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Evaluation of CXCR3 Gene Expression in Iraqi Women with Cervical Intraepithelial Neoplasia Induced By Human Papillomavirus

Mina M. Allos[1] *MSc*, **Hassan M. Naif**[2] *PhD*

[1]Dept. of Applied Sciences, University of Technology, Baghdad, Iraq, [2]Dept. of Medical Laboratory Technology, College of Health and Medical Technology, Al-Shaab University, Baghdad, Iraq

Abstract

Background Cytokines and chemokines play a crucial role in initiating the early immune response against High-Risk Human papillomavirus (HR-HPV) infection, hence preventing the progression of cervical lesions to cervical cancer (CC). The C-X-C motif chemokine ligand 10 (CXCL10) interacts with C-X-C motif chemokine receptor 3 (CXCR3) to facilitate immune responses by stimulating and attracting specific types of immune cells.

Objective To explore the role of the CXCR3 in women infected or uninfected with HR-HPV-induced tumors.

Methods The study included 100 female patients diagnosed with cervical intraepithelial neoplasia (CIN); twenty-two samples were shown to be HR-HPV positive, while thirteen samples were not. The presence of HR-HPV was confirmed using a real-time polymerase chain reaction (PCR) kit from Sacase, Italy and 20 apparently healthy women as a control group. Two-step reverse transcription PCR (RT-qPCR) was used to assess the expression of CXCR3 in both CC cells and cervical neoplasia in different stages {atypical squamous cells of undetermined significance (ASC-US), CIN I&II, and high-grade squamous intraepithelial lesion (HSIL)} samples.

Results Results showed that gene expression of CXCR3 level was substantially and significantly upregulated ($p < 0.001$) in the cervix tissue of women with CIN in (early and moderated) stages of disease-infected HR-HPV but not in the control group. On the other hand, at late stages of disease (HSIL) and CC stage, the level of CXCR3 was significantly downregulated ($p > 0.05$). Receiver operating characteristic analysis showed that CXCR3 had 73% sensitivity with 100% specificity.

Conclusion The results suggested that these molecules may serve as potential biomarkers for CIN progression of women infected with HPV and as an indicator of immune responses in surviving patients. Also, reductions in CXCR3 gene expression may reflect viral immune escape/or viral clearances.

Keywords HR-HPV, CXCR3, real time PCR, cervical neoplasia, cervical cancer, Iraqi women

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List of abbreviations: ASC-US = Atypical squamous cells of undetermined significance, CC = Cervical cancer, CIN = Cervical intraepithelial neoplasia, CXCL10 = C-X-C motif chemokine ligand 10, CXCR3 = C-X-C motif chemokine receptor 3, HSIL = High-grade squamous intraepithelial lesion, HR-HPV = High-Risk Human Papillomavirus.

Introduction

Chemokines and cytokines are recognized for their ability to interact with various types of immune cells, such as T cells, immature dendritic cells, and monocytes, in the inflamed region [(1)]. Chemokines such as C-

X-C motif chemokine receptor 3 (CXCR3) and C-X-C motif chemokine ligand 10 (CXCL10) play essential functions in attracting immune cells to the genital tract tissue. When CXCL10 binds to CXCR3, it stimulates several types of immune cells, including (monocytes, T-helper cell type 1, CD8+ T cells, natural killer T cells, NK cells, B cells, dendritic cells, and some cancer cells) [2]. Cells expressing CXCR3 include activated effector CD8+ T-cells, NK cells, differentiated CD4+ T cells, and epithelial cells [3].

The chemokine receptor CXCR3 is a G protein-coupled receptor that is mainly plays role in attracting certain types of immune cells, suppression of angiogenesis and activation of T helper immune response to infection [4]. Dendritic cells secreting CXCL10 are actively engaged with T cells, indicating that CXCL10/CXCR3 plays a significant role in the interactions between these cells [5].

Human Papillomavirus (HPV) is the most common sexually transmitted virus worldwide, and over 180 genotypes of the virus have been reported. HPV infection in men has been considered to be transient, with a main clinical presentation being warts in the external genitals [6]. Infection with HPV, stimulates the immune response and attracts the accumulation of leukocytes by chemokines that are the largest subfamily cytokines that stimulate the movement of leukocytes towards a specific target [7,8]. HPV has several mechanisms to inhibit the immune response, including the dysregulation of chemokine expression (up-regulation or down-regulation) [9]. High-Risk HPV (HR-HPV) oncoproteins E6 and E7, have a high interact affinity biding to regulatory immune proteins. E6/E7 oncoproteins block expression immune gene and signaling pathways of immune system, which creates an overall immunosuppressive environment [10,11].

Persisting infection with certain type of oncogenic HR-HPV is responsible for progression of precancerous lesions in the cervix, skin, head and neck, and esophageal head and neck. HPV DNA integrates into the genome of basal epithelial cells and infect keratinocytes; the major make-up of outer-layer of skin, outer surface of the cervix and oral mucosa [12]. Human immune system can eliminate approximately 90% of the circulation HPV within six months, and persistent infections can arise from cervical intraepithelial neoplasia I (CIN I), which subsequently advances to CIN III and ultimately invasive carcinoma. Hence, chronic HR-HPV infection and altered immune surveillance may potentially serve as the precursors to cervical cancer (CC) [13,14]. During the early stages, cervix lesions infected with HPV recruit Langerhans cells, NK T cells, NK cells, and dendritic cells, which play a crucial role in promoting an immune response against infection and pro-inflammatory through cytokines and chemokine [8].

This study aimed to assess the circulating levels of circular CXCR3 in tissue swabs from females with cervicovaginal intraepithelial neoplasia (CIN) infected with human papillomavirus (HPV), to examine the relationship between circular CXCR3 and HPV infection, and to evaluate its potential use as a biomarker for HPV infection.

Methods

Clinical samples

Thirty-five of exfoliated cervix swabs were included in this study. They were sub-grouped into three degrees with cervical lesion abnormalities with different stages of lesions {atypical squamous cells of undetermined significance (ASC-US), CIN I&II, and high-grade squamous intraepithelial lesion (HSIL)} and three cases with CC, they were all associated with HR-HPV infection, six with early stage of lesions (ASC-US) not infected with HR-HPV and twenty matched non-cervical lesions as a healthy control were included in this study. The identification of HR-HPVs was achieved through the extraction and purification of DNA from samples, followed by the amplification of 14 specific high-risk genotypes by using real-

time polymerase chain reaction (RT-PCR) with a kit provided by (Sacase, Italy).

They were collected from the Gynecological Oncology Department of Baghdad Teaching Hospital from February to December 2022. Ethical approval has been warranted by the Ministry of Health (4924, 31/1/2022) and the Scientific Committee of the College of Biotechnology, Al-Nahrain University. The CC patients included in this study underwent chemotherapy or radiotherapy prior to surgery or biopsy. All tissue samples were rapidly stored at -80°C in virus transport media (VTM) media until use. Samples obtained from pregnant women, those under treatment and negative for both Pap smear and HR-HPV were excluded or those who had a total uterine or cervical resection were also excluded.

RNA and RT-PCR

The total RNA (1 µg) was extracted from cervix swab by TransZol Up Plus RNA kit and all RNA convert to cDNA by EasyScript First-Strand cDNA Synthesis Super Mix and Luna Universal qPCR Master Mix (2x) using fluorescent dye SYBR-Green kit following the manufactures manual. Sequence of primers is shown in Table 1 and were prepared according to manufacturer (Macrogene, Korea) designed using the site https://www.ncbi.nlm.nih.gov/tools/primer-blast/primertool. Reaction components of total 20 µL consists of cDNA 5 µL (109 copies), nuclease free water 4 µL, reverse primer 0.5µL (0.25 µM), forward primer 0.5 µL (0.25 µM), master mix 10 µL (2x) (fluorescent day SYBR-Green). Thermocycler conditions initial-denaturation 95°C for 1 min, Denaturation 95°C for 15 seconds, extension 60°C for 30 seconds, melt curve 60-90°C for 1 sec, hold 4°C.

Table 1. Primer sequences designed for detection of CXCR3 and U6

Primer	Sequence (5'→3' direction)	
	CXCR3 primer pair	**Reference**
Forward	5'-TGCATCAGCTTTGACCGCTA-3'	This study
Reverse	5'-GAAGTCAGACTGTGGGCGAA-3'	
	U6 primer pair	
Forward	GCTTCGGCAGCACATATACTAAAAT	
Reverse	CGCTTCACGAATTTGCGTGTCAT	

Statistical analysis

The software GraphPad PRISM 8 and IBM statistical package for social sciences (SPSS) version 27 were used to calculate the mean and standard error and P <0.05 was considered as a significant difference. Incorporating one-way and two-way ANOVA to elucidate the effects of one or two independent variables on a dependent variable, respectively, while the addition of Receiver Operating Characteristic (ROC) curves would facilitate the evaluation of the diagnostic specificity and sensitivity ability of the test variables by calculating the area under the curve (AUC). Relative gene expression (Livak and Schmittgen, 2001) [15] was analyzed using CT value and 2-ΔΔCT method of target gene depending on a compared to endogenous control. The following formulae were used to determine the fold change: ΔCT = CT (target gene) – CT U6 gene)

- ΔΔCT = ΔCT (sample) – ΔCT (control average)
- Fold change = 2^(-ΔΔCT)
- Fold change = 2^(-ΔΔCT) of patients/Average 2^(-ΔΔCT) of Controls

It was found that the control value was held to be 1, and it was discovered that samples with values that are less than 1 are downregulated, while samples with values that are more than 1 are considered to be upregulated.

Results

Gene expression of chemokine receptor CXCR3

To understand of the role of CXCR3 as (pro-inflammatory or anti-inflammatory) in women with cervical lesions abnormalities, either with HR-HPV infection or without HR-HPV infection and CC, the mRNA expression was analyzed by using RT-qPCR. The gene expression was standardized to the level of a house-keeping gene (U6) was used as a standard for gene expression calculation by the fold change of 2−ΔΔCt method. Results showed a significant negative correlation between mRNA CXCR3 gene expression with disease progression, as shown in figure (1).

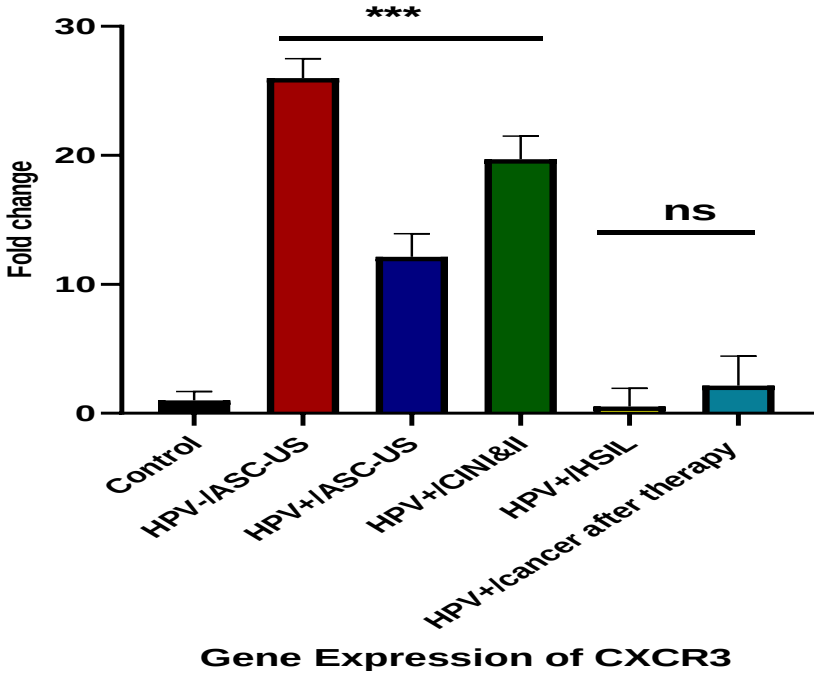

Figure 1. Quantitative gene expression of the CXCR3 at different stages of cervical lesions and cervical cancer. NS: P value >0.05, *= P value <0.05**= very high significant, HPV- = Not infected with human papilloma virus, HPV+= Infected with human papilloma virus. ASC-US = Atypical squamous cells of undetermined significance, CIN = Cervical intraepithelial neoplasia, HSIL = high-grade squamous intraepithelial lesion**

The expression level of CXCR3 was significantly increased at early stages of CIN of infected women with HR-HPV, the fold change values were 12 and 19, for ASC-US, CIN I/II, respectively. It was shown that the expression of this gene was significantly reduced with fold change of 0.5 and 2 at the stage of HSIL and CC patients respectively, compared to adjacent normal tissues. Interestingly, women with CIN early stage (ASC-US) and non-infected with HR-HPV had a higher level of CXCR3 with fold change value of 25. These results indicate that expression of CXCR3 was involved an anti-inflammatory chemokine in women infected or not infected HR-HPV-induced tumors and suffering from CIN associated with patient

survival, immune response, and viral clearance to HR-HPV infection at different stages of cervical lesions.

Sensitivity and Specificity of CXCR3

The predictive performance of CXCR3 gene expression was evaluated using the ROC curve analysis, as shown in figure (2). CXCR3 demonstrated significant potential as a biomarker for the early immune response in cervical lesions with HPV infection. The analysis yielded an AUC of 0.8148 with a 95% confidence interval (CI) ranging from 0.6684 to 0.9613. At a cut-off value greater than 8.078, CXCR3 expression achieved a sensitivity of 73.33% and a specificity of 100%.

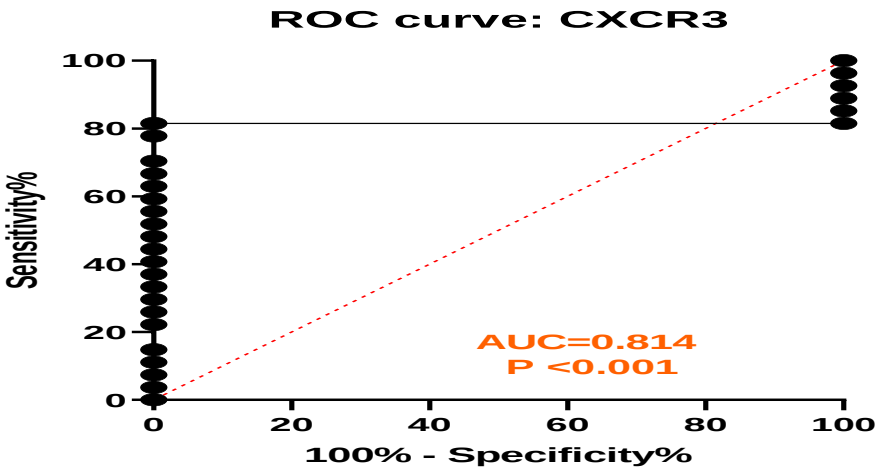

Figure 2. CXCR3 sensitivity and specificity of CXCR3 in infected women with HPV

Discussion

Cervical cancer caused by HR-HPV showed lower levels of CXCR3 and CXCL10 gene expression, which might indicate that the virus is avoiding the immune system or being cleared by it. HPV does not cause viremia, nor does it induce viral cytolysis or cell death, and viral replication and release are not associated with inflammation; this may explain why the level of CXCR3 at the ASC-US stage of HPV is higher than in CIN stages infected with HPV.

Chemokines and their receptors play physiological and pathological function in the activation of immune cells, organs development, inflammatory responses and pathogen infection [16]. They may also influence leukocyte movement and cell homing in tissues [7]. Chronic inflammation in the genital tract and alteration in the immune response may be associated with the cancer progression [17]. The elevation of CXCL10 in tumor tissues stimulates T-cell polarization into effector T cells, which aids in the destruction of cancer cells, by attracting CXCR3 on activated type 1 of T helper and CD8+ T cells to inflame at tumor sites [18]. The antiviral role of CXCR3 suppressing infection with some type of virus by stimulation T helper type 1 responses [19]. The upregulation or down regulation of CXCR3 and its ligands (CXCL9, CXCL10, CXCL11) could help progression of several types of cancers includes cervical cancer [20]. The present investigation revealed that CXCL10 expression is significantly increased in cervical tissues of patients infected with HR-HPV at different stages of disease or cervical cancer. The expression level of CXCR3 plays a crucial role in determining the CC TIME characteristics. Specifically, a decrease in CXCR3 expression correlates with a decreased presence of M1 macrophages as well as block memory CD8+ T cells and CD4+ T cells [20]. CXCR3-mediated T cell migration is important in clearing infections caused by percutaneous vaccinia virus,

protozoan parasite Toxoplasma gondii, and herpes simplex virus type 2 [21-23]. CXCL10-CXCR3 may well regulate PD-L1 expression through JAK and STAT signaling pathways in fibroblasts cells [24-26]. Analysis of CXCR3 expression levels in the cytoplasm and cytosol of 75 cancer cells from patients diagnosed with stage I/II breast cancer revealed that elevated CXCR3 expression correlates with reduced overall survival. Furthermore, CXCR3 expression levels exhibited a positive correlation with tumor size, metastasis, and the number of affected lymph nodes [27]. The expression level of CXCR3 in ovarian cancer was dramatically upregulated in both the main ovarian location and abdominal metastatic lesions. Indicating that CXCR3 may serve as a crucial biomarker for assessing the prognosis of ovarian cancer patients [28]. In cancer patients, the production of CXCR3 is more closely associated with the development of lymph node metastasis. The results of this study suggest that CXCR3 is an independent risk [29].

In conclusion, the results demonstrated that CXCR3 was highly expressed during HPV infection at early stages of cervical lesions. It has been demonstrated that, CXCR3 is helpful indictor for immune response for prevention of CIN development to late stage in the patient and prevent CC. The high expression of CXCR3 may serve as immune stimulators (antitumor and antiviral) for disease progression, proliferation of cervical lesions infection with HR-HPV or other sexually transmitted diseases (STD) and for viral clearance.

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Author contribution

Eshaq: Methodology, investigation, formal analysis, roles/writing: original draft and writing. Dr. Naif: Project idea and planning, supervision, writing-review and editing.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

1. Choi YW, Kang MC, Seo YB, et al. Intravaginal administration of Fc-fused IL7 suppresses the cervicovaginal tumor by recruiting HPV DNA vaccine-induced CD8 T cells. Clin Cancer Res. 2016; 22(23): 5898-908. doi: 10.1158/1078-0432.CCR-16-0423
2. Kuo PT, Zeng Z, Salim N, et al. The role of CXCR3 and its chemokine ligands in skin disease and cancer. Front Med. 2018; 5: 271. doi: 10.3389/fmed.2018.00271
3. Wang W, Uberoi A, Spurgeon M, et al. Stress keratin 17 enhances papillomavirus infection-induced disease by downregulating T cell recruitment. PLoS Pathog. 2020; 16(1): 1008206. doi: 10.1371/journal.ppat.1008206
4. Altara R, Mallat Z, Booz GW, et al. The CXCL10/CXCR3 axis and cardiac inflammation: Implications for immunotherapy to treat infectious and noninfectious diseases of the heart. J Immunol Res. 2016; 2016: 4396368. doi: 10.1155/2016/4396368.
5. Pontes Ferreira C, Moro Cariste L, Henrique Noronha I, et al. CXCR3 chemokine receptor contributes to specific CD8+ T cell activation by pDC during infection with intracellular pathogens. PLoS Negl Trop Dis. 2020; 14(6): 0008414. doi: 10.1371/journal.pntd.0008414.
6. Dawood MA, Al-Shuwaikh AM, Al-Kawaz UM. The association of reactive oxygen species with high-risk Human Papilloma virus infection and some sperm parameters in a sample of Iraqi idiopathic infertile males. Iraqi J Med Sci. 2024; 22(1): 178-187. doi: 10.22578/IJMS.22.1.20.
7. Kufareva I, Gustavsson M, Zheng Y, et al. What do structures tell us about chemokine receptor function and antagonism? Annu Rev Biophys. 2017; 46: 175-98. doi: 10.1146/annurev-biophys-051013-022942.
8. Bule P, Aguiar SI, Aires-Da-Silva F, et al. Chemokine-directed tumor microenvironment modulation in cancer immunotherapy. Int J Mol Sci. 2021; 22(18): 9804. doi: 10.3390/ijms22189804.
9. Attademo L, Tuninetti V, Pisano C, et al. Immunotherapy in cervix cancer. Cancer Treat Rev. 2020; 90: 102088. doi: 10.1016/j.ctrv.2020.102088.
10. Acevedo-Sánchez V, Rodríguez-Hernández RM, Aguilar-Ruíz SR, et al. Extracellular vesicles in cervical cancer and hpv infection. Membranes (Basel). 2021; 11(6): 453. doi: 10.3390/membranes11060453.
11. Zhou C, Tuong ZK, Frazer IH. Papillomavirus immune evasion strategies target the infected cell and the local immune system. Front Oncol. 2019; 9: 682. doi: 10.3389/fonc.2019.00682.

12. Chiang C, Pauli EK, Biryukov J, et al. The human papillomavirus e6 oncoprotein targets USP15 and TRIM25 to suppress RIG-I-mediated innate immune signaling. J Virol. 2018; 92(6): 1737-17. doi: 10.1128/JVI.01737-17.
13. Bashaw AA, Leggatt GR, Chandra J, et al. Modulation of antigen presenting cell functions during chronic HPV infection. Papillomavirus Res. 2017; 4: 58-65. doi: 10.1016/j.pvr.2017.08.002.
14. Smola S. Immunopathogenesis of HPV-associated cancers and prospects for immunotherapy. Viruses. 2017; 9(9): 254. doi: 10.3390/v9090254.
15. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001 Dec;25(4):402-8. doi: 10.1006/meth.2001.1262. PMID: 11846609.
16. Zhu H, Chen X, Hu Y, et al. Long non-coding RNA expression profile in cervical cancer tissues. Oncol Lett. 2017; 14(2): 1379-86. doi: 10.3892/ol.2017.6319.
17. Matsuzaki G, Yamasaki M, Tamura T, et al. Dispensable role of chemokine receptors in migration of mycobacterial antigen-specific CD4+ T cells into Mycobacterium-infected lung. Immunobiology. 2019; 224(3): 440-8. doi: 10.1016/j.imbio.2019.01.006.
18. Li X, Wu J, Wu Y, et al. Imbalance of vaginal microbiota and immunity: Two main accomplices of cervical cancer in chinese women. Int J Womens Health. 2023; 15: 987-1002. doi: 10.2147/IJWH.S406596.
19. Karin N. CXCR3 Ligands in cancer and autoimmunity, chemoattraction of effector t cells, and beyond. Front Immunol. 2020; 11: 976. doi: 10.3389/fimmu.2020.00976.
20. Spencer Clinton JL, Tran LL, Vogt MB, et al. IP-10 and CXCR3 signaling inhibit Zika virus replication in human prostate cells. PLoS One. 2020; 15(12): 1-30. doi: 10.1371/journal.pone.0244587.
21. Xu J, Huang Z, Wang Y, et al. Identification of novel tumor microenvironment regulating factor that facilitates tumor immune infiltration in cervical cancer. Front Oncol. 2022; 12: 846786. doi: 10.3389/fonc.2022.846786.
22. Hickman HD, Reynoso GV, Ngudiankama BF, et al. CXCR3 chemokine receptor enables local CD8(+) T cell migration for the destruction of virus-infected cells. Immunity. 2015; 42(3): 524-37. doi: 10.1016/j.immuni.2015.02.009.
23. Cohen SB, Maurer KJ, Egan CE, et al. CXCR3-dependent CD4$^+$ T cells are required to activate inflammatory monocytes for defense against intestinal infection. PLoS Pathog. 2013; 9(10): 1003706. doi: 10.1371/journal.ppat.1003706.
24. Thapa M, Welner RS, Pelayo R, Carr DJ. CXCL9 and CXCL10 expression are critical for control of genital herpes simplex virus type 2 infection through mobilization of HSV-specific CTL and NK cells to the nervous system. J Immunol. 2008; 180(2): 1098-106. doi: 10.4049/jimmunol.180.2.1098.
25. Chen X, He H, Xiao Y, et al. CXCL10 produced by hpv-positive cervical cancer cells stimulates exosomal PDL1 expression by fibroblasts via CXCR3 and JAK-STAT pathways. Front Oncol. 2021; 11: 629350. doi: 10.3389/fonc.2021.629350.
26. Allos MM, Naif HM. Association between Cervix intraepithelial neoplasia (CIN) and high-risk human Papillomavirus (HPV) genotypes in Iraqi Women. Malaysian J Fundam Appl Sci. 2024; 20(3): 639-47. doi: https://doi.org/10.11113/mjfas.v20n3.3382.
27. Ma X, Norsworthy K, Kundu N, et al. CXCR3 expression is associated with poor survival in breast cancer and promotes metastasis in a murine model. Mol Cancer Ther. 2009; 8(3): 490-8. doi: https://doi.org/10.1158/1535-7163.mct-08-0485
28. Windmüller C, Zech D, Avril S, et al. CXCR3 mediates ascites-directed tumor cell migration and predicts poor outcome in ovarian cancer patients. Oncogenesis. 2017; 6(5): 331. doi: 10.1038/oncsis.2017.29.
29. Wagener-Ryczek S, Schoemmel M, Kraemer M, et al. Immune profile and immunosurveillance in treatment-naive and neoadjuvantly treated esophageal adenocarcinoma. Cancer Immunol Immunother. 2020; 69(4): 523-33. doi: 10.1007/s00262-019-02475-w.

Correspondence to Mina M. Allos
E-mail: mina.m.eshaq@uotechnology.edu.iq
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