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ORIGINAL ARTICLE

Detection of HPV16 viral load in L2 gene as a related predictor of cervical cancer among women in Dhi-Qar province by qRT-PCR

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Abstract

Background The most common infection among young women that increases the risk of developing cervical cancer (CC) is human papillomavirus (HPV). In this study, we are going to assess whether HPV16 DNA concentration helps indicate cervical cancer progression ,As well as for age groups and their relationship to cervical cancer.

Methods Present study included 93 adult females suffering from cervical cancer during the period from 2017 to 2020. Molecular detection of HPV was done using amplification of *the L2* gene (minor capsid protein).

Results Present results showed that 60 (65%) of the patients from 93 cervical cancer cases were infected by HPV16 while only 5 (8%) of healthy patients from the control group were positive for HPV16. So, the current study revealed high HPV16 load in cervical cancer ranged from 1.09×10^2 IU/ml to 5.07×10^3 IU/ml with a mean ± SD of viral load was 1043.25 ± 8.50 IU/ml while in healthy individuals very low viral load ranging from 88 IU/ml to 101 IU/ml and mean ± SD of viral load was 91.25 ± 2.90 IU/ml was reported.

Conclusion HPV16 viral load is significantly associated with cervical carcinoma among women in Dhi-Qar Province.

Keywords HPV16 · Viralload · Cervical cancer · qRT-PCR · *L2* gene · hrHPV16

Introduction

Globally, cervical cancer is the second most common malignant condition found in women. For the development of cervical cancer, persistent oncogenic type Human papillomavirus HPV infection has been proved as a biological intermediate [1, 2]. There are 12 types of Human papillomavirus including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 that has been considered to have oncogenic properties [3]. A study conducted among women who are 16 years to 29 years of age has shown that yearly, 42% and 56% of the women are infected with HPV 16 and HPV 18 respectively [4]. In persistent infections, the viral load for HPV 16 and 18 remained higher and it was concluded that hrHPV viral load can be used as a marker for persisting infections. Probably it helps in differentiating between regressing intraepithelial neoplasia2 (CIN2) and CIN3 lesions [5]. This can also help in assessing the development of the same in cervical

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cancer [6] using serial High risk Human Papillomavirus viral load measurements. Previous studies have been able to diagnose the after effect of High risk Human Papillomavirus (hrHPV) infection. The degree of CIN based on 2–3 consecutive measurements also suggested the potential of using this parameter in HPV triage in women who are HPV positive [7]. The Increased HPV viral load has also been shown to be an alternative indicator for persistence. In predicting the risk of squamous intraepithelial lesions (SIL), the role of HPV viral load has also been reported [8, 9]. Besides, HPV 16 infections were revealed to last longer than other forms of oncogenic HPV. For measuring HPV viral load, there are many PCR assays. Recent studies use methods that target HPV16 E6 and L1 genes based on quantitative real-time PCR (qRT-PCR). In this study, we will concentrate on the genetics of the small capsid protein, L2, which plays a key role during the assembly of virions [10, 11]. L2, which is approximately 500 amino acids long have an estimated molecular mass of 55KDa [11]. Thus, the current study sought to determine whether hrHPV16 viral load can be used as a predictor of cervical cancer progression in Dhi-Qar Province via qRT-PCR amplification of minor capsid protein L2.

Materials and methods

Sample collection

Samples were collected from Al-Hussein Teaching Hospital in Dhi-Qar Province/Iraq in the Histopathology Laboratory, and 93 samples were taken from the cervical cancer tissue blocks they were stored in the period from 2017 to 2020. Samples were registered according to the name and age of the patient, histologically diagnosed, and divided according to the stage of cancer development. Another 60 healthy women were included in the control groups. The approval from the institutional ethics committee of Al-Hussein Teaching Hospital was taken, (Approval Number: 66 on 16.03.2020).

DNA extraction

The HPV16 DNA was extracted from formalin-fixed paraffin-embedded tissue blocks by using G-spinTM Total DNA Extraction Kit (Fixed tissues protocol) as per the company instruction. The viral DNA concentration and purification perform by using a Nanodrop spectrophotometer (THERMO. USA) by reading the absorbance at (260/280 nm).

Primer and qRT-PCR

Human papillomavirus qPCR primer for minor capsid protein *L2* gene (F: TGAAAATCCCGCCTTTGAGC and R: TGTGCCTTCAGGT GTTTCAC) were designed in this study using NCBI-Genbank database sequence (MH777342.2). PCR master mix was done (RealMODTM Green SF 2 × qPCR mix) as per the company instructions. The PCR master mix component was then centrifuged using Exispin vortex centrifuge at 3000 rpm for 3 min. The threshold cycle number (CT value) was used for real-time data analysis.

Statistical analysis

The data thus obtained were analyzed statistically. A student's *t*-test was performed for independent variables. *U* Mann-Whitney test was done for dependent variables. STATISTICA 10 PL software and Statistical Package for Social Sciences version 20 was used for statistical analysis with Microsoft Excel 2010. Only results that had a p-value < 0.05 were considered statically significant [12–15] .

Results

In the present study, The SYBR green qRT-PCR method was used for quantification of the HPV16 *L2* gene (Fig. 1) and the results showed that among 93 women with cervical cancer there are 60 (65%) cases that are positive for hrHPV16 and only 5 (8%) were positive in the healthy control group (Fig. 2). Depending on the viral copy number, viral load was calculated and significant differences appeared between the case and the control in the level of viral load ($P = 0.001$). The present study revealed high hrHPV16 load in cervical cancer ranging from 1.09×10^2

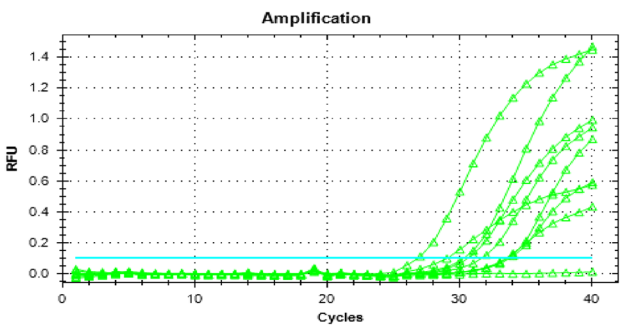

Fig. 1 Real-time PCR amplification plot of *L2* gene for detection of HPV-16

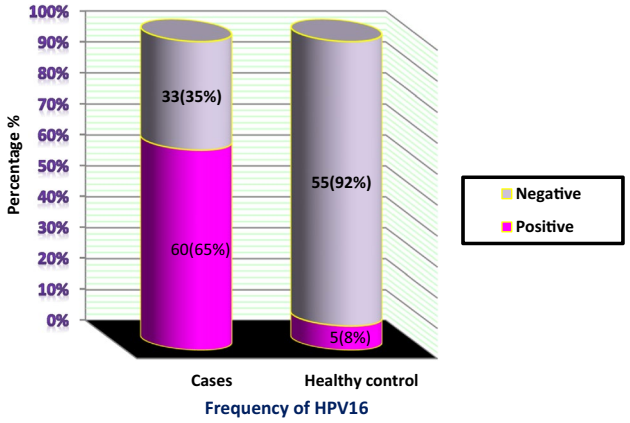

Fig. 2 Frequency of HPV16 in studied groups

Table 1 Frequency of HPV16 in studied groups

Viral load (IU/ml)	Case-control comparison		P-value
	Case	Control	
Range	1.09×10^2–5.07×10^3	88–101	
Mean ± SD	1043.25 ± 8.50	91.25 ± 2.90	0.001*
SE	1.097	0.374	
N.	60	60	

*Significant correlation ($p<0.05$)

IU/ml to 5.07×10^3 IU/ml with a mean ± SD of viral load of 1043.25 ± 8.50 IU/ml while healthy individuals have very low viral load ranging from 88 IU/ml to 101 IU/ml and mean ± SD of viral load of 91.25 ± 2.90 IU/ml was reported (Table 1).

Distribution of viral load according to patients' age groups in (Fig. 3) revealed that females with cervical cancer in the age group of 32–42 years and 43–52 years have the highest mean of hrHPV 16 loads but the mean of viral load was close in the age group 53–62 years and 63–78 years. However, these results showed statistically significant differences in the delivery of viral load according to patients' age groups (P = 0.021, $X^2 = 9.77$, DF = 3). Moreover, the Distribution of viral load according to cervical cancer stages also exposed statically significant differences (P = 0.001, $X^2 = 55.8$, DF = 4) when the highest mean (4022 IU/ml) of HPV16 load was detected in females who undergo from spread to distant cancer (stage III). The HPV16 load also showed an increase in patients in the cancer stages IV and II (3200 IU/ml and 3172 IU/ml respectively) whereas the lowest mean (987 IU/ml) of viral load appeared in stage I of cervical cancer as in (Fig. 4).

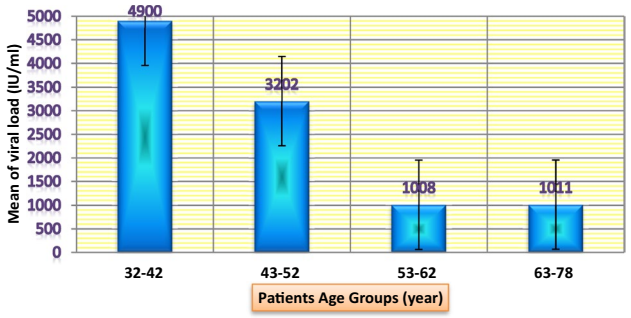

Fig. 3 Scattering viral load according to patients age groups (P = 0.021, $X^2 = 9.77$, DF = 3)

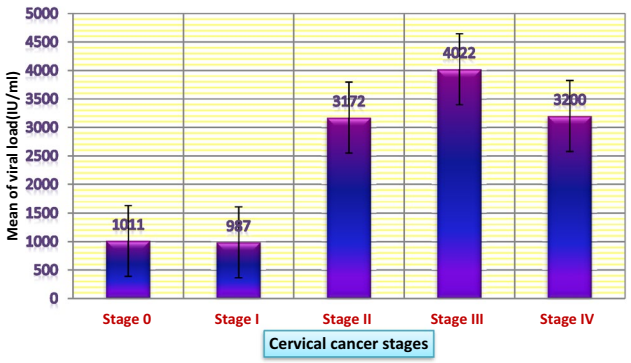

Fig. 4 Distribution viral load according to cervical cancerstages (P= 0.001, X^2= 55.8, DF= 4).

Discussion

Epidemiological studies have shown that the first step in preventing cervical cancer and minimization of costs is the initiation of screening in the early stage [16–18]. The most prominent types of HPV are 18, 16, and 52 [19–28]. Following the previous study finding, the present study reported that 60 cases out of 93 cervical cancer are positive for hrHPV16. In a study conducted on a Mexican population, it was reported that geographical area also influences the frequency of viral genotypes. Reported in a study conducted in Jalisco, Aguascalientes, and Zacatecas showed that HPV 51 and 16 are the most prevalent ones [29–39]. In another study conducted in Tlaxcala, the highly prevalent genotype was HPV 16 and 18 [40]. In present data, females in the age group 32–42 years and 43–52 years have the highest hrHPV16 copy number, and that is consistent with another Iraqi study by Pity et al., (2019) who found the highest HPV positivity samples in the 30–49 years age group, An exceptionally high prevalence of cervical HPV was reported in Iraq due to the lack of a coordinated vaccine program in place [41]. Several studies have shown that

the measurement of viral load in cervical specimens for oncogenic HPV forms (including HPV16) is an appropriate predictor of recurrent infection and the risk of SIL and CC incidence [42–47]. However, the cervical cancer progression and its association with the HR-HPV viral load remains controversial. In the present study, the Distribution of viral load according to cervical cancer stages also exposed statically significant differences when the highest mean (4022 IU/ml) of HPV16 load detected in females who undergo spread to distant cancer (stage III) also showed an increase in patients in the cancer stages IV and II (3200 IU/ml and 3172 IU/ml respectively) whereas the lowest mean (987 IU/ml) of viral load appeared in stage I of cervical cancer. This finding was confirmed by past studies that have reported the association of high HPV load in cytological experiments with the increased risk of developing carcinoma in situ [48, 49]. Moreover, the current result agrees with Moberg et al. (2004), where it was reported that with an increase in the viral load in cervical smear the risk of future cancer development increases [50–55].

The hypothesis presented by Peitsaro et al. also supports the present study's finding [56]. Variations in sampling result in differing cell numbers in a sample and thus affect the viral load. This lack of a norm has established a major barrier to the use of viral load [57, 58]. Several studies have shown that increase in the viral load, and the risk and severity of the lesions increase [59, 60]. Shen et al. used micro-cutting technology in different grades of cervical cancer to gather viral load. This study reported that in high-grade disease viral load can be a crucial independent predictor [60, 61]. In some of the recent studies for HPV primary screening, the viral load was used either in combination or alone as a triage strategy [60, 62–64]. In a study by Duan et al. BioPerfectus Multiplex Real-Time PCR assay (BMRT) was used to quantitatively evaluate the number of single-copy genes. This assay is capable of detecting 14 subtypes of high-risk HPV and 7 subtypes of low and medium-risk strains and gives a viral load per unit cell [57]. However, the implementation of the qRT-PCR-based approach targeting the HPV16 L2 gene revealed consistency in the results for viral load and SIL and cancer grade [65–71]. To demonstrate this, a protocol based on PCR was used in this study to check if the viral load was an indicator of HPV 16 burden.

Conclusion

To conclude, hrHPV16's viral load is associated with the severity of cervical lesions. In primary screening, qRT-PCR can be used for HPV-positive females. To determine their relative importance for cervical cancer growth, larger cohort studies are warranted.

Funding None.

Declarations

Conflict of interest None, has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were following the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Written informed consent was given by the patients and the control subjects for their tissue samples to be included in this study.

References

1. Wentzensen N, Schiffman M, Dunn T, Zuna RE, Gold MA, Allen RA, Wang SS (2009) Multiple human papillomavirus genotype infections in cervical cancer progression in the study to understand cervical cancer early endpoints and determinants. Int J Cancer 125(9):2151–2158. https://doi.org/10.1002/ijc.24528
2. Wentzensen N, Gravitt PE, Long R, Schiffman M, Dunn ST, Carreon JD, Wang SS (2012) Human papillomavirus load measured by linear array correlates with quantitative PCR in cervical cytology specimens. J Clin Microbiol 50(5):1564–1570. https://doi.org/10.1128/JCM.06240-11
3. de Sanjose S, Brotons M, Pavon MA (2018) The natural history of human papillomavirus infection. Best Pract Res Clin Obstet Gynecol 47:2–13. https://doi.org/10.1016/j.bpobgyn.2017.08.015
4. van der Weele P, van Logchem E, Wolffs P, van den Broek I, Feltkamp M, de Melker H, King AJ (2016) Correlation between viral load, the multiplicity of infection, and persistence of HPV16 and HPV18 infection in a Dutch cohort of young women. J Clin Virol 83:6–11. https://doi.org/10.1016/j.jcv.2016.07.020
5. Depuydt CE, Jonckheere J, Berth M, Salembier GM, Vereecken AJ, Bogers JJ (2015) Serial type-specific human papillomavirus (HPV) load measurement allows differentiation between regressing cervical lesions and serial virion productive transient infections. Cancer Med 4(8):1294–1302. https://doi.org/10.1002/cam4.473
6. Depuydt CE, Thys S, Beert J, Jonckheere J, Salembier G, Bogers JJ (2016) Linear viral load increase of a single HPV-type in women with multiple HPV infections predicts progression to cervical cancer. Int J Cancer 139(9):2021–2032. https://doi.org/10.1002/ijc.30238
7. Verhelst S, Poppe WA, Bogers JJ, Depuydt CE (2017) Serial measurement of type-specific human papillomavirus load enables classification of cervical intraepithelial neoplasia lesions according to occurring human papillomavirus-induced pathway. Eur J Cancer Prev 26(2):156–164. https://doi.org/10.1097/CEJ.0000000000000241
8. Schlecht NF, Trevisan A, Duarte-Franco E, Rohan TE, Ferenczy A, Villa LL, Franco EL (2003) Viral load as a predictor of the risk of cervical intraepithelial neoplasia. Int J Cancer 103(4):519–524. https://doi.org/10.1002/ijc.10846
9. Iftner T, Germ L, Swoyer R, Kjaer SK, Breugelmans JG, Munk C, Taddeo FJ (2009) Study comparing human papillomavirus (HPV) real-time multiplex PCR and Hybrid Capture II INNO-LiPA v2 HPV genotyping PCR assays. J Clin Microbiol 47(7):2106–2113. https://doi.org/10.1128/JCM.01907-08

10. Wang JW, Roden RB (2013) L2, the minor capsid protein of papillomavirus. Virology 445(1–2):175–186. https://doi.org/10.1016/j.virol.2013.04.017
11. Pal A, Kundu R (2020) Human papillomavirus E6 and E7: the cervical cancer hallmarks and targets for therapy. Front Microbiol 10:3116. https://doi.org/10.3389/fmicb.2019.03116
12. Jalil AT, Kadhum WR, Khan MUF, Karevskiy A, Hanan ZK, Suksatan W, Abdullah MM (2021) Cancer stages and demographical study of HPV16 in gene L2 isolated from cervical cancer in Dhi-Qar province, Iraq. Appl Nanosci. https://doi.org/10.1007/s13204-021-01947-9
13. Widjaja G, Jalil AT, Rahman HS, Abdelbasset WK, Bokov DO, Suksatan W, Ahmadi M (2021) Humoral immune mechanisms involved in protective and pathological immunity during COVID-19. Human Immunol. https://doi.org/10.1016/j.humimm.2021.06.011
14. Moghadasi S, Elveny M, Rahman HS, Suksatan W, Jalil AT, Abdelbasset WK, Jarahian M (2021) A paradigm shift in cell-free approach: the emerging role of MSCs-derived exosomes in regenerative medicine. J Transl Med 19(1):1–21. https://doi.org/10.1186/s12967-021-02980-6
15. Jasim SA, Catalan Opulencia MJ, Majdi A, Yusupova DZ, Mustafa YF, Hammid AT, Nezhad DK, P, (2022) Investigation of crotonaldehyde adsorption on pure and pd-decorated gan nanotubes: a density functional theory study. Solid State Commun. https://doi.org/10.1016/j.ssc.2022.114741
16. Oyervides-Muñoz MA, Pérez-Maya AA, Sánchez-Domínguez CN, Berlanga-Garza A, Antonio-Macedo M, Valdéz-Chapa LD, Garza-Rodríguez ML (2020) Multiple HPV infections and viral load association in persistent cervical lesions in Mexican women. Viruses 12(4):380. https://doi.org/10.3390/v12040380
17. Burger EA, Smith MA, Killen J, Sy S, Simms KT, Canfell K, Kim JJ (2020) Projected time to elimination of cervical cancer in the USA: a comparative modeling study. The Lancet Public Health 5(4):e213–e222. https://doi.org/10.1016/S2468-2667(20)30006-2
18. Baezconde-Garbanati L, Agurto I, Gravitt PE, Luciani S, Murphy S, Ochoa C, Rodríguez YL (2020) Barriers and innovative interventions for early detection of cervical cancer. salud pública de méxico 61:456–460. https://doi.org/10.21149/10425
19. Lu S, Cong X, Li M, Chang F, Ma L, Cao YT (2015) Distribution of high-risk human papillomavirus genotypes in HPV-infected women in Beijing, China. J Med Virol 87(3):504–507. https://doi.org/10.1002/jmv.24075
20. Salahdin OD, Majdi A, Opulencia MJ, Taban TZ, Hammid AT, Zhao X (2022) Oxygen reduction reaction on metal-doped nanotubes and nanocages for fuel cells. Ionics. https://doi.org/10.1007/s11581-022-04564-w
21. Saleh MM et al (2020) CD4/CD8 T lymphocyte ratio and interleukin-6 in COVID-19 patients. Turkish J Immunol 8(3):129–134. https://doi.org/10.25002/tji.2020.1347
22. Turki Jalil A, Hussain Dilfy S, Oudah Meza S, Aravindhan S, Kadhim M, Aljeboree M, A (2021) CuO/ZrO2 nanocomposites: facile synthesis, characterization and photocatalytic degradation of tetracycline antibiotic. J Nanostruct. https://doi.org/10.22052/JNS.2021.02.014
23. Sarjito EM, Jalil A, Davarpanah A, Alfakeer M, Awadh Bahajjaj A, Ouladsmane M (2021) CFD-based simulation to reduce greenhouse gas emissions from industrial plants. Int J Chem React Eng. https://doi.org/10.1515/ijcre-2021-0063
24. Majdi A, Vacareanu R (2021) Evaluation of seismic damage to Iraqi educational reinforced concrete building using FEMA P-58 methodology. IOP Conf Series: Earth Environ Sci. https://doi.org/10.1088/1755-1315/664/1/012110
25. Hussein AA, Majdi A, Alsodani ZA, Vacareanu R (2021) Analysis of relationship between design and implementation stages within construction projects in Iraq. IOP Conf Series: Earth Environ Sci. https://doi.org/10.1088/1755-1315/664/1/012069
26. Marofi F, Rahman HS, Al-Obaidi ZMJ, Jalil AT, Abdelbasset WK, Suksatan W, Jarahian M (2021) Novel CAR T therapy is a ray of hope in the treatment of seriously ill AML patients. Stem Cell Res Amp: Ther 12(1):1–23. https://doi.org/10.1186/s13287-021-02420-8
27. Jalil AT, Shanshool MT, Dilfy SH, Saleh MM, Suleiman AA (2022) Hematological and serological parameters for detection of COVID-19. J Microbiol Biotechnol Food Sci. https://doi.org/10.15414/jmbfs.4229
28. Vakili-Samiani S, Jalil AT, Abdelbasset WK, Yumashev AV, Karpisheh V, Jalali P, Jadidi-Niaragh F (2021) Targeting Wee1 kinase as a therapeutic approach in hematological malignancies. DNA repair. https://doi.org/10.1016/j.dnarep.2021.103203
29. Campos RG, Rosas M, Gutiérrez Santillán A, Delgado E, Gutiérrez M, Orozco T, García Martínez RE, Rosas Cabral ED, A (2019) Unusual prevalence of high-risk genotypes of human papillomavirus in a group of women with neoplastic lesions and cervical cancer from Central Mexico. PLoS ONE 14(4):e0215222. https://doi.org/10.1371/journal.pone.0215222
30. Assi LN, Majdi A, Alhamadani Y, Ziehl P (2021) Early properties of concrete with alkali-activated fly ash as partial cement replacement. Proc Inst Civil Eng Constr Mater 174(1):13–20. https://doi.org/10.1680/jcoma.19.00092
31. Hussein GM, Mohammed SM, Faris M, Mohammed A, Kadhim MJ, Awadh SA, Ajam WA, Jalil AT (2022) Find new channel for overcoming chemoresistance in cancers: Role of stem cells-derived exosomal microRNAs. Int J Biol Macromol 219:530–537. https://doi.org/10.1016/j.ijbiomac.2022.07.253
32. Ngafwan N, Al-Shawi s, Bokov SG (2021) Study on novel fluorescent carbon nanomaterials in food analysis. Food Sci Technol. https://doi.org/10.1590/fst.37821
33. Marofi F, Abdul-Rasheed OF, Rahman HS, Budi HS, Jalil AT, Yumashev AV, Jarahian M (2021) CAR-NK cell in cancer immunotherapy: a promising frontier. Cancer Sci 112(9):3427. https://doi.org/10.1111/cas.14993
34. Al-Hetty HRAK, Abdulameer SJ, Alghazali MW, Aljaberi M, Saleh MM, Suleiman AA, Jalil AT (2022) Contributions and therapeutic potential of tumor-derived microRNAs containing exosomes to cancer progression. Gene Rep 101672. https://doi.org/10.1016/j.genrep.2022.101672
35. Alghazali MW, Al-Hetty HRAK, Ali ZMM, Saleh MM, Suleiman AA, Jalil AT (2022) Non-coding RNAs, another side of immune regulation during Triple-Negative Breast Cancer. Pathol Res Pract 154132. https://doi.org/10.1016/j.prp.2022.154132
36. 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 semenstored at 4° c for 50 hours. Arch Razi Inst 76(4):923–931. https://doi.org/10.22092/ari.2021.355901.1735
37. Raya I, Chupradit S, Kadhim MM, Mahmoud MZ, Jalil AT, Surendar A, Bochvar AN (2021) Role of compositional changes on thermal magnetic and mechanical properties of Fe-PC-based amorphous alloys. Chin Phys B. https://doi.org/10.1088/1674-1056/ac3655
38. Chupradit S, Jalil AT, Enina Y, Neganov DA, Alhassan MS, Aravindhan S, Davarpanah A (2021) Use of organic and copper-based nanoparticles on the turbulatorinstallment in a shell tube heat exchanger: a CFD-based simulation approach byusing nanofluids. J Nanomater. https://doi.org/10.1155/2021/3250058
39. Faraj JA, Al-Athari AH, Mohie SED (2022) Reprogramming the tumor microenvironment to improve the efficacy of cancer immunotherapies. Med Oncol 39:239. https://doi.org/10.1007/s12032-022-01842-5

40. Rudolph SE, Lorincz A, Wheeler CM, Gravitt P, Lazcano-Ponce E, Torres-Ibarra L, Salmerón J (2016) Population-based prevalence of cervical infection with human papillomavirus genotypes 16 and 18 and other high-risk types in Tlaxcala, Mexico. BMC Infect Dis 16(1):1–10. https://doi.org/10.1186/s12879-016-1782-x
41. Pity IS, Abdo HM, Goreal AA (2019) Human papillomavirus genotyping among different cervical smears in Duhok/Iraq. Asian Pac J Cancer Prev: APJCP 20(7):2059. https://doi.org/10.31557/APJCP.2019.20.7.2059
42. Bulkmans NWJ, Berkhof J, Bulk S, Bleeker MCG, Van Kemenade FJ, Rozendaal L, Meijer CJLM (2007) High-risk HPV type-specific clearance rates in cervical screening. British J Cancer 96(9):1419–1424. https://doi.org/10.1038/sj.bjc.6603653
43. Kahrizi MS, Patra I, Jalil AT, Achmad H, Alesaeidi S, Al-Gazally ME, Alesaeidi S (2022) Leukocyte telomere length and obesity in children and adolescents: A systematic review and meta-analysis. Front Genet 13:861101. https://doi.org/10.3389/fgene.2022.861101
44. Mohaddeseh Rahbaran E, Razeghian MS, Maashi AT, Jalil G, Widjaja L, Thangavelu MY, Kuznetsova P, Nasirmoghadas F, Heidari F, Marofi M, Jarahian, (2021) Cloning and embryo splitting in mammalians: brief history, methods, and achievements. Stem Cells Int. https://doi.org/10.1155/2021/2347506
45. Jalil AT, Ashfaq S, Bokov DO, Alanazi AM, Hachem K, Suksatan W, Sillanpää M (2021) High-sensitivity biosensor based onglass resonance PhC cavities for detection of blood component and glucoseconcentration in human urine. Coatings 11:1555. https://doi.org/10.3390/coatings11121555
46. Chupradit S, Ashfaq S, Bokov D, Suksatan W, Jalil AT, Alanazi AM, Sillanpaa M (2021) Ultra-sensitive biosensor withsimultaneous detection (of cancer and diabetes) and analysis of deformationeffects on dielectric rods in optical microstructure. Coatings 11:1564. https://doi.org/10.3390/coatings11121564
47. Bokov D, Turki Jalil A, Chupradit S, Suksatan W, Javed Ansari M, Shewael IH, Kianfar E (2021) Nanomaterial by sol-gel method: synthesis and application. Adv Mater Sci Eng. https://doi.org/10.1155/2021/5102014
48. Dalstein V, Riethmuller D, Prétet JL, Le Bail Carval K, Sautière JL, Carbillet JP, Mougin C (2003) Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: a longitudinal French cohort study. Int J Cancer 106(3):396–403. https://doi.org/10.1002/ijc.11222
49. Moberg M, Gustavsson I, Gyllensten U (2004) Type-specific associations of human papillomavirus load with risk of developing cervical carcinoma in situ. Int J Cancer 112(5):854–859. https://doi.org/10.1002/ijc.20480
50. Moberg M, Gustavsson I, Wilander E, Gyllensten U (2005) High viral loads of human papillomavirus predict the risk of invasive cervical carcinoma. Br J Cancer 92(5):891–894. https://doi.org/10.1038/sj.bjc.6602436
51. Shabgah AG, Al-Obaidi ZMJ, Rahman HS, Abdelbasset WK, Suksatan W, Bokov DO, Navashenaq JG (2022) Does CCL19 act as a double-edged sword in cancer development? Clin Exp Immunol 20:1–12. https://doi.org/10.1093/cei/uxab039
52. Kartika R, Alsultany FH, Jalil AT, Mahmoud MZ, Fenjan MN, Rajabzadeh H (2021) Ca12O12 nanocluster as highly sensitive material for the detection of hazardous mustard gas: density-functional theory. Inorg Chem Commun. https://doi.org/10.1016/j.inoche.2021.109174
53. Jalil AT, Al-Khafaji AHD, Karevskiy A, Dilfy SH, Hanan ZK (2021) Polymerase chain reaction technique for molecular detection of HPV16 infections among women with cervical cancer in Dhi-Qar province. Mater Today: Proc. https://doi.org/10.1016/j.matpr.2021.05.211
54. Hanan ZK, Saleh MB, Mezal EH, Jalil AT (2021) Detection of human genetic variation in VAC14 gene by ARMA-PCR technique and relation with typhoid fever infection in patients with gallbladder diseases in Thi-Qar province/Iraq. Mater Today Proc. https://doi.org/10.1016/j.matpr.2021.05.236
55. Hachem K, Jasim SA, Al-Gazally ME, Riadi Y, Yasin G, Turki Jalil A, Dehno Khalaji A (2022) Adsorption of Pb (II) and Cd (II) by magnetic chitosan-salicylaldehyde Schiff base: synthesis, characterization, thermal study and antibacterial activity. J Chin Chem Soc. https://doi.org/10.1002/jccs.202100507
56. Peitsaro P, Johansson B, Syrjänen S (2002) Integrated human papillomavirus type 16 is frequently found in cervical cancer precursors as demonstrated by a novel quantitative real-time PCR technique. J Clin Microbiol 40(3):886–891. https://doi.org/10.1128/JCM.40.3.886-891.2002
57. Duan L, Du H, Wang C, Huang X, Qu X, Shi B, Wu R (2020) The application of BMRT-HPV viral load to secondary screening strategies for cervical cancer. PloS ONE. https://doi.org/10.1371/journal.pone.0232117
58. Carcopino X, Henry M, Mancini J, Giusiano S, Boubli L, Olive D, Tamalet C (2012) Significance of HPV 16 and 18 viral load quantitation in women referred for colposcopy. J Med Virol 84(2):306–313. https://doi.org/10.1002/jmv.23190
59. Luo H, Belinson JL, Du H, Liu Z, Zhang L, Wang C, Wu R (2017) Evaluation of viral load as a triage strategy with primary high-risk human papillomavirus cervical cancer screening. J Low Genit Tract Dis 21(1):12–16
60. Fu Xi L, Schiffman M, Ke Y, Hughes JP, Galloway DA, He Z, Kiviat NB (2017) Type-dependent association between risk of cervical intraepithelial neoplasia and viral load of oncogenic human papillomavirus types other than types 16 and 18. Int J Cancer 140(8):1747–1756. https://doi.org/10.1002/ijc.30594
61. Huang CG, Lee LA, Tsao KC, Liao CT, Yang LY, Kang CJ, Yen TC (2014) Human papillomavirus 16/18 E7 viral loads predict distant metastasis in oral cavity squamous cell carcinoma. J Clin Virol 61(2):230–236. https://doi.org/10.1016/j.jcv.2014.07.007
62. Dong L, Wang MZ, Zhao XL, Feng RM, Hu SY, Zhang Q, Zhao FH (2018) Human papillomavirus viral load as a useful triage tool for non-16/18 high-risk human papillomavirus positive women: a prospective screening cohort study. Gynecol Oncol 148(1):103–110. https://doi.org/10.1016/j.ygyno.2017.11.016
63. Kang L, Zhao F, Chen F, Chen W, Li J, Zhang X, Qiao Y (2014) Value of high-risk human papillomavirus viral load in predicting cervical lesions and triaging for high risk (HR)-HPV-positive women. Chin J Oncol 36(4):316–320
64. Luo H, Du H, Belinson JL, Wu R (2019) Evaluation of alternately combining HPV viral load and 16/18 genotyping in secondary screening algorithms. PLoS ONE 14(7):e0220200. https://doi.org/10.1371/journal.pone.0220200
65. Trevisan A, Schlecht NF, Ramanakumar AV, Villa LL, Franco EL, Ludwig-McGill Study Group (2013) Human papillomavirus type 16 viral load measurement as a predictor of infection clearance. J Gen Virol 94(Pt 8):1850. http://observatorio.fm.usp.br/handle/OPI/4011
66. Widjaja G, Jalil AT, Budi HS, Abdelbasset WK, Efendi S, Suksatan W, Yumashev AV (2022) Mesenchymal stromal/stem cells and their exosomes application in the treatment of intervertebral disc disease: a promising frontier. Int Immunopharmacol. https://doi.org/10.1016/j.intimp.2022.108537
67. Khan MUF, Ali BR, Mohammed HQ, Al-Shammari HMT, Jalil AT, Hindi NKK, Kadhim MM (2022) Serum level estimation of some biomarkers in diabetic and non-diabetic COVID-19

infected patients. Appl Nanosci. https://doi.org/10.1007/s13204-021-02167-x
68. Olegovich Bokov D, Jalil AT, Alsultany FH, Mahmoud MZ, Suksatan W, Chupradit S, Nezhad DKp (2022) Ir-decorated gallium nitride nanotubes as a chemical sensor for recognition of mesalamine drug: a DFT study. Mol Sim. https://doi.org/10.1080/08927022.2021.2025234
69. Khaki N, Fosshat S, Pourhakkak P, Thanoon RD, Jalil AT, Wu L (2022) Sensing of acetaminophen drug using Zn-doped boron nitride nanocones: a DFT inspection. Appl Biochem Biotechnol. https://doi.org/10.1007/s12010-022-03830-x
70. Hussein HK, Aubead M, Kzar HH, Karim YS, Amin AH, Al-Gazally ME, Heydari H (2022) Association of cord blood asprosin concentration with atherogenic lipid profile and anthropometric indices. Diabetol Amp: Metab Syndr 14(1):1–6. https://doi.org/10.1186/s13098-022-00844-7
71. Yaghoubi S, Karimi MH, Lotfinia M, Gharibi T, Mahi-Birjand M, Kavi E, Hosseini F, Sineh Sepehr K, Khatami M, Bagheri N, Abdollahpour-Alitappeh M (2020) Potential drugs used in the antibody–drug conjugate (ADC) architecture for cancer therapy. J Cell Physiol 235(1):31–64. https://doi.org/10.1002/jcp.28967