0.39	978	
6	SNRPF	12	0.01	0.34	170	
7	PKM	11	0.01	0.38	246	
8	RPS14	11	0.01	0.33	126	
9	SYNCRIP	11	0.00	0.34	126	
10	EBNA1BP2	10	0.00	0.33	92	
11	PAICS	10	0.00	0.37	122	
12	PGK1	9	0.00	0.37	168	
13	PRMT5	9	0.03	0.33	382	
14	PSMD2	9	0.01	0.37	326	
15	ALYREF	7	0.03	0.32	514	
16	CFL1	7	0.03	0.36	404	
17	PDIA6	7	0.09	0.33	1608	
18	RANBP1	7	0.00	0.32	10	
19	SNRPA	6	0.00	0.31	24	
20	GLTSCR2	5	0.00	0.30	2	
21	GSTP1	5	0.00	0.35	44	
22	HSPA6	5	0.00	0.32	8	
23	PYGL	4	0.00	0.33	0	
24	UPP1	4	0.03	0.33	392	
25	PDIA4	3	0.00	0.30	118	
26	GLRX3	2	0.03	0.25	518	
27	MANF	2	0.00	0.25	0	
28	NPM3	2	0.00	0.29	2	
29	POLD2	1	0.00	0.24	0	
30	SH3PXD2A	1	0.00	0.27	0	
31	TXNDC17	1	0.00	0.20	0	
32	TYMP	1	0.00	0.25	0	
33	UHRF1	1	0.00	0.25	0	

Note: R, BtwC, and Cc refer to row, betweenness centrality, and closeness centrality respectively.

Table 2. Nodes of Cluster-2

R	Display Name	Degree	BtwC	Cc	Stress	FC
1	S100A7	14	0.25	0.34	4110	
2	KRT16	10	0.03	0.27	952	
3	PI3	10	0.15	0.33	2498	
4	KRT6A	9	0.05	0.27	760	
5	SPRR2D	9	0.04	0.27	358	
6	SPRR2A	8	0.00	0.27	64	
7	SPRR2B	8	0.00	0.27	46	
8	SPRR2G	8	0.01	0.27	466	
9	SERPINB3	6	0.01	0.27	444	
10	SERPINB4	6	0.00	0.27	24	
11	LCE5A	5	0.00	0.26	0	
12	S100A7A	5	0.00	0.26	12	
13	DSG3	4	0.00	0.22	10	
14	KRT6C	4	0.00	0.22	16	
15	S100A2	4	0.00	0.26	10	
16	S100A9	4	0.44	0.40	7008	
17	S100A8	3	0.01	0.31	202	
18	SLPI	3	0.00	0.26	12	
19	DSC3	2	0.00	0.22	0	
20	S100A11	2	0.00	0.26	0	
21	KRT77	1	0.00	0.21	0	

Note: R, BtwC, and Cc refer to row, betweenness centrality, and closeness centrality respectively.

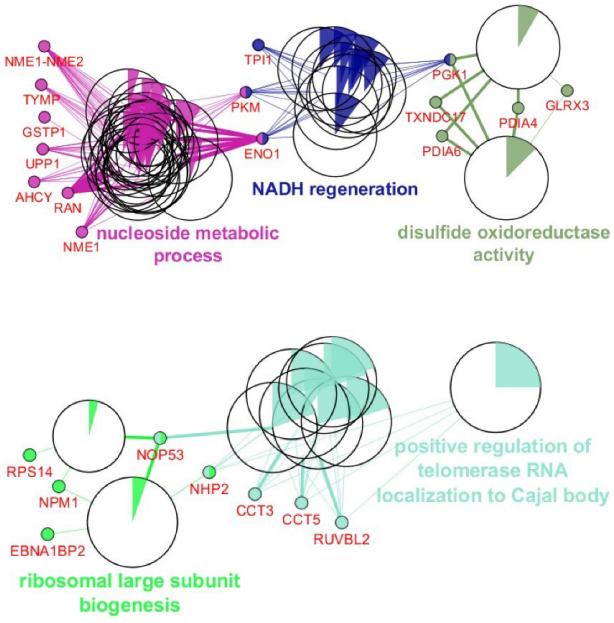

Figure 6. Biological Terms Related to the Nodes of Cluster-1. The Kappa score was considered to be 0.4

are depicted in the volcano plot (see Figure 3). Many significant up- and down-regulated genes appear in the volcano plot. This plot as a useful tool is applied to discover differences between the studied gene expression profiles.[14]

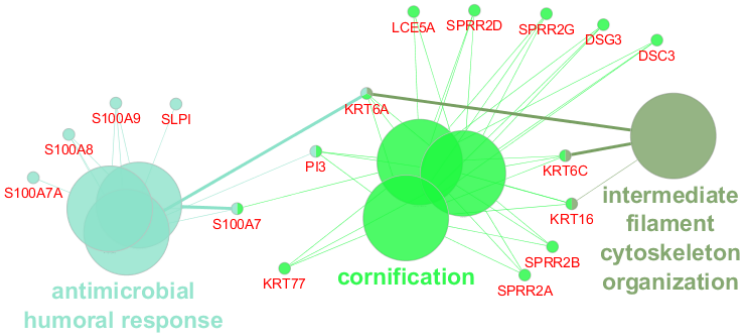

Figure 7. Biological Terms Related to the Nodes of Cluster-1. The Kappa score = 0.4

The assessment revealed that 89 DEGs discriminate the gene expression profiles of the two compared groups of samples. PPI network analysis led to the highlight of 63 DEGs which were organized in the main connected component of the constructed interactome. Interactome networks are applied widely to evaluate diseases.[15] As it is depicted in Figure 4, the main connected component includes two clusters: the major (cluster-1) and the minor (cluster-2). Cluster-2 includes S100, SPRR, and KRT families of genes plus the other 7 genes which are connected to cluster-1 via ENO1 (see Figure 5). The nodes of the two clusters are presented in Tables 1 and 2. As it is shown in Table 1, ENO1 is the potent bottleneck node (it is characterized by BtwC = 0.49) and the third-ranked hub.

CCT5 and CCT3 are two top nodes based on degree value in cluster-1 (see Table 1). These two hubs are related to the "positive regulation of telomerase RNA localization to Cajal body" class of biological terms (see Figure 6). Investigations indicate that the accumulation of human telomerase in Cajal bodies facilitates telomere elongation.[16] Positive regulation of this process leads to more elongation of the telomere. The role of telomeres in diseases is discussed in many documents. Yeh et al pointed out the role of telomeres in heart disease. Based on this document, telomeres are considered therapeutic targets in heart disease.[17] The important class of the biological term, which is related to the nine DEGs of cluster-1 is the "nucleoside metabolic process". Nucleosides are known as intermediates of nucleotide metabolism. Nucleotide *de novo* synthesis leads to producing nucleoside monophosphates AMP and UMP. These products will be processed and included in all purine and pyrimidine nucleotides. The important cellular reactions such as the synthesis of nucleic acids depend on these products.[18]

Cornification is the main class of biological terms, which is related to the 13 genes of cluster-2. The stratum corneum of the epidermis makes the skin a physical and permeability barrier. Terminal differentiation of epidermal keratinocytes, which is known as cornification, leads to the provision of stratum corneum.[19] It seems that cluster-2 is involved mostly in skin regeneration.

Conclusion

In conclusion, two significant results were pointed out in this investigation. First, our findings indicate that the full effects of laser application on human skin require several days. Second, two classes of genes, which are involved in DNA biosynthesis and skin regeneration, are affected by laser radiation. ENO1 as a critical gene function as a linker between the gene sets that are involved in DNA biosynthesis and the individuals that are related to skin regeneration. It can be suggested that a standard protocol is required for each mode of laser therapy.

Acknowledgments

This project was supported by Shahid Beheshti University of Medical Sciences.

Authors' Contribution

Conceptualization: Rezaie Tavirani, Zahra Razzaghi, Reza Mohamoud Robati.
Data curation: Zahra Razzaghi, Mohammad Rostami Nejad, Babak Arjmand.
Formal analysis: Rezaie Tavirani, Maryam Hamzeloo-Moghadam.
Funding acquisition: Zahra Razzaghi, Mohammad Rostami Nejad.
Investigation: Zahra Razzaghi, Babak Arjmand, Mostafa Rezaei Tavirani, Maryam Hamzeloo-Moghadam, Mohammad Rostami Nejad, Reza Mohamoud Robati.
Methodology: Zahra Razzaghi.
Project administration: Rezaie Tavirani.
Resources: Zahra Razzaghi.
Software: Rezaie Tavirani.
Supervision: Hamzeloo, Rezaie, Reza Mohamoud Robati.
Validation: Zahra Razzaghi.
Visualization: Rezaie Tavirani.
Writing–original draft: Babak Arjmand, Rezaie Tavirani.
Writing–review & editing: Zahra Razzaghi, Babak Arjmand, Mostafa Rezaei Tavirani, Maryam Hamzeloo-Moghadam, Mohammad Rostami Nejad, Reza Mohamoud Robati.

Competing Interests

The authors declare that they have no conflict of interest.

References

1. Gonçalves RV, Novaes RD, do Carmo Cupertino M, Moraes B, Leite JP, do Carmo Gouveia Peluzio M, et al. Time-dependent effects of low-level laser therapy on the morphology and oxidative response in the skin wound healing in rats. Lasers Med Sci. 2013;28(2):383-90. doi: 10.1007/s10103-012-1066-7.

2. Bernardi S, Giudici F, Cesareo R, Antonelli G, Cavallaro M, Deandrea M, et al. Five-year results of radiofrequency and laser ablation of benign thyroid nodules: a multicenter study from the Italian minimally invasive treatments of the thyroid group. Thyroid. 2020;30(12):1759-70. doi: 10.1089/thy.2020.0202.
3. Nagtegaal S, David S, Philippens M, Leemans A, Verhoeff J. Any radiation dose to the hippocampus leads to local volume loss in a dose-time-dependent way. Int J Radiat Oncol Biol Phys. 2019;105(1):E104-E5. doi: 10.1016/j.ijrobp.2019.06.2302.
4. Sherrill JD, Finlay D, Binder RL, Robinson MK, Wei X, Tiesman JP, et al. Transcriptomic analysis of human skin wound healing and rejuvenation following ablative fractional laser treatment. PLoS One. 2021;16(11):e0260095. doi: 10.1371/journal.pone.0260095.
5. Mussttaf RA, Jenkins DFL, Jha AN. Assessing the impact of low level laser therapy (LLLT) on biological systems: a review. Int J Radiat Biol. 2019;95(2):120-43. doi: 10.1080/09553002.2019.1524944.
6. Dompe C, Moncrieff L, Matys J, Grzech-Leśniak K, Kocherova I, Bryja A, et al. Photobiomodulation-underlying mechanism and clinical applications. J Clin Med. 2020;9(6):1724. doi: 10.3390/jcm9061724.
7. Arjmand B, Safari-Alighiarloo N, Razzaghi M, Rezaei Tavirani M, Rostami Nejad M, Rezaei Tavirani M. Assessment of post-radiation time effect on gene expression profiles of *Saccharomyces cerevisiae* samples after appling a UV laser. J Lasers Med Sci. 2021;12:e91. doi: 10.34172/jlms.2021.91.
8. Hossain MA, Saiful Islam SM, Quinn JMW, Huq F, Moni MA. Machine learning and bioinformatics models to identify gene expression patterns of ovarian cancer associated with disease progression and mortality. J Biomed Inform. 2019;100:103313. doi: 10.1016/j.jbi.2019.103313.
9. Xiao Q, Wang L, Zhang J, Zhong X, Guo Z, Yu J, et al. Activation of Wnt/β-catenin signaling involves 660 nm laser radiation on epithelium and modulates lipid metabolism. Biomolecules. 2022;12(10):1389. doi: 10.3390/biom12101389.
10. Rezaei-Tavirani M, Rezaei Tavirani M, Zamanian Azodi M, Moravvej Farshi H, Razzaghi M. Evaluation of skin response after erbium:yttrium-aluminum-garnet laser irradiation: a network analysis approach. J Lasers Med Sci. 2019;10(3):194-9. doi: 10.15171/jlms.2019.31.
11. Tian R, Li F, Che S, Song M, Liu L, Guo R, et al. Hub gene screening associated with early glaucoma: an integrated bioinformatics analysis. Comput Math Methods Med. 2022;2022:8030243. doi: 10.1155/2022/8030243.
12. Manuskiatti W, Punyaratabandhu P, Tantrapornpong P, Yan C, Cembrano KAG. Objective and long-term evaluation of the efficacy and safety of a 1064-nm picosecond laser with fractionated microlens array for the treatment of atrophic acne scar in Asians. Lasers Surg Med. 2021;53(7):899-905. doi: 10.1002/lsm.23368.
13. Drnovšek-Olup B, Beltram M, Pizem J. Repetitive Er:YAG laser irradiation of human skin: a histological evaluation. Lasers Surg Med. 2004;35(2):146-51. doi: 10.1002/lsm.20080.
14. Li W, Freudenberg J, Suh YJ, Yang Y. Using volcano plots and regularized-chi statistics in genetic association studies. Comput Biol Chem. 2014;48:77-83. doi: 10.1016/j.compbiolchem.2013.02.003.
15. Vidal M, Cusick ME, Barabási AL. Interactome networks and human disease. Cell. 2011;144(6):986-98. doi: 10.1016/j.cell.2011.02.016.
16. Cristofari G, Adolf E, Reichenbach P, Sikora K, Terns RM, Terns MP, et al. Human telomerase RNA accumulation in Cajal bodies facilitates telomerase recruitment to telomeres and telomere elongation. Mol Cell. 2007;27(6):882-9. doi: 10.1016/j.molcel.2007.07.020.
17. Yeh JK, Lin MH, Wang CY. Telomeres as therapeutic targets in heart disease. JACC Basic Transl Sci. 2019;4(7):855-65. doi: 10.1016/j.jacbts.2019.05.009.
18. Möhlmann T, Bernard C, Hach S, Ekkehard Neuhaus H. Nucleoside transport and associated metabolism. Plant Biol (Stuttg). 2010;12 Suppl 1:26-34. doi: 10.1111/j.1438-8677.2010.00351.x.
19. Murata T, Honda T, Mostafa A, Kabashima K. Stratum corneum as polymer sheet: concept and cornification processes. Trends Mol Med. 2022;28(5):350-9. doi: 10.1016/j.molmed.2022.02.008.