

# The Open Dentistry Journal

Content list available at: <https://opendentistryjournal.com>



## RESEARCH ARTICLE

### Combination of Metformin and Laser against Head and Neck Cancer Cells *In Vitro*

Ghasem Ebrahimi<sup>1,2,#</sup>, Elaheh Dalir Abdolahinia<sup>3,#</sup>, Parnia Pouya<sup>3</sup>, Amirabbas Jalili Bolhasani<sup>1</sup>, Simin Sharifi<sup>4,\*</sup> and Solmaz Maleki Dizaj<sup>4,#</sup>

<sup>1</sup>Department of Biochemistry and Clinical Laboratories, Faculty of Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>2</sup>Department of Dental Biomaterials, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>3</sup>Research Center for Pharmaceutical Nanotechnology, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>4</sup>Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

#### Abstract:

##### Introduction:

Metformin is a common drug for the treatment of diabetic patients. This drug has been shown to have anti-cancer effects. Metformin acts through the AMPK/mTOR pathway against cancer cells. We hypothesized that metformin, in combination with laser as photodynamic therapy (PDT), may increase cytotoxicity for cancer treatment. This study aimed to evaluate the anti-cancer effect and mechanism of metformin combined with laser irradiation on head and neck cancer cells.

##### Methods:

The anti-cancer effect of metformin was evaluated using head and neck cancer cells (HN5 cells) and oral cavity normal cells (HUGU) cells *in vitro*. Cell viability and phototoxicity were assessed with the MTT assay. Evaluation of protein expression of p-mTOR and mTOR genes was evaluated with the western blot method.

##### Results:

The results showed that the toxic effect of metformin on HN5 cells was dose- and time-dependent. Metformin had significantly more cytotoxic effects on cancer cells than HUGU normal cells. The laser increased the cytotoxic effect of metformin in HUGU and HN5 cells significantly ( $p < 0.05$ ). Metformin down-regulated the level of p-mTOR/mTOR protein, and despite the decreased ratio of p-mTOR/mTOR, the laser has affected the performance of metformin on p-mTOR/mTOR.

##### Discussion:

In this study, we used the laser without using a photosensitizer, but the simultaneous use of laser light and metformin increased the lethality of metformin on cells, which requires further research to determine whether metformin itself is a functional photosensitizer.

##### Conclusion:

: This study introduces the combination of metformin and laser as a new anti-tumor treatment that needs further studies.

**Keywords:** Photodynamic therapy, Metformin, Mtor, Head and neck cancer, Cell viability, Phototoxicity.

#### Article History

Received: August 08, 2023

Revised: October 07, 2023

Accepted: October 17, 2023

## 1. INTRODUCTION

Cancer is a common disease in which uncontrolled and abnormal proliferation of cells occurs that can involve other nearby tissues [1 - 3]. Conventional cancer treatment is chemotherapy, which has many adverse side effects [4 - 6].

Photodynamic therapy (PDT) is utilized to treat various types of cancer [7 - 9]. In PDT, a photosensitizer is administered that irradiates the tumor and produces reactive oxygen species (ROS), which eradicate cancer cells [10 - 13].

The tumor may recur after PDT treatment, so this method is not always completely effective [14]. For energy production, normal cells depend mainly on mitochondrial oxidative phosphorylation, but cancerous cells switch to enhanced aerobic glycolysis, which is known as the Warburg effect [15].

\* Address correspondence to this author at the Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran;  
E-mail: [sharifi.ghazi@gmail.com](mailto:sharifi.ghazi@gmail.com)

#These authors contributed equally to this work

The Warburg effect can affect the response to anticancer drugs [16 - 18]. Pyruvate kinase M2 (PKM2) expression is strongly increased in cancer and promotes the survival of cells. Also, phosphoinositide 3-kinase signaling by protein kinase B (AKT) can regulate the expression of glucose transporter (GLUT1) and increase glucose capture [19, 20]. In the metabolic reprogramming of cancerous cells, the mammalian target of rapamycin (mTOR) has an important role that is activated in most cancers [21].

A possible appropriate solution to prevent cancer recurrence in PDT is to combine it with other treatment methods. Because alterations in cellular metabolism happen in cancerous cells, the usage of agents that control the metabolism of glucose can be a great choice to decrease recurrence. In this regard, metformin is considered a promising drug in the treatment of cancer, which acts by inhibiting the AMP-activated protein kinase (AMPK)/mTOR pathway [22, 23]. Metformin inhibits tumor growth in skin cancer in obese and overweight mice by activation of AMPK and weakened signaling *via* mTOR complex and p70S6K [24]. Another study has shown that metformin increases the toxicity of 5-aminolevulinic acid-mediated photodynamic therapy in lung cancer cells [25]. Also, metformin combined with 20-tetra-sulfophenyl-porphyrin-PDT inhibits inflammation and angiogenesis of tumors in carcinosarcoma in rats [26]. Considering that metformin indirectly prevents the proliferation of cancer cells and reduces the risk of cancer, therefore, the simultaneous use of laser and metformin on cancer cells may have a favorable effect in the treatment of cancer patients. The present study evaluates the effect of the combination of metformin and photodynamic therapy without photosensitizer against head and neck cancer cells *in vitro*.

## 2. MATERIALS AND METHODS

### 2.1. Cell Culture

The HN5 cells (head and neck cancer cells) and HUGU cells (oral cavity; gingiva normal cells) were purchased from the Pasteur Institute of Iran. They were cultured in a supplemented DMEM medium (Gibco) with 10% FBS (Gibco) at 37 °C in an incubator with 5% CO<sub>2</sub>.

### 2.2. Photodynamic Therapy

For PDT, cells were seeded in 96-well microplates (10,000 cells/well). After 24 h, the cells were exposed to several concentrations of metformin (0, 5, 10, 20, 40, 80, and 160 mM) for 4 hours at 37 °C, then the cells were irradiated with laser light (630 nm) once for 60 seconds. After irradiation, the microplates were incubated for 72 hours until further examination. In this study, we didn't use a photosensitizer.

### 2.3. Cytotoxicity

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) test (Sigma-Aldrich) was used for the evaluation of cell viability. The fresh MTT solution was added

to wells and incubated for 4 h. Then, well's contents were exchanged with DMSO and absorbance was read at 570 nm. Cytotoxicity was calculated by  $[A]_{\text{test}}/[A]_{\text{control}} \times 100$  [27]. The experiment was repeated three times.

### 2.4. Western Blots

Western blot analysis was performed 72 hours after treatment of cells with IC50 concentration of metformin. For western blotting, RIPA buffer with Triton (Bioworld) with pH 7.4 was used for the preparation of cellular extract. The concentration of protein was evaluated by Bradford Protein Assay. The cellular extract was heated at 98 °C for 5 min. Electrophoresis was performed *via* acrylamide/bisacrylamide gel in denatured condition (SDS-PAGE) and moved to the membrane of polyvinylidene difluoride (PVDF) (Bio-Rad), was done *via* a Transblot Turbo system (Bio-Rad). The skimmed milk in 0.1% TBS-Tween 20 was used for blocking membranes and then incubated with antibodies. Then, the membrane was incubated with an anti-p-mTOR antibody (Ser 2481) (sc-2930,89), anti-mTOR antibody (30) and anti-GAPDH antibody ((sc-365062) (all Santa Cruz Biotechnology, Santa Cruz, CA), at 4°C overnight. Then, a secondary antibody conjugated with peroxidase (Thermo Fisher) was used for washing and incubating. The visualization of protein bands was performed by chemiluminescence (ECL Plus Kit) by the high-resolution ChemiDoc XRS system (Bio-Rad). The bands corresponding to the different proteins were evaluated *via* Image Lab software (Bio-Rad).

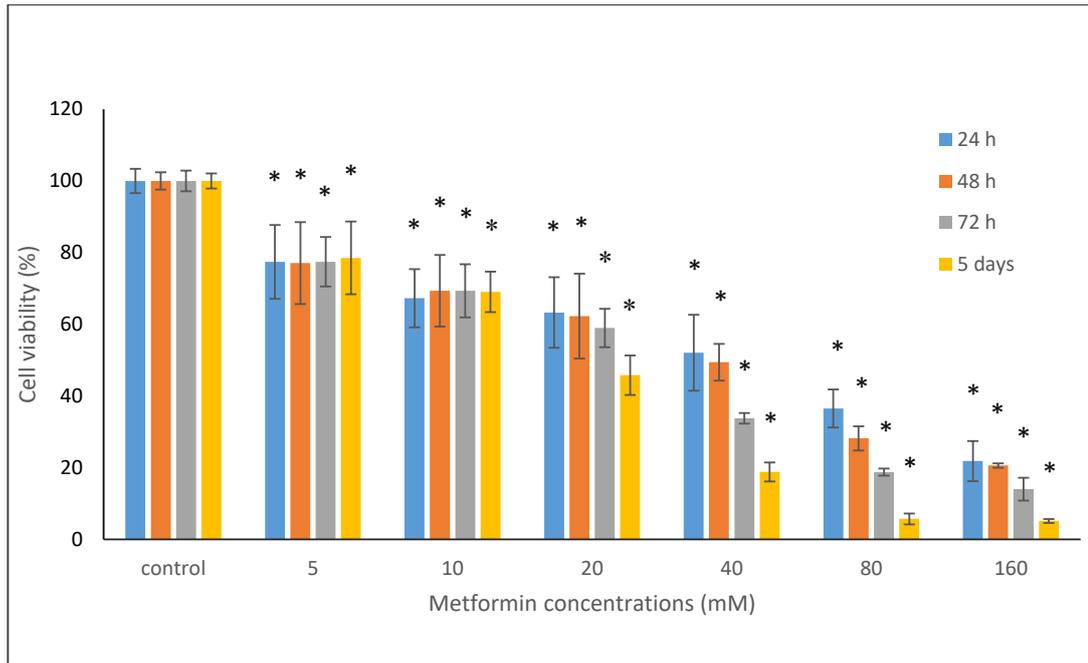
### 2.5. Statistical Analysis

Two-way ANOVA was used for analyzing the MTT test results, and One-way ANOVA was used for evaluating the western blot results, and differences were considered significant at  $p < 0.05$ .

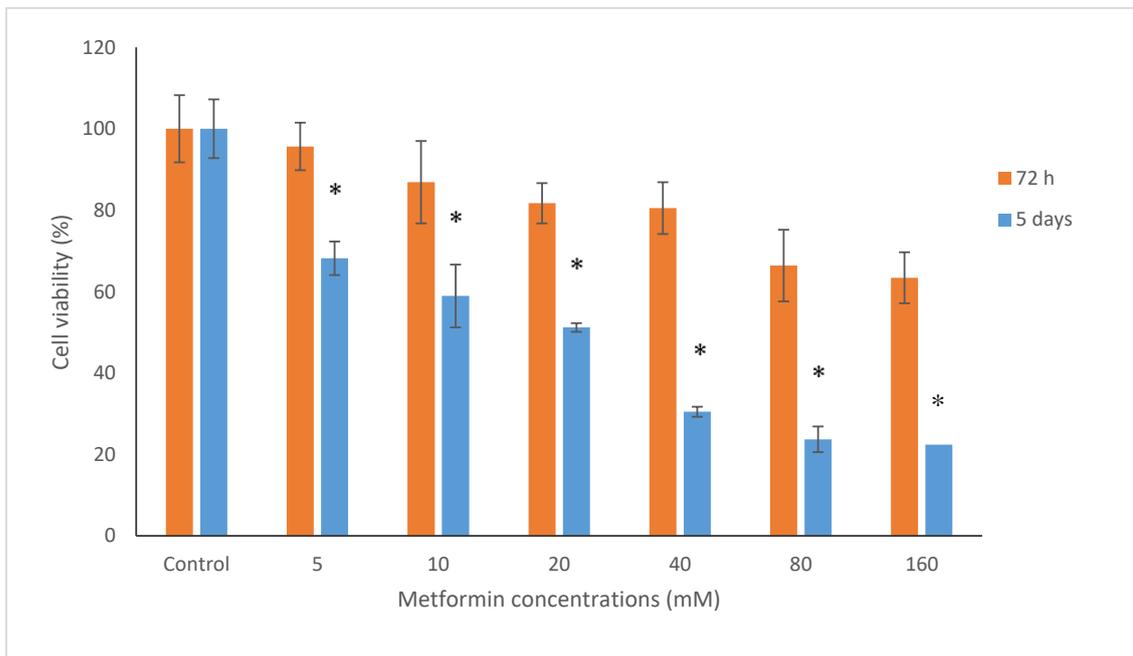
## 3. RESULTS

In this study, the MTT method was used to investigate the cytotoxicity of metformin on HN5 oral cancer cells and HUGU normal and non-cancerous cells. In this test, HN5 cells were exposed to metformin for 24, 48, 72 hours and 5 days. The results displayed that the toxic effect of metformin on HN5 cells was dose- and time-dependent (Fig. 1). HUGU cells were treated with metformin for 72 hours and 5 days. The results of the toxic effect of metformin on these cells are shown in Fig. (2). The results showed that metformin had significantly more cytotoxic effects on cancer cells than normal cells.

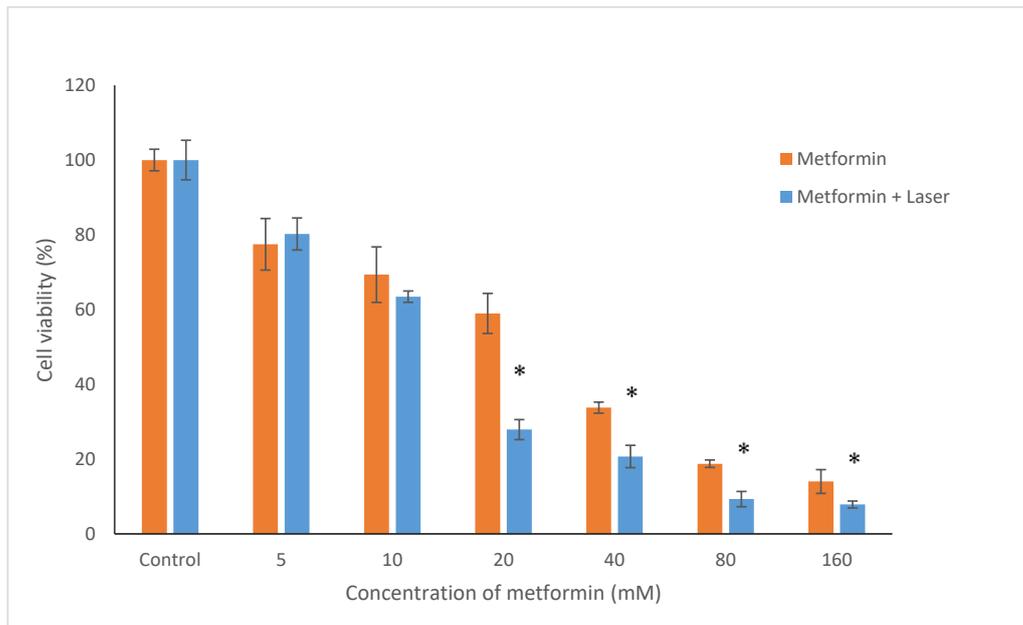
To evaluate the effects of Metformin and laser combination by light radiation energy intensities, HUGU and HN5 cells were incubated with different concentrations of Metformin. Cells were exposed to laser for 1 min. Cell survival was examined *via* an MTT test 72 hours after irradiation. The obtained results showed that with the increase in metformin concentration, the rate of cell survival gradually decreases (Figs. 3 and 4). The laser increased the cytotoxic effect of metformin in HUGU and HN5 cells significantly ( $p < 0.05$ ).



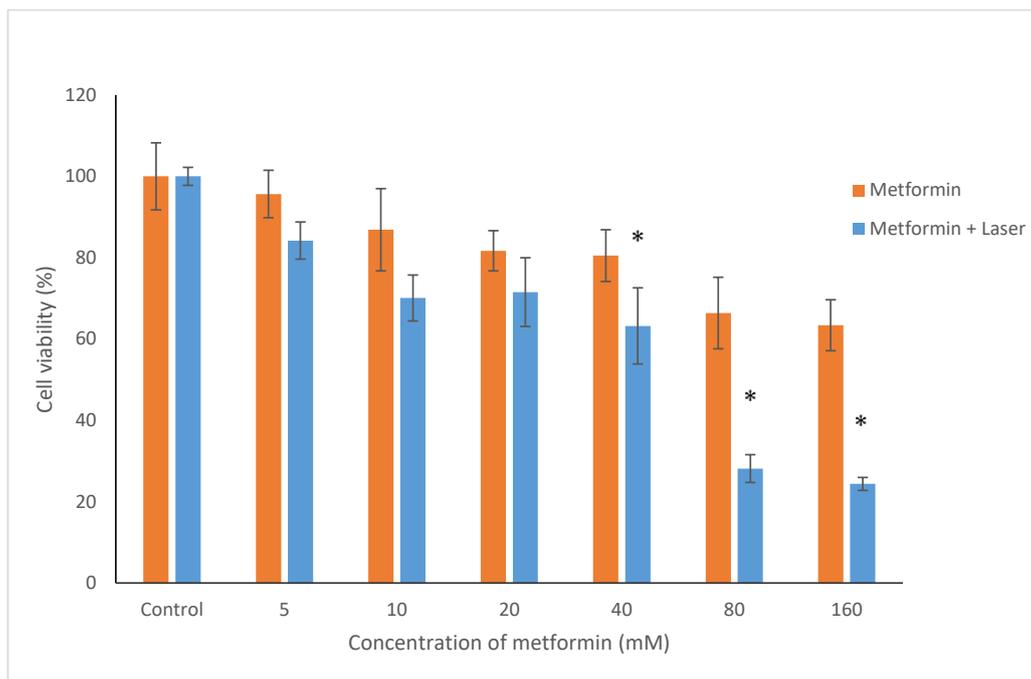
**Fig. (1).** The results of MTT assay on metformin-treated HN5 cells in 24, 48, 72 h and 5 days. \* Statistical significance compared with control group ( $p < 0.05$ ).



**Fig. (2).** The results of MTT assay on metformin-treated HUGU cells in 72 h and 5 days. \* Statistical significance between the 72 h and 5 days ( $p < 0.05$ ).



**Fig. (3).** The results of MTT assay on metformin-treated HN5 cells with and without laser radiation in 72 h. \* Statistical significance between the laser-radiated and non-radiated groups ( $p < 0.05$ ).



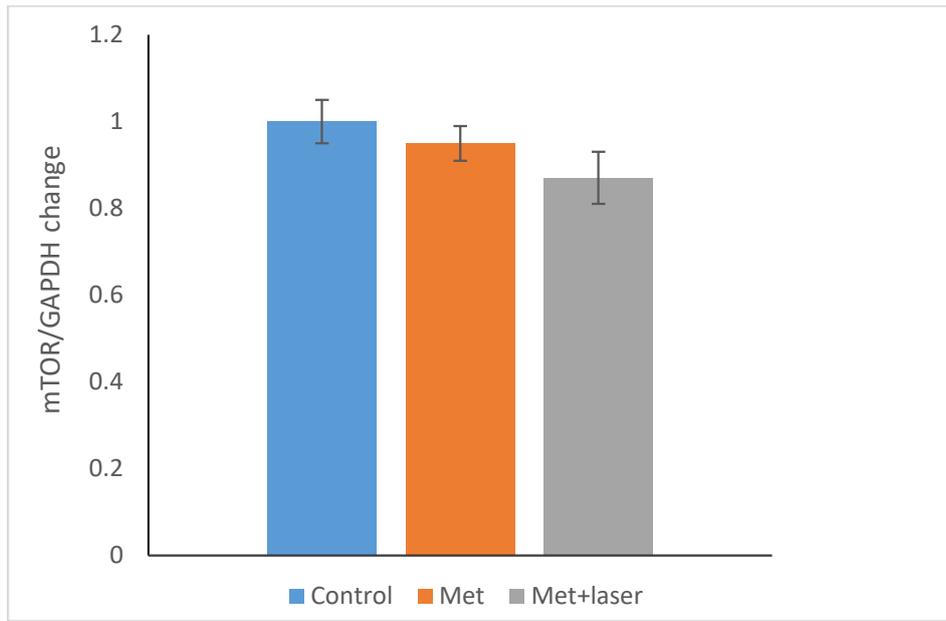
**Fig. (4).** The results of MTT assay on metformin-treated HUGU cells with and without laser radiation in 72 h. \* Statistical significance between the laser-radiated and non-radiated groups ( $p < 0.05$ ).

### 3.1. Effect of Metformin on AMPK/ mTOR Pathway in HN5 Cell Line

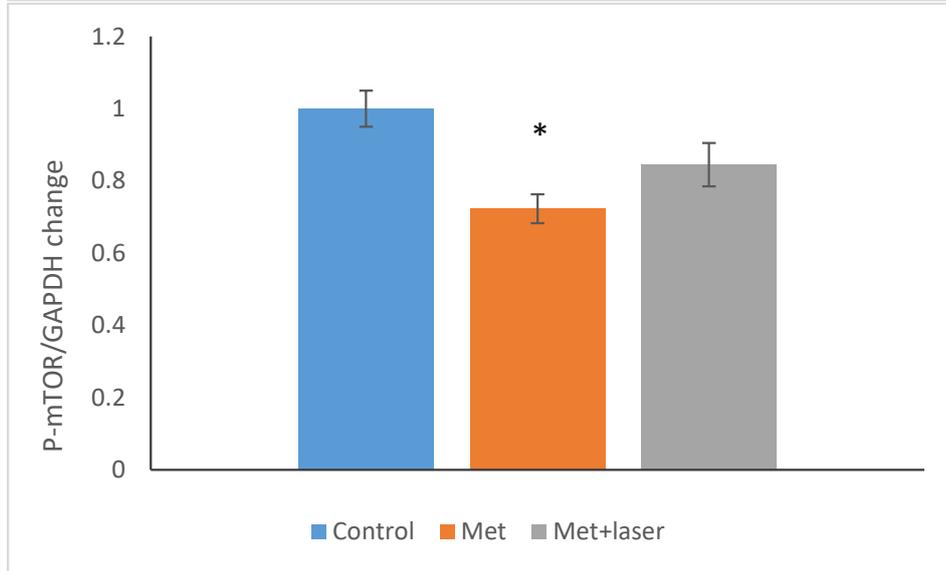
The anticancer property of metformin is multifunctional and it has the ability to control cancer through different pathways, one of which is the mTOR/AMPK pathway [28 - 30]. In this study, the expression of mTOR and p-mTOR

protein after 48 hours of treatment on HN5 cells with MET, MET+ Laser was done by western blotting (Fig. 5a - d) shows that the level of p-mTOR protein compared to mTOR protein in the group treated with metformin showed a significant decrease ( $p < 0.05$ ), while in the group treated with metformin along with laser, the decrease observed is not significant compared to the control group.

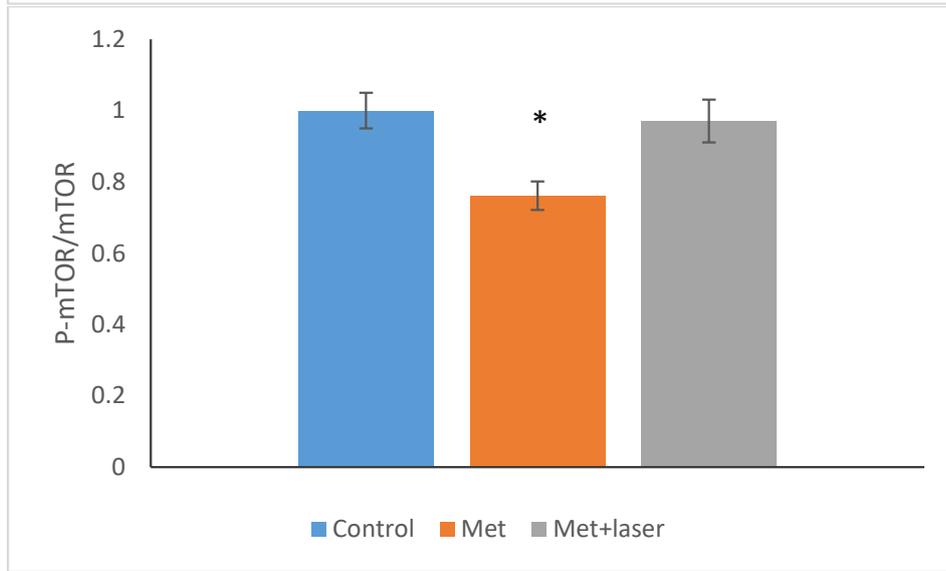
**a**

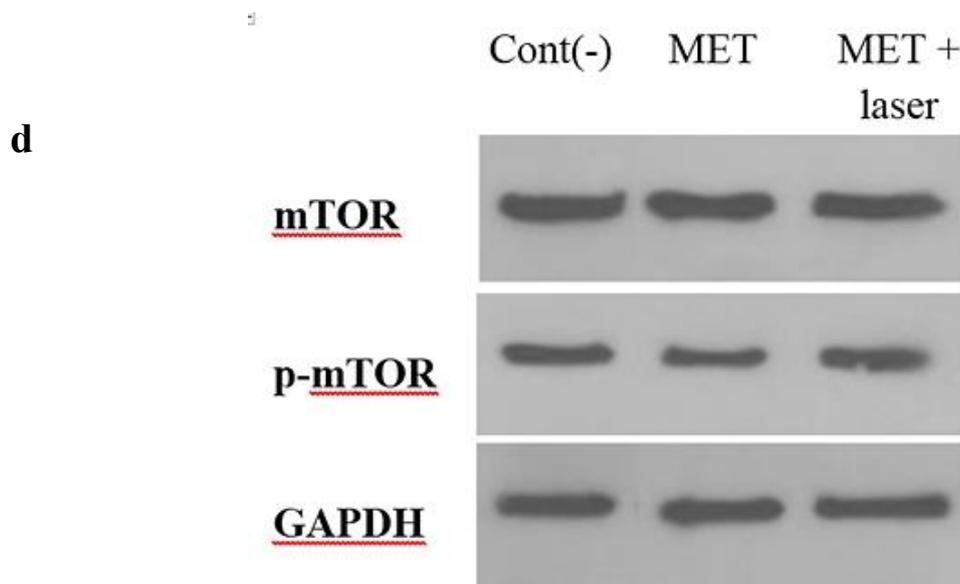


**b**



**c**





**Fig. (5).** HN5 cells were treated without or with metformin for 48 h. Metformin decreases both the mTOR and p-mTOR proteins in HN5 cells. Western blot showing expression of mTOR and p-mTOR (a, b). \* Statistical significance between the laser-radiated and control groups ( $p < 0.05$ ).

#### 4. DISCUSSION

PDT is a new method of cancer treatment [31]. In PDT, photosensitizers can rapidly decompose intracellular oxygen under laser irradiation and keep cells in a hypoxic state [32]. Metformin is a classic hypoglycemic drug, and it also has an anticancer effect through the AMPK signaling pathway [33]. However, the anticancer effect of metformin is very low in conventional therapeutic doses. Studies have confirmed that metformin concentrations in the millimolar range in preclinical models have the greatest anticancer potential [34], which is more than that of serum metformin with conventional antidiabetic doses. Therefore, metformin combination therapy with PDT for PDT-resistant tumors can be utilized for maximum therapeutic efficacy [35].

In the present study, we showed the simultaneous use of laser and metformin significantly increased the death of cancer cells, which means that these two had a synergistic effect. This effect was observed in high concentrations of metformin (20-160 mM). Also, the simultaneous use of laser and metformin increased cell death in normal HUGU cells, but the effect was greater on HN5 cancer cells. In this study, we used laser without using photosensitizer, but the simultaneous use of laser light and metformin increased the lethality of metformin, which requires further research to determine whether metformin itself is a functional photosensitizer.

Several previous studies have elucidated the mechanism of metformin's anti-tumor effects. Metformin can decrease cancer cell proliferation by altering numerous signaling pathways, the most well-studied of which is the mTOR/AMPK signaling system [30, 36]. Metformin, as an anti-diabetic biguanide is presumed to inhibit mTOR *via* AMPK stimulation of TSC1/2-dependent or -independent mechanisms by increasing the cellular AMP/ATP ratio [37]. In this investigation, we found that metformin activated the mTOR/AMPK signaling pathway

in HN5 cells consistent with previous studies [38].

#### CONCLUSION

It was proven that mTOR signaling regulates cell growth, proliferation, and metabolism, which has been linked to a number of human pathologies [39 - 41]. Also, mTOR signaling has a vital role in autophagy that is controlled by numerous factors such as mitogens, nutrients, and energy [42 - 45]. Our findings showed that metformin down-regulated the level of p-mTOR/mTOR protein, which was consistent with the results of other studies related to oral cancer [46 - 49] and other cancers [50]. According to previous studies, cells exposed to the laser can induce an increase in mTOR signaling [51]. As a result, in our study, despite the decreased ratio of p-mTOR/mTOR, the laser has affected the performance of metformin on p-mTOR/mTOR. Thus, this decrease was not significant in comparison to the control group. This study introduces the combination of metformin and laser as a new anti-tumor treatment, but it requires extensive studies for their anti-cancer effects in various types of cancer cells and *in vivo* studies.

#### LIST OF ABBREVIATIONS

<b>PKM2</b>	=	Pyruvate kinase M2
<b>GLUT1</b>	=	Glucose transporter
<b>mTOR</b>	=	Mammalian target of rapamycin
<b>PVDF</b>	=	Polyvinylidene difluoride

#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ethics code was received from the Ethics Committee of Tabriz University of Medical Sciences, and all the steps were performed after obtaining the code (IR.TBZMED.VCR.REC.1400.436).

## HUMAN AND ANIMAL RIGHTS

No humans/animals were used for studies that are the basis of this research.

## CONSENT FOR PUBLICATION

Not applicable.

## DATA AVAILABILITY STATEMENT

The raw/processed data can be shared upon request from the corresponding author [S.S].

## FUNDING

The Vice Chancellor for Research at Tabriz University of Medical Sciences provided financial support for this research that is greatly acknowledged (grant number 68889).

## CONFLICT OF INTEREST

Dr. S.Simin Sharifi and Solmaz Maleki Diza are the Editorial Advisory Board Members of The Open Dentistry Journal.

## ACKNOWLEDGEMENTS

Declared none.

## REFERENCES

- Samadi N, Tabasinezhad M, Ghanbari P, *et al.* Sphingosin 1-phosphate contributes in tumor progression. *J Cancer Res Ther* 2013; 9(4): 556-63. [http://dx.doi.org/10.4103/0973-1482.126446] [PMID: 24518696]
- Bakhshaiesh TO, Armat M, Shانهbandi D, *et al.* Arsenic trioxide promotes paclitaxel cytotoxicity in resistant breast cancer cells. *Asian Pac J Cancer Prev* 2015; 16(13): 5191-7. [http://dx.doi.org/10.7314/APJCP.2015.16.13.5191] [PMID: 26225652]
- Schiliro C, Firestein BL. Mechanisms of metabolic reprogramming in cancer cells supporting enhanced growth and proliferation. *Cells* 2021; 10(5): 1056. [http://dx.doi.org/10.3390/cells10051056] [PMID: 33946927]
- Sharifi S, Dalir Abdolahinia E, Ghavimi MA, *et al.* Effect of curcumin-loaded mesoporous silica nanoparticles on the head and neck cancer cell line, HN5. *Curr Issues Mol Biol* 2022; 44(11): 5247-59. [http://dx.doi.org/10.3390/cimb44110357] [PMID: 36354669]
- Maleki Dizaj S, Alipour M, Dalir Abdolahinia E, *et al.* Curcumin nanoformulations: Beneficial nanomedicine against cancer. *Phytother Res* 2022; 36(3): 1156-81. [http://dx.doi.org/10.1002/ptr.7389] [PMID: 35129230]
- Armat M, Oghabi Bakhshaiesh T, Sabzichi M, *et al.* The role of Six1 signaling in paclitaxel-dependent apoptosis in MCF-7 cell line. *Bosn J Basic Med Sci* 2016; 16(1): 28-34. [PMID: 26773176]
- Collier NJ, Haylett AK, Wong TH, *et al.* Conventional and combination topical photodynamic therapy for basal cell carcinoma: systematic review and meta-analysis. *Br J Dermatol* 2018; 179(6): 1277-96. [http://dx.doi.org/10.1111/bjd.16838] [PMID: 29889302]
- Chen B, Xiong L, Chen WD, *et al.* Photodynamic therapy for middle-advanced stage upper gastrointestinal carcinomas: A systematic review and meta-analysis. *World J Clin Cases* 2018; 6(13): 650-8. [http://dx.doi.org/10.12998/wjcc.v6.i13.650] [PMID: 30430120]
- Wang L, Yang H, Li B. Photodynamic therapy for prostate cancer: A systematic review and meta-analysis. *Prostate Int* 2019; 7(3): 83-90. [http://dx.doi.org/10.1016/j.pnil.2018.12.002] [PMID: 31485431]
- Agostinis P, Berg K, Cengel KA, *et al.* Photodynamic therapy of cancer: An update. *CA Cancer J Clin* 2011; 61(4): 250-81. [http://dx.doi.org/10.3322/caac.20114] [PMID: 21617154]
- Ang JM, Riaz IB, Kamal MU, Paragh G, Zeitouni NC. Photodynamic therapy and pain: A systematic review. *Photodiagn Photodyn Ther* 2017; 19: 308-44. [http://dx.doi.org/10.1016/j.pdpdt.2017.07.002] [PMID: 28716738]
- Calixto G, Bernegossi J, de Freitas L, Fontana C, Chorilli M. Nanotechnology-based drug delivery systems for photodynamic therapy of cancer: A review. *Molecules* 2016; 21(3): 342. [http://dx.doi.org/10.3390/molecules21030342] [PMID: 26978341]
- Cohen D, Lee P. Photodynamic therapy for non-melanoma skin cancers. *Cancers* 2016; 8(10): 90. [http://dx.doi.org/10.3390/cancers8100090] [PMID: 27782043]
- Lanoue J, Goldenberg G. Basal cell carcinoma: A comprehensive review of existing and emerging nonsurgical therapies. *J Clin Aesthet Dermatol* 2016; 9(5): 26-36. [PMID: 27386043]
- Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell* 2011; 144(5): 646-74. [http://dx.doi.org/10.1016/j.cell.2011.02.013] [PMID: 21376230]
- Bhattacharya B, Mohd Omar MF, Soong R. The Warburg effect and drug resistance. *Br J Pharmacol* 2016; 173(6): 970-9. [http://dx.doi.org/10.1111/bph.13422] [PMID: 26750865]
- Sánchez-Aragó M, Cuezva JM. The bioenergetic signature of isogenic colon cancer cells predicts the cell death response to treatment with 3-bromopyruvate, iodoacetate or 5-fluorouracil. *J Transl Med* 2011; 9(1): 19. [http://dx.doi.org/10.1186/1479-5876-9-19] [PMID: 21303518]
- Santamaria G, Martinez-Diez M, Fabregat I, Cuezva JM. Efficient execution of cell death in non-glycolytic cells requires the generation of ROS controlled by the activity of mitochondrial H<sup>+</sup>-ATP synthase. *Carcinogenesis* 2006; 27(5): 925-35. [http://dx.doi.org/10.1093/carcin/bgi315] [PMID: 16361271]
- Dong G, Mao Q, Xia W, *et al.* PKM2 and cancer: The function of PKM2 beyond glycolysis. *Oncol Lett* 2016; 11(3): 1980-6. [http://dx.doi.org/10.3892/ol.2016.4168] [PMID: 26998110]
- Courtney R, Ngo DC, Malik N, Ververis K, Tortorella SM, Karagiannis TC. Cancer metabolism and the Warburg effect: The role of HIF-1 and PI3K. *Mol Biol Rep* 2015; 42(4): 841-51. [http://dx.doi.org/10.1007/s11033-015-3858-x] [PMID: 25689954]
- Yecies JL, Manning BD. mTOR links oncogenic signaling to tumor cell metabolism. *J Mol Med (Berl)* 2011; 89(3): 221-8. [http://dx.doi.org/10.1007/s00109-011-0726-6] [PMID: 21301797]
- Coyle C, Cafferty FH, Vale C, Langley RE. Metformin as an adjuvant treatment for cancer: A systematic review and meta-analysis. *Ann Oncol* 2016; 27(12): 2184-95. [http://dx.doi.org/10.1093/annonc/mdw410] [PMID: 27681864]
- Heckman-Stoddard BM, DeCensi A, Sahasrabudhe VV, Ford LG. Repurposing metformin for the prevention of cancer and cancer recurrence. *Diabetologia* 2017; 60(9): 1639-47. [http://dx.doi.org/10.1007/s00125-017-4372-6] [PMID: 28776080]
- Checkley LA, Rho O, Angel JM, *et al.* Metformin inhibits skin tumor promotion in overweight and obese mice. *Cancer Prev Res* 2014; 7(1): 54-64. [http://dx.doi.org/10.1158/1940-6207.CAPR-13-0110] [PMID: 24196830]
- Osaki T, Yokoe I, Takahashi K, *et al.* Metformin enhances the cytotoxicity of 5-aminolevulinic acid-mediated photodynamic therapy *in vitro*. *Oncol Lett* 2017; 14(1): 1049-53. [http://dx.doi.org/10.3892/ol.2017.6237] [PMID: 28693272]
- Nenu I, Popescu T, Aldea MD, *et al.* Metformin associated with photodynamic therapy-a novel oncological direction. *J Photochem Photobiol B* 2014; 138: 80-91. [http://dx.doi.org/10.1016/j.jphotobiol.2014.04.027] [PMID: 24911275]
- Hamidi A, Sharifi S, Davaran S, Ghasemi S, Omidi Y, Rashidi MR. Novel aldehyde-terminated dendrimers; synthesis and cytotoxicity assay. *Bioimpacts* 2012; 2(2): 97-103. [PMID: 23678447]
- Vancura A, Bu P, Bhagwat M, Zeng J, Vancurova I. Metformin as an anticancer agent. *Trends Pharmacol Sci* 2018; 39(10): 867-78. [http://dx.doi.org/10.1016/j.tips.2018.07.006] [PMID: 30150001]
- Misiricki Marjanovic MS, Vuicevic LM, Despotovic AR, Stamenkovic MM, Janjetovic KD. Dual anticancer role of metformin: an old drug regulating AMPK dependent/independent pathways in metabolic, oncogenic/tumorsuppressing and immunity context. *Am J Cancer Res* 2021; 11(11): 5625-43. [PMID: 34873484]
- Olokpa E, Mandape SN, Pratap S, Stewart LMV. Metformin regulates multiple signaling pathways within castration-resistant human prostate cancer cells. *BMC Cancer* 2022; 22(1): 1025.

- [31] [http://dx.doi.org/10.1186/s12885-022-10115-3] [PMID: 36175875]  
Anand S, Yasinchak A, Bullock T. A non-toxic approach for treatment of breast cancer and its metastases: capecitabine enhanced photodynamic therapy in a murine breast tumor model. *J Cancer Metastasis Treat* 2019; 5: 6.  
[http://dx.doi.org/10.20517/2394-4722.2018.98]
- [32] Varol M. Photodynamic therapy assay. *Methods Mol Biol* 2020; 2109: 241-50.
- [33] Howell JJ, Hellberg K, Turner M, *et al.* Metformin inhibits hepatic mTORC1 signaling *via* dose-dependent mechanisms involving AMPK and the TSC complex. *Cell Metab* 2017; 25(2): 463-71.  
[http://dx.doi.org/10.1016/j.cmet.2016.12.009]
- [34] Chandel NS, Avizonis D, Reczek CR, *et al.* Are metformin doses used in murine cancer models clinically relevant? *Cell Metab* 2016; 23(4): 569-70.  
[http://dx.doi.org/10.1016/j.cmet.2016.03.010]
- [35] Pei X, Wang X, Xian J, *et al.* Metformin and oxyphotodynamic therapy as a novel treatment approach for triple-negative breast cancer. *Ann Transl Med* 2020; 8(18): 1138.  
[http://dx.doi.org/10.21037/atm-20-5704]
- [36] Lei Y, Yi Y, Liu Y, *et al.* Metformin targets multiple signaling pathways in cancer. *Chin J Cancer* 2017; 36(1): 17.  
[http://dx.doi.org/10.1186/s40880-017-0184-9] [PMID: 28126011]
- [37] Hu D, Xie F, Xiao Y, *et al.* Metformin: A potential candidate for targeting aging mechanisms. *Aging Dis* 2021; 12(2): 480-93.  
[http://dx.doi.org/10.14336/AD.2020.0702] [PMID: 33815878]
- [38] Lu R, Yang J, Wei R, *et al.* Synergistic anti-tumor effects of liraglutide with metformin on pancreatic cancer cells. *PLoS One* 2018; 13(6): e0198938.  
[http://dx.doi.org/10.1371/journal.pone.0198938] [PMID: 29897998]
- [39] Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell* 2017; 168(6): 960-76.  
[http://dx.doi.org/10.1016/j.cell.2017.02.004] [PMID: 28283069]
- [40] Zou Z, Tao T, Li H, Zhu X. mTOR signaling pathway and mTOR inhibitors in cancer: Progress and challenges. *Cell Biosci* 2020; 10(1): 31.  
[http://dx.doi.org/10.1186/s13578-020-00396-1] [PMID: 32175074]
- [41] Fernandes SA, Demetriades C. The multifaceted role of nutrient sensing and mTORC1 signaling in physiology and aging. *Frontiers in Aging* 2021; 2: 707372.  
[http://dx.doi.org/10.3389/fragi.2021.707372] [PMID: 35822019]
- [42] Sengupta S, Peterson TR, Sabatini DM. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell* 2010; 40(2): 310-22.  
[http://dx.doi.org/10.1016/j.molcel.2010.09.026] [PMID: 20965424]
- [43] Wang X, Proud CG. The mTOR pathway in the control of protein synthesis. *Physiology* 2006; 21(5): 362-9.  
[http://dx.doi.org/10.1152/physiol.00024.2006] [PMID: 16990457]
- [44] Mafi S, Mansoori B, Taeb S, *et al.* mTOR-mediated regulation of immune responses in cancer and tumor microenvironment. *Front Immunol* 2022; 12: 774103.  
[http://dx.doi.org/10.3389/fimmu.2021.774103] [PMID: 35250965]
- [45] Sun Y, Wang H, Qu T, *et al.* mTORC2: A multifaceted regulator of autophagy. *Cell Commun Signal* 2023; 21(1): 4.  
[http://dx.doi.org/10.1186/s12964-022-00859-7] [PMID: 36604720]
- [46] Gutkind JS, Molinolo AA, Wu X, *et al.* Inhibition of mTOR signaling and clinical activity of metformin in oral premalignant lesions. *JCI Insight* 2021; 6(17): e147096.  
[http://dx.doi.org/10.1172/jci.insight.147096] [PMID: 34255745]
- [47] Chomanicova N, Gazova A, Adamickova A, Valaskova S, Kyselovic J. The role of AMPK/mTOR signaling pathway in anticancer activity of metformin. *Physiol Res* 2021; 70(4): 501-8.  
[http://dx.doi.org/10.33549/physiolres.934618] [PMID: 34062070]
- [48] Seo Y, Kim J, Park SJ, *et al.* Metformin suppresses cancer stem cells through AMPK activation and inhibition of protein prenylation of the mevalonate pathway in colorectal cancer. *Cancers* 2020; 12(9): 2554.  
[http://dx.doi.org/10.3390/cancers12092554] [PMID: 32911743]
- [49] Wang Y, Xu W, Yan Z, *et al.* Metformin induces autophagy and G0/G1 phase cell cycle arrest in myeloma by targeting the AMPK/mTORC1 and mTORC2 pathways. *J Exp Clin Cancer Res* 2018; 37(1): 63.  
[http://dx.doi.org/10.1186/s13046-018-0731-5] [PMID: 29554968]
- [50] Sinnen-Smith J, Kisfalvi K, Kui R, Rozengurt E. Metformin inhibition of mTORC1 activation, DNA synthesis and proliferation in pancreatic cancer cells: Dependence on glucose concentration and role of AMPK. *Biochem Biophys Res Commun* 2013; 430(1): 352-7.  
[http://dx.doi.org/10.1016/j.bbrc.2012.11.010] [PMID: 23159620]
- [51] Pelliccioli ACA, Martins MD, Dillenburg CS, Marques MM, Squarize CH, Castilho RM. Laser phototherapy accelerates oral keratinocyte migration through the modulation of the mammalian target of rapamycin signaling pathway. *J Biomed Opt* 2014; 19(2): 028002.  
[http://dx.doi.org/10.1117/1.JBO.19.2.028002] [PMID: 24531144]

