How to Cite:
Kassid, L. M., & Hamied, A. S. (2022). Sequencing of ca-int-l gene of Candida Spp. In infected urinary tract among Iraqi women. *International Journal of Health Sciences*, *6*(S7), 461–472.
https://doi.org/10.53730/ijhs.v6n7.11088

Sequencing of CA-INT-L gene of Candida Spp. In infected urinary tract among Iraqi women

Lina Mohammed Kassid
College of Education for Pure Science Ibn-Al-Haitham, University of Baghdad, Baghdad, Iraq

Atyaf Saied Hamied
College of Education for Pure Science Ibn-Al-Haitham,University of Baghdad, Baghdad, Iraq
Email: atyaf.s.h@ihcoedu.uobaghdad.edu.iq

***Abstract*---**The most common nosocomial fungal infection in hospitals is urinary tract candidiasis. Candida albicans is the most prevalent cause of nosocomial fungal urinary tract infections, however Candida species distribution is changing rapidly. At the same time, the rise in urinary tract candidiasis has resulted in the emergence of antifungal-resistant Candida species. This study aimed to diagnose Candida Spp. In women with UTI and reveal the nucleotides sequences of CA-INT-L Gene to look for mutation within the gene. This study included 100 women patients suffering from urinary tract infections and vaginal swabs samples from those individuals were taken to identify the presence of Candida. They were between the ages of 22 and 67. Candida identification has been accomplished using a variety of chemical and microscopically techniques. 100 samples have been clinically diagnosed with Candida Spp. But only 62 of the samples were positive by Vitik. This number has also reduced to only 25 after using the PCR. The sequence of the CA-INT-L showed different variations within the gene of the isolates.

***Keywords*---**Sequencing, urinary, among Iraqi

Introduction

Candida is a kind of microbe found in the gastrointestinal tract. (Crook, 1984; Truss, 1981). Candida spp. infections are commonly referred to as candidiasis (Schuster and Fisher 2022 ; Hamied , 2021)

Bloating, gas, diarrhea, and stomach cramps are some of the symptoms of candidiasis. Other symptoms include headaches, sinusitis, palpitations, and food and environmental sensitivities (Kogan, Castillo, and Barber 2016). When

Candida is present as a fungus in the intestines, the rhizomes pierce the gut walls, and endotoxins from the Candida overgrowth begin to permeate the rest of the body, this is known as polysystemic candidiasis (Basmaciyan et al. 2019). Candidiasis is the most common fungal infection, especially in people who have a weakened immune system. The use of long-acting antibiotics, immunosuppressive medications, and patients with HIV infection all contribute to a high prevalence of candidiasis (Arya N. R and Naureen B. Rafiq, 2022).

According to Abi-Said et al. (1997), the genus Candida contains approximately 154 species. Six species are the most often isolated from human illnesses. Candida albicans is the most common of these species. Candida tropicalls, Candida glabrata, Candida parapsilosis, Candida krusie, and Candida lusitaniae have all been identified as Candida infection causal agents. (Mohsin and Ali, 2021)

Candida albicans is the most prevalent pathogen isolated from urine, accounting for 50-70 percent of all pathogens identified. The next most prevalent species, Candida glabrata and Candida tropicalis, respectively account for 10-35 percent of cases. Candidaemia caused by C. glabrata is becoming more common in specific types of individuals, such as those with hematological malignancies and transplant recipients. Approximately 50% of all identified species in the biggest prospective study of kidney transplant recipients were C. glabrata, and 30% were C. albicans (Kim et al. 2019 ; Othman et al , 2018).

Method

This study comprised 100 women patients with urinary tract infections who visited the urology department at Al-yarmook teaching hospital, and vaginal swabs samples from those individuals were taken to identify the presence of Candida. They were between the ages of 22 and 67. Candida identification has been accomplished using a variety of chemical and microscopically techniques.

DNA extraction

The vaginal swabs samples have been briefly centrifuged. Then the DNA have been extracted from the swabs samples by using the commercial kit Fungal/Bacterial/ Yeast DNA MiniPrep™, (Catalog No. D6005). After the completion of the DNA extraction the eluted DNA have subjected to electrophoresis in order to ensure the accuracy of DNA extraction technique.

Polymerase chain reaction of INT gene

In order to amplify the target region within the CA-INT-L gene, a mixture prepared in a new sterile tube for each sample. The mixture reaction contains the following components; 5µl of Taq PCR PreMix, 10 picomols/µl (1 µl) of each sense primer and its anti-sense, 1.5µl of extracted DNA, then the final volume completed to 25µl by adding 16.5 µl of nuclease free water. The primers sequences that have been used to target the specific region are listed in the table (1). After the addition of each components, the tubes have sealed and placed in the thermal cycler machine and programmed as stated in table (2).

Table 1
Primer pair sequence targets the CA-INT-L gene

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5′ATAAGGGAAGTCGGCAAAATAGATCCGTA3′	59.3	40 %	532 base pair
Reverse	5′CCTTGGCTGTGGTTTCGCTAGATAGTAGAT3′	60.9	46 %	

Table 2
The cycle steps and holdings conditions of the PCR programs for the CA-INT-L gene amplification

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	3 min.	1 cycle
2-	Denaturation -2	95°C	45sec	35 cycle
3-	Annealing	72°C	45sec	
4-	Extension-1	72°C	1 min	
5-	Extension -2	72°C	7 min.	1 cycle

Results

The samples that have been collected were reached 100 samples that have already diagnosed with Candidiasis, but after usin the Vitik system it has shown that only 62 of the samples were positive. This number has also reduced to only 25 after using the PCR. This distribution of the samples are shown in the figure (1).

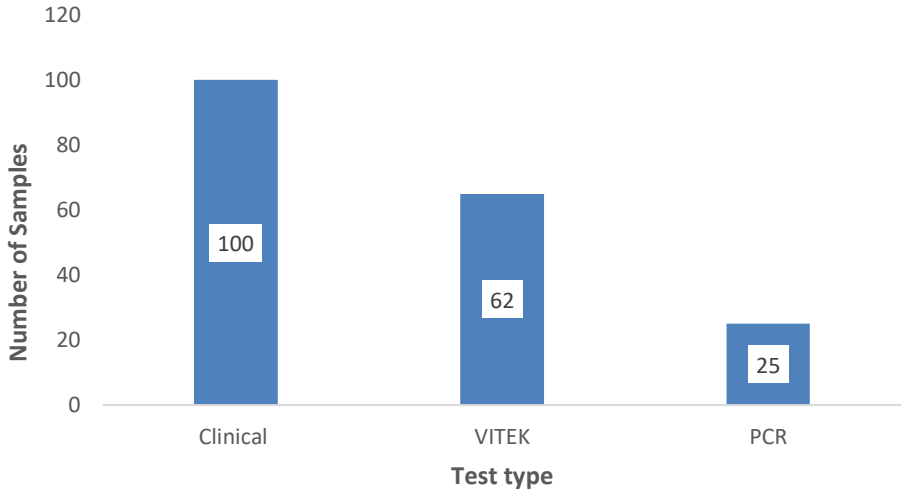

Figure (1); distribution of the positive samples according to the test that have been used

The amplified products have went through gel electrophoresis and the results of gel documentation are shown in figure (2). The results showed sharp single band with 532 base pair long.

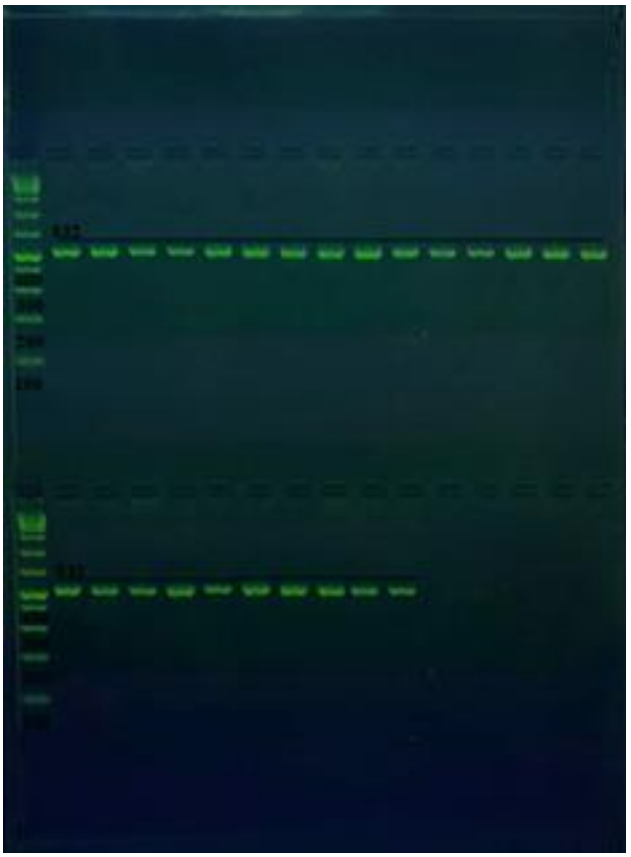

Figure (2) PCR product the band size 532 bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder (1000Plus).

The amplified products have been sent to Macrogene company for sequencing, and once the results have received the nucleotides sequences have been analyzed by BLASTn tool from NCBI database and the results are shown in the figure (3). The results of sequences have been aligned to plus strand of Candida albicans isolate region internal transcribed spacer 1 under the ID; ON010023.1 and scored 187 bits the length of database sequence showed 95% similartities with the sequences of that obtained from this study.

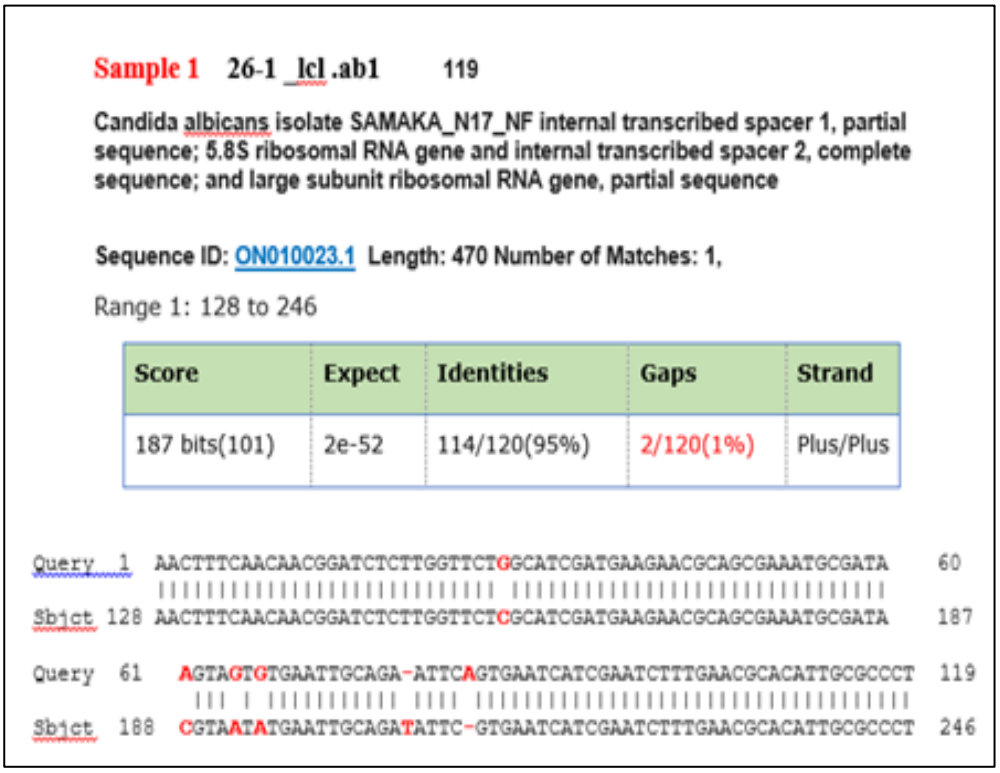

Figure (3); sequence alignment for one of the isolated samples in this study

The sequences alignment of all the samples are summarized in table (3). All the samples have compared to the same database sequence which can be reached online by the ID; ON010023.1.

The first sample showed 6 variations and included, three which are G/C at 56, G/A in both 65 and 67bp. And one transversion at nucleotide 61 which is A/C. Two gaps are also shown that correlated to the nucleotide T at position 79bp and to A at position 84bp. The second group showed eight variations which include 4 transitions; G/A at 194bp, A/G at 204bp, G/A at 223bp, and G/A 259bp. One transversion and one transition also shown one in nucleotide 188 A/C and C/T at 246. Two gaps shown in 206 and 211bp. The third group (sample6) showed five variation with no gaps between the two strands. The forth group (sample 7) showed the higher number of variations, nine that included; five; A/G at 140, G/C at 156, C/G at 189, G/A at 194, G/A at 224. Two trans version C/T at 155 and A/C at 188, two gaps include T at 206. And at 211. The fifth group (sample 8) showed four transition, and three trans version. And also showed the same positions of gaps as the other samples (positions 206bp and 211bp). The sixth group (sample 9) six transversion, and one C/T at 246.

Candida albicans						
No.	**Type of substitution**	**Location**	**Nucleotide**	**Sequence ID with compare**	**Source**	**Identities**
1	**Transversion**	56	G/C	ID: ON010023.1	Candida albicans isolate SAMAKA_N17_NF internal transcribed spacer 1	95%
	Transversion	**61**	**A/C**			
	Transition	**65**	**G/A**			
	Transition	**67**	**G/A**			
	Gap	**79**	**T**			
	Gap	**84**	**A**			
4	**Transversion**	**188**	**A/C**	**ID: ON010023.1**	**Candida albicans isolate SAMAKA_N17_NF internal transcribed spacer 1**	**95%**
	Transition	**194**	**G/A**			
	Transition	**204**	**A/G**			
	Gap	**206**	**T**			
	Gap	**211**	**A**			
	Transition	**223**	**G/A**			
	Transition	**246**	**C/T**			
	Transition	**259**	**G/A**			
6	**Transition**	**134**	**T/C**	**ID: ON010023.1**	**Candida albicans isolate SAMAKA_N17_NF internal transcribed spacer 1**	**95%**
	Transition	**140**	**T/C**			
	Transition	**174**	**T/C**			

	Transition	177	A/G			
	Transition	180	G/A			
7	Transition	140	A/G	ID: ON010023.1	Candida albicans isolate SAMAKA_N17_NF internal transcribed spacer 1	95%
	Transition	155	C/T			
	Transversion	156	G/C			
	Transition	188	A/C			
	Transversion	189	C/G			
	Transversion	194	G/A			
	Gap	206	T			
	Gap	211	A			
	Transversion	224	G/A			
8	Transition	155	C/T	ID: ON010023.1	Candida albicans isolate SAMAKA_N17_NF internal transcribed spacer 1	95%
	Transversion	156	G/C			
	Transversion	174	C/A			
	Transversion	175	C/G			
	Transition	188	A/C			
	Transitio	194	G/A			

	n					
	Gap	**206**	**T**			
	Gap	**211**	**A**			
	Transition	**224**	**G/A**			
9	**Transversion**	**188**	**A/C**	**ID: ON010023.1**	**Candida albicans isolate SAMAKA_N17_NF internal transcribed spacer 1**	**95%**
	Transversion	**189**	**C/G**			
	Transition	**192**	**G/A**			
	Transition	**194**	**G/A**			
	Gap	**245**	**T**			
	Gap	**249**	**C**			
	Transition	**223**	**G/A**			
	Transition	**246**	**C/T**			
	Transition	**259**	**G/A**			

Discussion

Invasive candidiasis (IC) is a leading cause of death and morbidity in the United States. Candida spp. account for 8 to 9% of all nosocomial bloodstream hospital acquired infection, with the risk being greater in the intensive care unit (ICU) and among cancer patients. Premortem diagnosis is not made in about half of the instances (Edmond et al., 1999).

Blood culture is the current gold standard for diagnosing IC, and it takes 24 to 48 hours for it to turn positive. It's possible that identifying the exact Candida sp. will take much longer, delaying antifungal therapy (Horn et al., 2009). Studies consistently demonstrate that delaying proper antifungal medication for 12 to 48 hours is linked with considerably higher all-cause mortality that is independent of other risk factors; adjusted odds ratios vary from 2.17 to 4.75 (Bassetti et al., 2007).

Various non-culture laboratory approaches for quick IC diagnosis have been developed during the last few decades. The polymerase chain reaction (PCR) is a promising technique for identifying fungal diseases, with high throughput, sensitivity, and specificity. A meta-analysis of 54 trials found that the pooled sensitivity and specificity for the diagnosis of candidemia were 0.95 and 0.92, respectively (Avni et al., 2011). This study succeeded to detect the presence of Candida in vaginal swabs samples, in contrast to what have recommended by previous study using the whole blood as the optimal sample for the detection of *Candida* DNA (He et al., 2020).

The results of PCR failed to showed false negative samples, this results disagree with previous study that showed two false-negative results by PCR assay (sensitivity: 94.1%) (Jiang et al. 2016). Targeting the 18sRNA gene have been previously showed numerous benefits upon using other regions. Previous study has demonstrated the efficacy of the 18S rRNA gene to detect fungal pathogen in clinical samples (Embong et al. 2008).

The sequence alignment results of the isolated samples to the NCBI database showed high number of variations in the isolated samples of this study, as it has shown previously by earlier study that loss-of-heterozygosity events were commonly associated with acquired resistance (Ford et al., 2005).

References

- Ford, C. B., Funt, J. M., Abbey, D., Issi, L., Guiducci, C., Martinez, D. A., Delorey, T., Li, B. Y., White, T. C., Cuomo, C., Rao, R. P., Berman, J., Thompson, D. A., & Regev, A. (2015). The evolution of drug resistance in clinical isolates of Candida albicans. eLife, 4, e00662. https://doi.org/10.7554/eLife.00662
- Arya N. R, and Naureen B. Rafiq. "Candidiasis - StatPearls - NCBI Bookshelf." https://www.ncbi.nlm.nih.gov/books/NBK560624/ (March 27, 2022).
- Basmaciyan, Louise et al. 2019. "'Candida Albicans Interactions With The Host: Crossing The Intestinal Epithelial Barrier.'" Tissue Barriers 7(2): 1612661. /pmc/articles/PMC6619947/ (March 27, 2022).
- Embong, Zunaina et al. 2008. "Specific Detection of Fungal Pathogens by 18S RRNA Gene PCR in Microbial Keratitis." BMC Ophthalmology 8(1): 1–8. https://bmcophthalmol.biomedcentral.com/articles/10.1186/1471-2415-8-7 (April 21, 2022).
- Jiang, Xiaoqun et al. 2016. "Rapid Detection of Candida Albicans by Polymerase Spiral Reaction Assay in Clinical Blood Samples." Frontiers in Microbiology 7(JUN): 916.
- Kim, Sang Jin et al. 2019. "Management of Candida Urinary Tract Infection in the Elderly." Urogenit Tract Infect 14(2): 33–2019. http://crossmark.crossref.org/dialog/?doi
- =10.14777/uti (March 27, 2022).
- Hamied, A. S. (2021) Candida and Candidiasis – A review of Virulence Factors . J. Biotechno. Res. Cen. Vol.15(2) : 13 – 20 PP.

- Kogan, Mikhail, Carlos Cuellar Castillo, and Melissa S. Barber. 2016. “Chronic Rhinosinusitis and Irritable Bowel Syndrome: A Case Report.” Integrative Medicine: A Clinician’s Journal 15(3): 44. /pmc/articles/PMC4982648/ (March 27, 2022).
- Schuster, Jennifer E., and Brian T. Fisher. 2022. “Candidiasis.” Pediatric Transplant and Oncology Infectious Diseases: 195-205.e3. https://www.ncbi.nlm.nih.gov/books/NBK560624/ (March 27, 2022).
- Mohsin, Z. A. and Ali, W. Sh. (2021) . Antagonistic Activity of Bacteriocin-producing Lactobacillus Against Candida spp . Iraqi J. Sci. Vol. 62(7) : 2153 – 2162 PP.
- Othman, K. I. , Abdullah, Sh. M. , Ali, B. and Majid, M. (2018) . Isolation and Identification Candida spp from Urine and Antifungal
- Susceptibility Test . Iraqi J. Sci. Vol. 59(4B) : 1981 – 1988 PP.
- He, Zheng-Xin, Zhao, Hui-Hai and Wang, Fu-Kun. "PCR-detectable Candida DNA exists a short period in the blood of systemic candidiasis murine model" Open Life Sciences, vol. 15, no. 1, 2020, pp. 677-682
- Edmond M. B., et al. 1999. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. Clin. Infect. Dis. 29:239–244
- Horn D. L., et al. 2009. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin. Infect. Dis. 48:1695–1703
- Jalil, A. T., Kadhum, W. R., Khan, M. U. F., Karevskiy, A., Hanan, Z. K., Suksatan, W., ... & Abdullah, M. M. (2021). Cancer stages and demographical study of HPV16 in gene L2 isolated from cervical cancer in Dhi-Qar province, Iraq. Applied Nanoscience, 1-7. https://doi.org/10.1007/s13204-021-01947-9
- Widjaja, G., Jalil, A. T., Rahman, H. S., Abdelbasset, W. K., Bokov, D. O., Suksatan, W., ... & Ahmadi, M. (2021). Humoral Immune mechanisms involved in protective and pathological immunity during COVID-19. Human Immunology. https://doi.org/10.1016/j.humimm.2021.06.011
- Moghadasi, S., Elveny, M., Rahman, H. S., Suksatan, W., Jalil, A. T., Abdelbasset, W. K., ... & Jarahian, M. (2021). A paradigm shift in cell-free approach: the emerging role of MSCs-derived exosomes in regenerative medicine. Journal of Translational Medicine, 19(1), 1-21. https://doi.org/10.1186/s12967-021-02980-6
- Saleh, M. M., Jalil, A. T., Abdulkereem, R. A., & Suleiman, A. A .(2020). Evaluation of Immunoglobulins, CD4/CD8 T Lymphocyte Ratio and Interleukin-6 in COVID-19 Patients. TURKISH JOURNAL of IMMUNOLOGY, 8(3), 129-134. https://doi.org/10.25002/tji.2020.1347
- Turki Jalil, A., Hussain Dilfy, S., Oudah Meza, S., Aravindhan, S., M Kadhim, M., & M Aljeboree, A. (2021). CuO/ZrO2 nanocomposites: facile synthesis, characterization and photocatalytic degradation of tetracycline antibiotic. Journal of Nanostructures. https://doi.org/10.22052/JNS.2021.02.014
- Sarjito, Elveny, M., Jalil, A., Davarpanah, A., Alfakeer, M., Awadh Bahajjaj, A. & Ouladsmane, M. (2021). CFD-based simulation to reduce greenhouse gas emissions from industrial plants. International Journal of Chemical Reactor Engineering, 20210063. https://doi.org/10.1515/ijcre-2021-0063

- Marofi, F., Rahman, H. S., Al-Obaidi, Z. M. J., Jalil, A. T., Abdelbasset, W. K., Suksatan, W., ... & Jarahian, M. (2021). Novel CAR T therapy is a ray of hope in the treatment of seriously ill AML patients. Stem Cell Research & Therapy, 12(1), 1-23. https://doi.org/10.1186/s13287-021-02420-8
- Jalil, A. T., Shanshool , M. T. ., Dilfy, S. H. ., Saleh, M. M., & Suleiman, A. A. . (2022). HEMATOLOGICAL AND SEROLOGICAL PARAMETERS FOR DETECTION OF COVID-19. Journal of Microbiology, Biotechnology and Food Sciences, e4229. https://doi.org/10.15414/jmbfs.4229
- Vakili-Samiani, S., Jalil, A. T., Abdelbasset, W. K., Yumashev, A. V., Karpisheh, V., Jalali, P., ... & Jadidi-Niaragh, F. (2021). Targeting Wee1 kinase as a therapeutic approach in Hematological Malignancies. DNA repair, 103203. https://doi.org/10.1016/j.dnarep.2021.103203
- NGAFWAN, N., RASYID, H., ABOOD, E. S., ABDELBASSET, W. K., Al-SHAWI, S. G., BOKOV, D., & JALIL, A. T. (2021). Study on novel fluorescent carbon nanomaterials in food analysis. Food Science and Technology. https://doi.org/10.1590/fst.37821
- Marofi, F., Abdul-Rasheed, O. F., Rahman, H. S., Budi, H. S., Jalil, A. T., Yumashev, A. V., ... & Jarahian, M. (2021). CAR-NK cell in cancer immunotherapy; A promising frontier. Cancer Science, 112(9), 3427. https://doi.org/10.1111/cas.14993
- Abosaooda, M., Wajdy, J. M., Hussein, E. A., Jalil, A. T., Kadhim, M. M., Abdullah, M. M., ... & Almashhadani, H. A. (2021). Role of vitamin C in the protection of the gum and implants in the human body: theoretical and experimental studies. International Journal of Corrosion and Scale Inhibition, 10(3), 1213-1229. https://dx.doi.org/10.17675/2305-6894-2021-10-3-22
- Jumintono, J., Alkubaisy, S., Yánez Silva, D., Singh, K., Turki Jalil, A., Mutia Syarifah, S., ... & Derkho, M. (2021). Effect of Cystamine on Sperm and Antioxidant Parameters of Ram Semen Stored at 4° C for 50 Hours. Archives of Razi Institute, 76(4), 923-931. https://dx.doi.org/10.22092/ari.2021.355901.1735
- Raya, I., Chupradit, S., Kadhim, M. M., Mahmoud, M. Z., Jalil, A. T., Surendar, A., ... & Bochvar, A. N. (2021). Role of Compositional Changes on Thermal, Magnetic and Mechanical Properties of Fe-PC-Based Amorphous Alloys. Chinese Physics B. https://doi.org/10.1088/1674-1056/ac3655
- Chupradit, S., Jalil, A. T., Enina, Y., Neganov, D. A., Alhassan, M. S., Aravindhan, S., & Davarpanah, A. (2021). Use of Organic and Copper-Based Nanoparticles on the Turbulator Installment in a Shell Tube Heat Exchanger: A CFD-Based Simulation Approach by Using Nanofluids. Journal of Nanomaterials. https://doi.org/10.1155/2021/3250058
- Mohaddeseh Rahbaran, Ehsan Razeghian, Marwah Suliman Maashi, Abduladheem Turki Jalil, Gunawan Widjaja, Lakshmi Thangavelu, Mariya Yurievna Kuznetsova, Pourya Nasirmoghadas, Farid Heidari, Faroogh Marofi, Mostafa Jarahian, "Cloning and Embryo Splitting in Mammalians: Brief History, Methods, and Achievements", Stem Cells International, vol. 2021, Article ID 2347506, 11 pages, 2021. https://doi.org/10.1155/2021/2347506

- Jalil, A.T.; Ashfaq, S.; Bokov, D.O.; Alanazi, A.M.; Hachem, K.; Suksatan, W.; Sillanpää, M. High-Sensitivity Biosensor Based on Glass Resonance PhC Cavities for Detection of Blood Component and Glucose Concentration in Human Urine. Coatings **2021**, 11, 1555. https://doi.org/10.3390/coatings11121555
- Chupradit, S.; Ashfaq, S.; Bokov, D.; Suksatan, W.; Jalil, A.T.; Alanazi, A.M.; Sillanpaa, M. Ultra-Sensitive Biosensor with Simultaneous Detection (of Cancer and Diabetes) and Analysis of Deformation Effects on Dielectric Rods in Optical Microstructure. Coatings **2021**, 11, 1564. https://doi.org/10.3390/coatings11121564
- Bassetti M., et al. 2007. Incidence, risk factors, and predictors of outcome of candidemia. Survey in 2 Italian university hospitals. Diagn. Microbiol. Infect. Dis. 58:325–331
- Avni T, Leibovici L, Paul M. PCR diagnosis of invasive candidiasis: systematic review and meta-a
- Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. International Journal of Health Sciences, 6(1), i-v. https://doi.org/10.53730/ijhs.v6n1.5949