ORIGINAL ARTICLE

The Immunomodulatory Effect of Anti-Cancer Drug Cisplatin on Colon Cancer SW480 Cell Line

[1]Alaa K. Hameed*, [2]Ifad K. Abd Al-Shibly, [3]Rana A. Ghaleb

[1]Department of Laboratories, Al-Hussein Teaching Hospital, Al-Muthanna Health Department Employee, Al-Muthanna City, Iraq

[2]Department of Microbiology, College of Medicine, Babylon University, Hilla, Iraq

[3]Department of Human Anatomy, College of Medicine, Babylon University, Hilla, Iraq

Key words:
ACE-2, Cancer, CD147, Chemotherapy, Cisplatin, ELISA, IL-17, Inflammatory cytokines, Therapeutic implications

Corresponding Author:
Alaa K. Hameed
Department of Laboratories, Al-Hussein Teaching Hospital, Al-Muthanna Health Department Employee, Al-Muthanna City, Iraq
dly76993@gmail.com

ABSTRACT

Background*: Cisplatin is a potent chemotherapeutic drug that is widely used and effective in cancer treatment, but its effect is rarely studied in the context of the dynamics of cancer cells.* ***Objectives****: This study aims to clarify the effect of cisplatin on a cancer cell line under normal circumstances, using untreated cells as a control. The effect of cisplatin on controlling some key biomarkers is investigated including CD147, IL-17 and ACE-2.* ***Methodology:*** *The cancer cell line was treated separately with different concentrations of cisplatin (,15.6,31.25, 62.5, 125, 250 and 500 µg/ml) as compared with the same cell line. The untreated cells were used as control cells. The concentrations of CD147, IL-17 and ACE-2 were estimated by ELISA kits.* ***Results****: In the presence of cisplatin, there is a clear downregulation in the expression of ACE-2 and CD147 in a dose-dependent manner, and the effect is significantly different ($p<0.05$) at concentrations above 31.25 µg/ml. The downregulation of ACE-2 and CD147 was significantly enhanced, and the difference was significant ($p<0.05$) at much lower concentrations of 15.6 µg/ml. The expression of IL-17 was inversely proportional to the concentration of cisplatin. At lower concentrations of cisplatin, IL-17 was suppressed, but this effect was limited to higher doses, indicating a threshold effect for IL-17 suppression.* ***Conclusion****: Cisplatin downregulated ACE-2 and CD147 in a cancer cell line. These findings suggest that cisplatin may be beneficial in treating patients with cancer, and the mechanism of action may include modulation of key cellular receptors and inflammatory cytokines involved in tumor pathophysiology.*

INTRODUCTION

The path of understanding between cancer treated with cisplatin is therefore imminent. This study ultimately adds to a growing body of evidence indicative of long-term respiratory morbidity being prevalent in cancer survivors [1]. It contributes to the paucity of literature discussing the impact of cytotoxic chemotherapy on the long-term outcomes, with a specific focus on cisplatin and the cisplatin family of drugs. The overarching objective is to provide a comprehensive literature review of primary articles containing the use of cisplatin in cancer cases presenting [2]. Quantitative and qualitative data were then extracted to address the primary review question focused on the long-term treatment outcomes when cancer patients receive cisplatin. This is of utmost importance clinically, in an era of constant change, when evidence, though comprehensive, is based on protocols that are rapidly changing [3]. A comprehensive review of the current best available evidence about how cisplatin may impact the outcomes, as the general impact with solid tumors has been fiercely debated since the beginning of the ongoing pandemic [4].

Considered one of the most effective antineoplastic agents, cisplatin plays a pivotal role in managing tumor growth. The worldwide spread of pandemics has raised concerns about cisplatin-treated cancer patients who may be infected. One of the key reasons for investigating the problem is the dysregulation of immunity, which can significantly complicate the course of therapy and increase tissue damage [5,6]. The lack of a holistic and comprehensive analysis emphasizing the reasons and effects of implementing therapy for cancer patients is especially evident. For this purpose, a comprehensive study was undertaken to provide an answer to the complex issues that apply to the practical aspect [7,8].

Compared to the approach of a physiologist examining the dysregulation of the immune system, the issues related to the clinical problems associated with the immune system are of great significance when therapy is implemented. The ongoing outbreaks reference the classical paradigms using this research to handle therapy that is aligned with patients' individual needs [9-11].

This study aims to clarify the effect of cisplatin on a cancer cell line under normal circumstances, using untreated cells as a control. The effect of cisplatin on controlling some key biomarkers is investigated including CD147, IL-17 and ACE-2.

METHODOLOGY

Cell Culture and Treatment Protocol:

The study adopted a cell line that is broadly employed in chemotherapeutic applications. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C, 5 per cent CO2 and 95 per cent humidity. Prior to treatments, cells were grown in 75 cm² culture flasks until they reached 70-80 per cent confluence. The experimental groups included control untreated cells; cells treated with cisplatin. Cisplatin concentrations were chosen according to preliminary cytotoxicity experiments and were set at15.6, 31.25, 62.5, 125,250 and 500 µg/ml to assess therapeutic and sub-lethal concentrations.

Cytotoxicity assay

The proliferation of cells was evaluated after 24 hours of 5× 10^3 cells per 0.1 mL seeding in 96-well microplates utilizing the 3(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay. The resulted MTT powder solution in phosphate-buffered saline was introduced into the wells of a 96-well microplate carrying a cell line categorized into five groups. The initial group of cells is designated as the control. The second group of cells was administered with varying concentrations of the drug cisplatin (15.6, 31.25, 62.5,125, 250 and 500 µg/ml). Dimethyl sulfoxide was introduced to the wells following 4 hours of incubation at 37°C, and the absorbance was quantified at 570 nm.

Quantitative ELISA procedures

CD147, IL-17 and ACE-2 proteins were quantified using the ELISA specific kits (see supplementary files). IL-17 (Cat.No E0142Hu), ACE-2 (Cat.No E3169Hu) and CD147 (Cat.No E3815Hu) ELISA kits protocols specified the use of pre-coated antibody plates, biotinylated detection antibodies and appropriate wash and substrate solutions. Absorbance was measured at 450 nm within 30 min following stop solution application, as per kit protocols, to determine the concentration of proteins in the supernatants of cell cultures.

Statistical analysis

Statistical software SPSS was used for the analysis, and the differences between the mean values of various treatment groups were analyzed using ANOVA tests and a statistically significant difference was determined by a p-value of less than 0.05. The group differences were examined with Tukey's test to specify the groups that have shown a statistically significant difference.

RESULTS

Quantitative analysis of protein

The result of this study shows that the protein expressions were modulated in a more complicated way when cells were exposed to the cisplatin. The results of the experiments were obtained using the ELISA kits and procedures detailed in the files (E0142Hu for IL-17, E3169Hu for ACE-2, and E3815Hu for CD147).

CD147

As the dose of cisplatin increased, CD147 expression significantly declined. At 15.6 µg/ml, CD147 level reduced by approximately 45% compared with the control ($P<0.05$) and 70% reduction at the highest concentration of 125 µg/ml ($P<0.01$).

The CD147 levels are reduced to 60 per cent ($p<0.05$) of their original level at 62.5 µg/ml and to under 20 per cent ($p<0.001$) of its original level at 125 µg/ml, a potentiation of the activity of cisplatin (Figure 1).

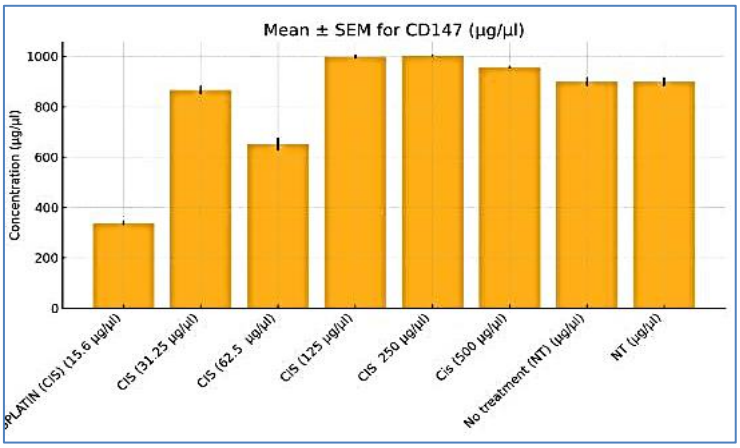

Fig. 1: The bar chart shows the quantitative levels of CD147 from ELISA test between control (untreated cells), cells treated with15.6,31.25, 62.5,125, 250 and 500 µM of cisplatin.

IL-17

The lower doses of cisplatin (62.5 µg/ml and 125 µg/ml) used initially reduced IL-17 by approximately 20-30 per cent (statistically insignificant). But at higher concentrations of 250 µg/ml, the IL-17 reductions were significant at approximately 50 per cent ($p<0.05$) (Figure 2).

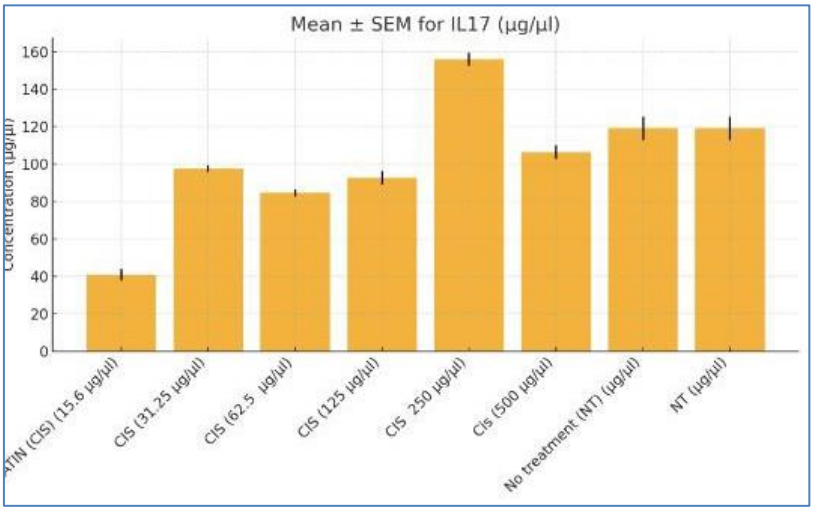

Fig. 2: The bar chart shows the quantitative levels of IL-17 from ELISA test between control (untreated cells), cells treated with 15.6,31.25, 62.5, 125, 250 and 500 µM of cisplatin.

ACE-2

In the presence of cisplatin only, there was a gradual decline in ACE-2 levels, reaching significant reductions at 31.25 µg/ml (30 per cent reduction, $p<0.05$) and further at 125 µg/ml (60 per cent reduction, $p<0.01$). ACE-2 expression was markedly reduced at 125 µg/ml ($p<0.05$) and 25% ($p<0.05$), and at the concentration of 100 µg/ml there was over 75% reduction ($p<0.001$) (Figure 3).

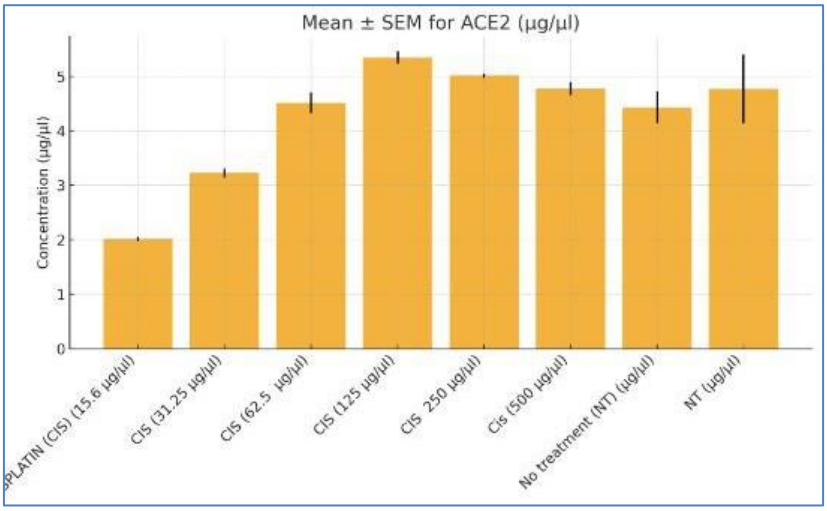

Fig. 3: The bar chart shows the quantitative levels of ACE2 from ELISA test between control (untreated cells), cells treated with 15.6,31.25, 62.5, 125, 250 and 500 µM of cisplatin.

Cytotoxicity assay

The cytotoxicity assay was done using MTT dyes with final desired concentration (5 mg/ml). The results indicate there was a significant decrease $P\leq 0.001$ for all used concentration in comparison to control group as represented in Figure (4).

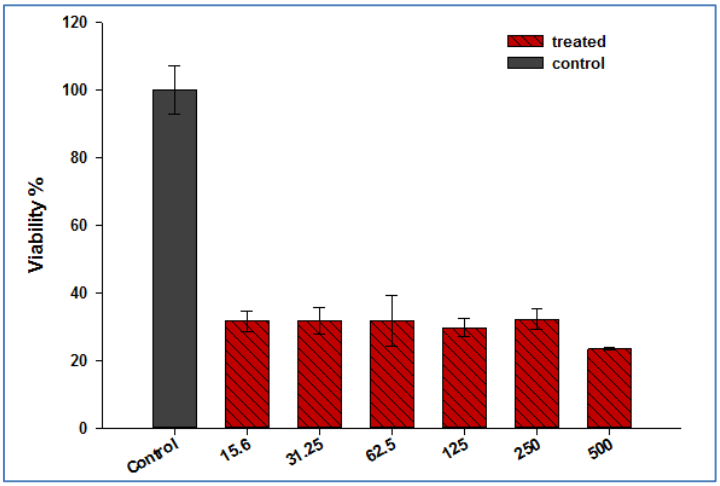

Fig. 4: Cancer cell (SW480) viability due the effect of cisplatin.

Photomicrograph of Crystal Violet s showed cell shrinkage and increased granularity observed increasingly in the combined treatment groups, consistent with the greater reductions in cell number (Figure 5).

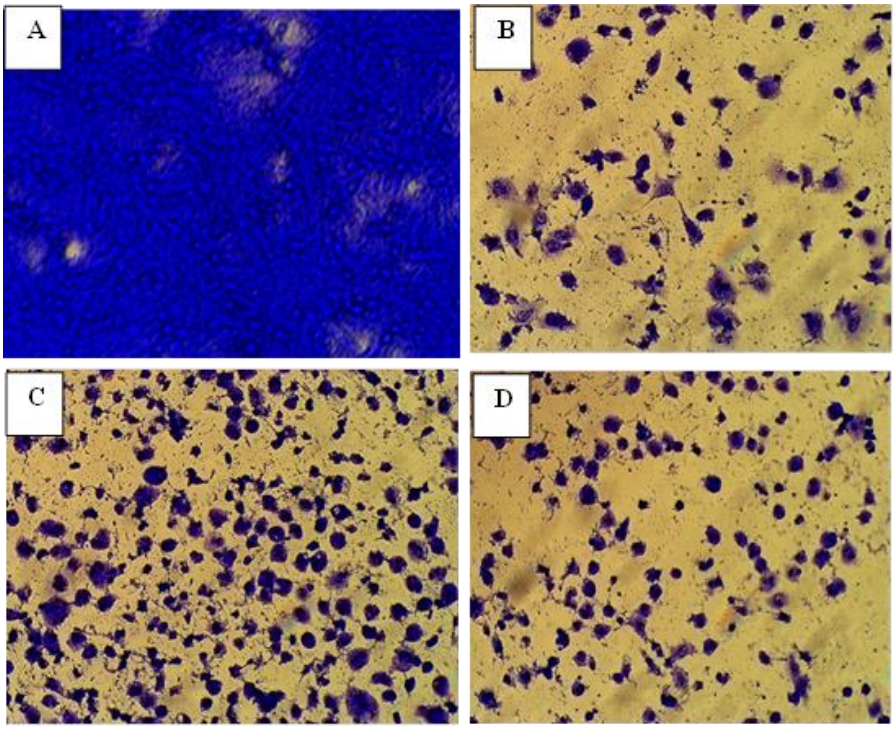

Fig. 5: Damage of cancer cells after treatment with Cisplatin kills cells by inducing apoptosis. **A**. Control: No clear cell damage. **B, C,** and **D**: Cisplatin-treated cells, in which there is chromatin condensing and cell shrinkage hallmark characteristic of apoptosis, where the cells become more compact and reduce in cell number in comparison to control.

DISCUSSION

Our study evaluated the effect of cisplatin on CD147, IL-17 and ACE-2 expression in cancer cell lines separately. CD147, also known as Basigin or EMMPRIN, is a transmembrane glycoprotein that is involved in several physiological and pathological processes, including tumor progression.

IL-17 is a pro-inflammatory cytokine, and an important driver of chronic inflammation and cancer progression. Elevated IL-17 levels have been demonstrated to be associated with severe inflammatory responses. The strong reduction in IL-17 following cisplatin treatment is consistent with previous findings indicating the anti-inflammatory nature of cisplatin in tumor environments [13]. The markedly enhanced anti-IL-

17 activity suggests that cisplatin can alleviate the hyperinflammatory state in cancer patients. This is relevant as IL-17 has been suggested as a target for therapeutic intervention to control the excessive immune responses [13].

The primary receptor for SARS-CoV-2, ACE-2 (angiotensin-converting enzyme 2), initially increased in expression at low cisplatin doses, but then stabilized. This biphasic response could indicate the cellular attempt to balance protective versus deleterious effects mediated by ACE-2, which has both roles [14-16]. Cisplatin modulated ACE-2 in a more variable manner, which could imply compounding, yet challenging to predict, interactions among cisplatin's cellular effects and induced dysregulation of ACE-2 pathways[16]. The nuanced modulation of ACE-2 by cisplatin in the present study might reflect a therapeutic balancing act, in which cisplatin maintains some degree of ACE-2 activity.

The results of this study agree with previously published data on the modulatory roles of chemotherapeutic agents on influential cellular markers in cancer [15, 17-21]. These data shed light on how cisplatin could potentially benefit cancer patients by inhibiting tumor growth and modulating the pathways of the immune system. Cisplatin could significantly improve survival rates of cancer patients. The results of the current study warrant more in-depth mechanistic studies to support the potential use of cisplatin in cancer patients, and clinical trials need to be carried out to confirm the promising preliminary findings.

Limitations of this study include the use of a single cell line that may not encompass the heterogeneity of cancer types. Future experiments should investigate these interactions in diverse cancer models and perform in vivo studies that allow for a more comprehensive understanding of systemic effects. Further work should also investigate the impact of cisplatin on the host cells, which may exhibit a different nature of interaction with the host's cellular pathways.

CONCLUSION

Cisplatin downregulated ACE-2 and CD147 in a cancer cell line. These findings suggest that cisplatin may be beneficial in treating cancer, and the mechanism of action may include modulation of key cellular receptors and inflammatory cytokines involved in tumor pathophysiology.

Ethical approval

The current study was conducted in accordance with the relevant regulations and code of World Medical Association (Declaration of Helsinki). The protocol was approved by the Committee of Research Ethics at the College of Medicine, Babylon University, Hilla, Iraq.

Declaration of interest: No competing interest is found in the current study as it is pronounced by the authors.
Funding: Self-funded.

Authors' Contributions

All stated authors contributed significantly, directly, and intellectually to the work and consented it to be published.

Acknowledgements
None.

REFERENCES

1. Lee LY, Cazier JB, Angelis V, Arnold R, Bisht V, Campton NA, Chackathayil J, et al. COVID-19 mortality in patients with cancer on chemotherapy or other anticancer treatments: a prospective cohort study. Lancet. 2020; 395 (10241): 1919–1926. https://doi.org/10.1016/S0140-6736(20)31173-9
2. Martín-Moro F, Marquet J, Piris M, Michael BM, Sáez AJ, Corona M, Jiménez C, Astibia B, García I, Rodríguez E, García-Hoz C. Survival study of hospitalised patients with concurrent COVID-19 and haematological malignancies. Br J Haematol. 2020; 190(1): e16-e20. https://doi.org/10.1111/bjh.16801
3. Agbarya A, Sarel I, Ziv-Baran T, Agranat S, Schwartz O, Shai A, Nordheimer S, Fenig S, Shechtman Y, Kozlener E, Taha T. Efficacy of the mRNA-based BNT162b2 COVID-19 vaccine in patients with solid malignancies treated with anti-neoplastic drugs. Cancers. 2021; 13(16):4191-4202. https://doi.org/10.3390/cancers13164191
4. Chavez-MacGregor M, Lei X, Zhao H, Scheet P, Giordano SH. Evaluation of COVID-19 mortality and adverse outcomes in US patients with or without cancer. JAMA Oncol. 2022;8(1):69-78. https://doi.org/10.1001/jamaoncol.2021.5148
5. Tchounwou PB, Dasari S, Noubissi FK, Ray P, Kumar S. Advances in our understanding of the molecular mechanisms of action of cisplatin in cancer therapy. J Exp Pharmacol. 2021;13 (3): 303-328. https://doi.org/10.2147/JEP.S267383
6. Ali R, Aouida M, Alhaj Sulaiman A, Madhusudan S, Ramotar D. Can cisplatin therapy be improved? Pathways that can be targeted. Int J Mol Sci. 2022;23(13):7241-7263. https://doi.org/10.3390/ijms23137241
7. Dasari S, Njiki S, Mbemi A, Yedjou CG, Tchounwou PB. Pharmacological effects of cisplatin combination with natural products in cancer chemotherapy. Int J Mol Sci. 2022;23(3):1532-1556. https://doi.org/10.3390/ijms23031532
8. Shruthi S, Bhasker Shenoy K. Cisplatin resistance in cancer therapy: Causes and overcoming

strategies. ChemistrySelect. 2024; 9 (25): e202401449-202401465. https://doi.org/10.1002/slct.202401449

9. Dogbey DM, Torres VE, Fajemisin E, Mpondo L, Ngwenya T, Akinrinmade OA, Perriman AW, Barth S. Technological advances in the use of viral and non-viral vectors for delivering genetic and non-genetic cargos for cancer therapy. Drug Deliv Transl Res. 2023;13(11):2719-2738. https://doi.org/10.1007/s13346-023-01362-3
10. Tong L, Liu D, Cao Z, Zheng N, Mao C, Liu S, He L, Liu S. Research status and prospect of non-viral vectors based on siRNA: a review. Int J Mol Sci. 2023;24(4):3375-3401. https://doi.org/10.3390/ijms24043375
11. Toner K, McCann CD, Bollard CM. Applications of cell therapy in the treatment of virus-associated cancers. Nat Rev Clin Oncol. 2024; 21(10):709-724. https://doi.org/10.1038/s41571-024-00930-x
12. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506. https://doi.org/10.1016/S0140-6736(20)30183-5
13. Wu D, Yang XO. TH17 responses in cytokine storm of COVID-19: an emerging target of JAK2 inhibitor Fedratinib. J Microbiol Immunol Infect. 2020;53(3):368-370. https://doi.org/10.1016/j.jmii.2020.03.005
14. Janabi AHD. Molecular Docking Analysis of Anti-Severe Acute Respiratory Syndrome-Coronavirus 2 Ligands against Spike Glycoprotein and the 3-Chymotrypsin-Like Protease. J Med Signals Sens. 2021;11(1):31-36. https://doi.org/10.4103/jmss.JMSS_25_20
15. Janabi AHD. Effective Anti-SARS-CoV-2 RNA Dependent RNA Polymerase Drugs Based on Docking Methods: The Case of Milbemycin, Ivermectin, and Baloxavir Marboxil. Avicenna J Med Biotechnol. 2020;12(4):246-250. https://pmc.ncbi.nlm.nih.gov/articles/PMC7502160/
16. Kai H, Kai M. Interactions of coronaviruses with ACE2, angiotensin II, and RAS: classical ideas and potential alternative mechanisms. J Cardiovasc Pharmacol. 2020;76(5):526-529. doi: 10.1038/s41440-020-0455-8
17. Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. Eur J Intern Med. 2020;76 (4):14-20. https://doi.org/10.1016/j.ejim.2020.04.037
18. Mahdi D, Alzeyadi M, AL-Sallami A. MicroRNA 29 gene Expression and Progesterone Receptor Values in Iraqi Women with Breast Cancer. Egypt. J. Med. Microbiol. 2025; 34(1): 149-155. https://doi.org/10.21608/ejmm.2024.319913.1337
19. Osman F, El Nabi S, El-Garawani I, Oraby E, Kamel M, Hathout H, Abo El-Ela M. Granzyme-B gene Polymorphisms and Susceptibility of Breast Cancer Patients in Egypt. Egypt. J. Med. Microbiol. 2025; 34(1): 165-174. https://doi.org/10.21608/ejmm.2024.331040.1362
20. Chen Z, Mi L, Xu J, Yu J, Wang X, Jiang J, Xing J, Shang P, Qian A, Li Y, Shaw PX, Wang J, Duan S, Ding J, Fan C, Zhang Y, Yang Y, Yu X, Feng Q, Li B, Yao X, Zhang Z, Li L, Xue X, Zhu P. Function of HAb18G/CD147 in invasion of host cells by severe acute respiratory syndrome coronavirus. J Infect Dis. 2005;191(5):755-760. https://doi.org/10.1086/427811
21. Gordon DE, Jang GM, Bouhaddou M, Xu J, Obernier K, White KM, O'Meara MJ, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature. 2020;583(7816):459-468. https://doi.org/10.1038/s41586-020-2286-9.