Original Article

# Elevated levels of CD56+ T Cells, CD16+ CD56+ T Cells, and CD56dim NK Cells in herpes simplex virus type 1 seropositive healthy individuals

*Mazen Almehmadi, PhD, Mamdouh Allahyani , PhD, Abdulelah Aljuaid PhD, Meshari A. Alsuwat, PhD, Mustafa Halawi, PhD.*

## ABSTRACT

**الأهداف**: دراسة أعداد خلايا NK، والخلايا T +CD56 وخلايا CD56، ومستويات CD16 لدى الأفراد الأصحاء المصابين بفيروس HSV–1. وعلى وجه التحديد، يسعى إلى قياس مستويات هذه الخلايا للتعرف على الأهمية المناعية المحتملة أثناء الحالة الإيجابية لفيروس HSV–1.

**المنهجية**: استخدمت هذه الدراسة تصميمًا بحثيًا مقطعيًا لفحص مستويات خلايا CD56+ وCD16+ بين الأفراد المصابين بفيروس الهربس البسيط من النوع 1 (HSV–1) في مدينة الطائف. تم تسجيل ما مجموعه 112 مشاركًا، وتم تحديد الحالة المصلية لفيروس HSV–1 عبر ELISA، وتم إجراء التقييم الخلوي باستخدام قياس التدفق الخلوي. أجريت الدراسة خلال الفترة من يناير 2023م ويوليو 2023م.

**النتائج**: وجدت دراستنا أن نسبة الإصابة بفيروس HSV–1هي 36%، على النقيض من المعدلات الأعلى في المملكة العربية السعودية. لم يلاحظ أي اختلافات كبيرة في العمر أو الجنس. أظهر الأفراد المصابون بفيروس HSV–1 ارتفاعًا في خلايا CD56 NK الخافتة وخلايا CD56+T، بما يتماشى مع الأبحاث السابقة حول تسلل الخلايا الليمفاوية أثناء تنشيط HSV–1. هناك ما يبرر إجراء مزيد من التحقيق لخلايا CD56– T وخلايا NK الساطعة.

**الخلاصة**: أظهر الأفراد المصابون بفيروس HSV–1 ارتفاعًا في خلايا CD56 NK المعتمة وخلايا CD56+ ، بما يتوافق مع أنشطة الخلايا الليمفاوية أثناء تنشيط الفيروس. يشير تعبير CD16 على خلايا CD56+T إلى اشتراكها في الدفاع الفيروسي، مما يؤكد الحاجة إلى مزيد من الدراسة في الاستجابات المناعية ضد HSV–1.

**Objectives:** To investigate the numbers of natural killer (NK) cells, CD56+ T-cells and CD56- T cells, and the levels ofCD16 in healthy individuals seropositive for herpes simplex virus Type 1 (HSV-1). Specifically, it seeks to measure the levels of these cells to learn about the possible immunological significance during HSV-1 seropositive status.

**Methods:** This study employed a cross-sectional research design to examine the levels of CD56+ T-cells and CD16+ among individuals seropositive for herpes simplex virus type 1 (HSV-1) in Taif city. A total of 112 participants were enrolled, with HSV-1 serostatus determined via ELISA, and cellular evaluation conducted using flow cytometry. The study was performed between January 2023 to July 2023.

**Results:** Our study found 36% HSV-1 seropositivity, contrasting with higher rates in Saudi Arabia. No significant age or gender differences were observed. HSV-1 seropositive individuals showed elevated dim CD56 NK cells and CD56+ T-cells, aligning with prior research on lymphocyte infiltration during HSV-1 activation. Further investigation is warranted for CD56- T-cells and bright NK cells.

**Conclusion:** HSV-1 seropositive individuals showed elevated dim CD56 NK cells and CD56+ T-cells, consistent with lymphocyte activities during viral activation. CD16 expression on CD56+ T-cells suggests their involvement in viral defence, emphasizing the need for further investigation into immune responses against HSV-1.

**Keywords:** CD56+, T-cells, HSV-1

***Saudi Med J 2024; Vol. 45 (12): 1312-1317***
***doi: 10.15537/smj.2024.45.12.20240498***

*From the Department of Clinical Laboratory Sciences (Almehmadi, Allahyani, Aljuaid, Alsuwat), College of Applied Medical Sciences, Taif University, Taif; and from the College of Nursing and Health Sciences (Halawi), Jazan University, Jazan, Kingdom of Saudi Arabia.*

*Received 9th June 2024. Accepted 4th November 2024.*

*Address correspondence and reprint request to: Dr. Mazen Almehmadi, Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, Taif, Kingdom of Saudi Arabia.*
*E-mail: Mazenn@tu.edu.sa*
*ORCID ID: https://orcid.org/0000-0002-7580-8667*

**Disclosure.** This study was funded by Taif University, Kingdom of Saudi Arabia. Project No.: TU-DSPP-2024-02

Herpes simplex virus type 1 (HSV-1) poses a significant health concern, causing cold sores and fever blisters in healthy individuals.[1] These lesions, resulting from periodic viral reactivation, can lead to discomfort and social stigma.[2] In immunocompromised individuals, such as those with human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), HSV-1 can cause severe and prolonged infections, including potentially life-threatening conditions like herpes simplex encephalitis.[1,3] Besides its direct health effects, HSV-1 can also have psychosocial implications, impacting individuals' quality of life and interpersonal relationships due to the stigma associated with visible lesions.[2] Preventing HSV-1 transmission is challenging due to its contagious nature through direct contact or asymptomatic shedding. However, measures such as practicing good hand hygiene and using barrier methods during sexual activity can reduce transmission risk.[1] Overall, HSV-1 infection represents a complex health issue, affecting both physical and emotional well-being, and necessitates comprehensive strategies for prevention and management.

This viral infection prompts immune responses involving natural killer (NK) cells and T-cells. Natural killer cells play a crucial role in early defence against HSV-1 by directly targeting infected cells and secreting antiviral cytokines.[4] CD56+ T-cells, a subset of T-cells expressing the CD56 marker, also participate in immune surveillance against other type of Herpesviridae family such as cytomegalovirus.[5] These cells exhibit cytotoxic activity against virus-infected cells and contribute to the viruses' clearance.[5,6] However, in immunocompromized individuals, these immune responses may be impaired, leading to more severe and prolonged HSV-1 infections.[7,8] CD16, also known as FcγRIII, is crucial during viral infections, primarily through its role in antibody-dependent cellular cytotoxicity (ADCC). When antibodies bind to viral antigens on infected cells, CD16 on NK cells recognizes these complexes, activating the NK cells to release cytotoxic granules containing perforin and granzymes. This results in the apoptosis of infected cells, thereby controlling viral spread and reducing viral load. Additionally, CD16 engagement enhances cytokine production, such as interferon-gamma (IFN-γ), which further boosts the immune response by activating other immune cells and contributing to the body's overall antiviral defence.[9,10] Understanding the interplay between HSV-1 and NK and T-cells is essential for elucidating immune mechanisms and developing targeted therapies for HSV-1 infections.

**Methods.** The study received ethical approval from the Taif University Institutional Review Board and carried out between January 2023 to July 2023. The study was performed at Taif University, with participants from different age groups from 21 to 46 years. The participants included 48 females and 64 males and were provided an informed consent prior samples collection. The study excluded all patients with any disease that could interfere with the findings such as HSV-1 reactivation symptoms, diabetes, asthma, HIV, Hepatic infection,autoimmune diseases, transplantation patients or recently vaccinated, pregnancy for female participants, fever, and those with abnormal hormonal disease. The included should be older than 18 years, and do not have any of the exclusion conditions.

Sample collection and preparation

A total of 112 participants were enrolled, and blood samples were collected into EDTA tubes. Additionally, 2 mL of venous blood was drawn into serum separator tubes and centrifuged at 2500 RPM for 10 minutes. The serum was then separated, transferred into Eppendorf tubes, and stored at -80°C. The serum was thawed once immediately before testing in a 37°C water bath for 20 minutes, for the purpose of evaluating HSV-1 serostatus.

*Qualitative detection of HSV-1 immunoglobulin G (IgG).* The detection of IgG antibodies was performed by ELISA, the kit was purchased from Abcam (ab108737) and evaluated through the Bio-Rad xMarkTM microplate spectrophotometer which was used to read and incubate the plate after the kit was processed according to manufacturer protocol.

*Phenotypical analysis.* This study employed a cross-sectional research design to examine the levels of NK cells, CD3+ CD56- T-cells, and CD56+ T-cells. Natural killer cells were evaluated according to the density of CD56, dim NK cells are the higher number of NK cells, while bight NK cells are 8 to 9 times less than dim NK cells. CD16+ expression levels were estimated to study the cells' ability to communicate during the antibody-dependent cellular cytotoxicity.

Fresh PBMCs were utilized in different experimental conditions. Specific protocols for each sample type were followed to ensure consistency and accuracy. Blood samples were collected in EDTA tubes and PBMCs were isolated within 2 hours of collection using a standard Ficoll-Paque gradient by overlaying method then centrifugation with brake-off for 25 minutes at 1500 RPM. Isolated PBMCs were analyzed immediately on the same day of collection. Flow cytometry was performed using the BD FACSCanto™ (BD Biosciences). Daily quality control of the cytometer was

performed using BD Cytometer Setup and Tracking (CS&T) beads, which were used to check instrument performance, including sensitivity and compensation settings. The titer of antibodies used was determined empirically through titration experiments to identify the optimal concentration that provided the best signal-to-noise ratio without non-specific binding. Antibodies used are CD16 (clone NKP15) is used at a final 1 µg/µL concentration, CD56 (clone NCAM16.2) is used at a final 1 µg/µL concentration, CD3 (clone OKT3) is used at a final 1 µg/µL concentration. The number of cells per sample tube were 1X106cells. The acquired number of events collect were between 100,000 and 200,000 events per sample during acquisition, which is standard practice for routine analysis of PBMCs. This number of events allows for sufficient analysis of major populations while maintaining comparability across samples, the acquisition was consistent and robust. Gates for flow cytometry analysis were determined based on control samples, including unstained and single-color controls.

***Statistical analysis.*** The data were analyzed using GraphPad Prism software, version 6.04, based in La Jolla, CA, USA. To compare seropositive and seronegative individuals regarding NK cells, CD56+ T cells and CD56- T cells levels, the unpaired T-test was employed. Statistical significance set at $p \leq 0.05$. Flow cytometry analysis utilized FACS Diva software, incorporating double discrimination to eliminate doublets.

**Results.** Following the IgG immunoassay for HSV-1, our findings showed 41 (36%) seropositive and 71 (64%) seronegative participants. Among them 30 (73%) seropositive male and 11 (27%) females. Upon evaluating the differences among gender groups, no significant findings were identified. Our findings showed statistical significance difference between the cells (**Figure 1 & Table 1**), dim NK cells were statistically significant ($p$=0.04) as higher levels were detected in HSV-1 seropositive than seronegative. Moreover, CD56+ T-cells have dim CD56 only and were also higher in HSV-1 seropositive than seronegative ($p$=0.003).

CD16 levels of expression was evaluated between HSV-1 seronegative and seropositive individuals (**Figure 2 & Table 2**). Histogram was used measure the levels of a single receptor expression after only the specified gate was applied, thus, only the population of cells at the gate is viewed, and percentage was calculated from the total number of the same type of cells. CD16 supports the NK cells function during antibody-dependent cellular cytotoxicity, which boost their ability to target and kill infected cells that have been labelled by immunoglobulins. By evaluating the expression of these cells using flowcytometry and gating strategies on specified population of lymphocytes, CD16+ was higher and statistically significant ($p$=0.031) on CD56+ T-cells in HSV-1 seropositive (76%) than seronegative (62%). The other cells NK cells, dim NK, bright NK, and CD56- T-cells tend to have more CD16 expression in HSV-1 seropositive is higher than seronegative, however, the difference is not statistically significant.

**Discussion.** Understanding immune cells specifically lymphocytes during viral infection is crucial for several reasons. Firstly, T-cells play a pivotal role in adaptive immunity, recognizing and targeting specific viral antigens, thus orchestrating an effective immune response. NK cells provide rapid innate defence, targeting virus-infected cells. Studying these cells helps resolve the mechanisms underlying immune evasion and pathology, aiding vaccine and therapeutic development. Additionally, insights into T-cell and NK cell responses inform prognostic markers for disease severity and outcomes. Moreover, elucidating their interplay sheds light on immune memory and long-term protection against viral pathogens, contributing to the broader understanding of immunology and infectious diseases.

Our findings reported 36% HSV-1 seropositive and 64% seronegative among our study group, which were inconsistent with other study that reported higher prevalence of HSV-1 in Saudi Arabia.[11,12] This study reported about 88.8%, however, they have covered high number of participants from different regions for the purpose of studying the prevalence of different viral infections with different inclusion and exclusion criteria than our study. Also, another study, reported different findings to ours, as we reported 36% and they reported 20%, and they evaluated HSV-1 seroprevalence among blood bank donors.[12] The different prevalence rate can be due to several factors according to objectives and aims of the studies that target different study groups. Also, varying prevalence rates in research due to several other factors such as socioeconomic status, cultural practices, and age demographics. Additionally, differences in study methodologies, sample sizes, and populations contribute to the variations observed. Socioeconomic factors may influence access to healthcare and education, impacting awareness and testing rates. Cultural practices and age play a role as younger individuals may engage in activities that promote transmission. Consequently, these multifaceted influences result in fluctuating HSV-1 prevalence rates across different research studies and populations.

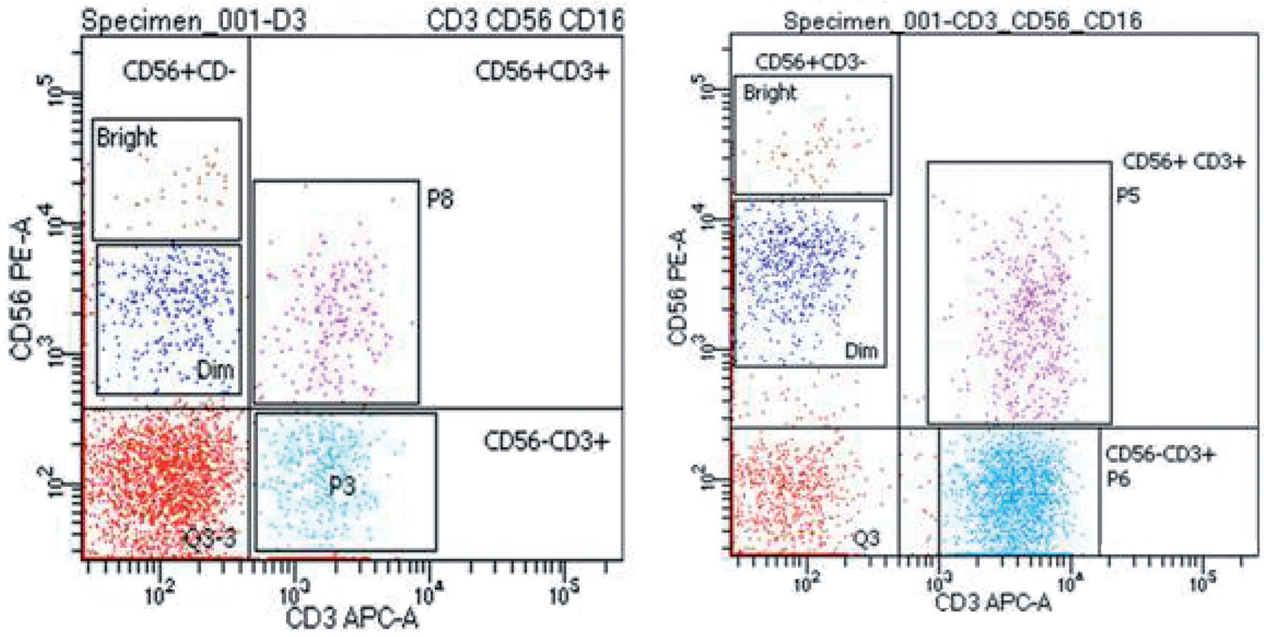


**Figure 1 -** The density plots were extracted by FACs Diva software and showed the different levels between HSV-1 seropositive and seronegative participants. A is extracted from seronegative while B was from seropositive individual. The percentage for the gated populations is as follows; A density plot dim NK: 4%, bright NK: 0.5%, CD56+CD3+: 2.5%, and CD56-T-cells: 48.2%. B density plot dim NK: 9.2%, bright NK: 1.1%, CD56+CD3+: 8.4%, and CD56-T-cells: 47.5%. NK: natural killer cell, HSV-1: Herpes simplex virus type 1

**Table 1 -** Unpaired T-test was applied to compare the means between seronegative and seropositive individuals.

| Variables | HSV-1 | | |
|---|---|---|---|
| | Seronegative % | Seropositive % | *P*-value |
| Bright NK | 1.6 | 1.8 | 0.67 |
| Dim NK | 4.6 | 9.2 | 0.04* |
| NK | 11.6 | 15 | 0.11 |
| CD56+ T-cells | 2.9 | 8.9 | 0.003* |
| CD56- T-cells | 51.2 | 48.8 | 0.87 |

*Indicates statistical significance set at $p \leq 0.05$. NK: natural killer cell, HSV-1: Herpes simplex virus type 1

Our findings revealed no significant differences between age groups or genders with respect to the study's objective. We have detected expansion of cells with dim CD56 receptor including dim NK cells, and CD56+ T-cells in HSV-1 seropositive population than seronegative population, this is consistent with a another(13), and due to nature of HSV-1 reactivation episodes this can explain why HSV-1 have higher levels of dim CD56 NK cells, and CD56+ T-cells to response to this viral infection. Other studies also found that CMV which is from the same family of HSV-1 can led to expansion of these cells in healthy ad kidneys transplant patients.[5,14] These viral infection tend to be dormant types and last for a lifetime with reactivating episodes that trigger the immune system continuously.

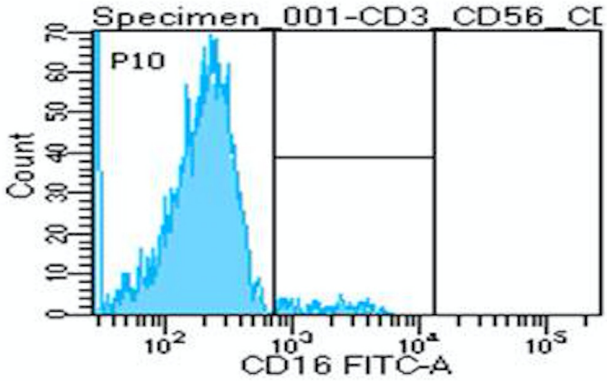


**Figure 2 -** Histograms were extracted by FACs diva software and showed the different levels between HSV-1 seropositive and seronegative participants in terms of CD16 expression. The percentage of expression was extracted by creating statics table, the mean was calculated and compared between the cell's populations.

Our findings also supported by several other studies that detected an immunogenic roles of NK cells and CD56+ T-cells against Herpesviridae family that have some similar nature between them in terms of infection.[13,15-18] However, our study is inconsistent with them for the levels of bright NK cells, CD56- T-cells which were reported insignificant differences.

**Table 2 -** UnpairedT-test was applied to compare the means between seronegative and seropositive individuals.

| | HSV-1 | | |
|---|---|---|---|
| Variables | Seronegative % | Seropositive % | *P*-value |
| CD16+ Bright NK | 51 | 56 | 0.67 |
| CD16+ Dim NK | 96 | 97 | 0.89 |
| CD16+ NK | 91 | 96 | 0.18 |
| CD16+ CD56+ T-cells | 62 | 76 | 0.031* |
| CD16+ CD56- T-cells | 4.1 | 5.4 | 0.09 |

*Indicates statistical significance set at $p \leq 0.05$. Only CD56+ T-cells showed significant difference in terms of CD16 expression. The rest have higher levels but statistical insignificant. NK: natural killer cell, HSV-1: herpes simplex virus type 1

CD16 is necessary during viral infections, principally through its role in ADCC. Labelling of virally infected cells by immunoglobulins play as a bridge of other immune cells to recognise the cells under stress and promote their apoptosis via the release of cytotoxic granules containing perforin and granzymes. Studying the expression of this CD on different type of immune cells at different occasion can place insight into their role. Our findings reported significant expression of this receptor on CD56+ T-cells in HSV-1 seropositive than seronegative population, and the other types of cells express CD16, but no significant difference were detected. CD16 was reported to be higher among CD56+ bright NK cells in HSV-1 seropositive which is like our findings, however, this study used study group with recurrent and sever cases of HSV-1.[19] But they were inconsistent to us in terms of the reduced levels of CD16 in bright CD56 NK cells. Another study focused on thrombocytopenia patients have reported inconsistent findings to ours where no significant differences was detected on their study group that focused on patients with different age groups.[20]

In conclusion, our study revealed a 36% HSV-1 seropositivity rate, contrasting with higher rates reported in other studies within Saudi Arabia. Discrepancies can be attributed to varied sample sizes, demographics, and study objectives. Interestingly, we observed no significant age or gender differences in HSV-1 seropositivity. Notably, HSV-1 seropositive individuals exhibited elevated levels of dim CD56 NK cells and CD56+ T-cells, consistent with lymphocyte infiltration during HSV-1 activation. This aligns with prior research indicating similar expansions during CMV infection. While our findings underscore the immunogenic roles of NK cells and CD56+ T-cells against Herpesviridae, discrepancies in CD56- T-cells and bright NK cells warrant further investigation. Particularly, significant CD16 expression on CD56+ T-cells in HSV-1 seropositive individuals suggests their involvement in viral defence, corroborating previous studies. However, reduced CD16 levels in bright CD56 NK cells diverge from some previous findings. Our study contributes to understanding immune responses to HSV-1, highlighting the complex interplay between different immune cell subsets and their role in antiviral defence mechanisms. Further research is warranted to elucidate these intricate dynamics and their implications for HSV-1 pathogenesis and immunity. This study has some limitations, CMV serostatus was not evaluated for the participants, and our future work will evaluate the effect of viral co-infections of those cells phenotype.

**Acknowledgment.** *The authors extend their appreciation to Taif University, Taif, Kingdom of Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-02).*

## References

1. Arduino PG, Alovisi M, Petti S. Herpes simplex virus type 1 in periapical pathoses: Systematic review and meta-analysis. *Oral Dis* 2024; 30: 865–876.
2. Arduino PG, Cabras M, Lodi G, Petti S. Herpes simplex virus type 1 in subgingival plaque and periodontal diseases. Meta-analysis of observational studies. *J Periodontal Res* 2022; 57: 256–268.
3. Piperi E, Papadopoulou E, Georgaki M, Dovrat S, Bar Illan M, Nikitakis NG, et al. Management of oral herpes simplex virus infections: The problem of resistance. A narrative review. *Oral Dis* 2024; 30: 877–894.
4. Laing KJ, Campbell VL, Dong L, Koelle DM. HLA-B*57:01 Complexed to a CD8 T-Cell Epitope from the HSV-2 ICP22 Protein Binds NK and T Cells through KIR3DL1. *Viruses* 2022; 14: 1019.
5. Almehmadi M, Flanagan BF, Khan N, Alomar S, Christmas SE. Increased numbers and functional activity of CD56+ T cells in healthy cytomegalovirus positive subjects. *Immunology* 2014; 142: 258-268.
6. Almehmadi M, Hammad A, Heyworth S, Moberly J, Middleton D, Hopkins MJ, et al. CD56+ T cells are increased in kidney transplant patients following cytomegalovirus infection. *Transpl Infect Dis* 2015; 17: 518-526.
7. Cohen PR. Clinical features of atypical presentations of mucocutaneous herpes simplex virus infection observed in immunosuppressed individuals. Part II: the knife-cut sign. *Dermatol Online J* 2024; 30.
8. Alshammari A, Alotaibi J, Almaghrabi R, Bawazeer R, Althawadi S, Tayeb H. First parechovirus reported case in Saudi Arabia in hospitalized immunocompromised adult patient. *Virol J* 2024; 21: 102.
9. Razizadeh MH, Zafarani A, Taghavi-Farahabadi M, Khorramdelazad H, Minaeian S, Mahmoudi M. Natural killer cells and their exosomes in viral infections and related therapeutic approaches: where are we? *Cell Commun Signal* 2023; 21: 1–22.
10. La Rosa F, Agostini S, Bianchi A, Nemni R, Piancone F, Marventano I, et al. Herpes simplex virus-1 (HSV-1) infection induces a potent but ineffective IFN-λ production in immune cells of AD and PD patients. *J Transl Med* 2019; 17: 1—0.

11. Memish ZA, Almasri M, Chentoufi AA, Al-Tawfiq JA, Al-Shangiti AM, Al-Kabbani KM, et al. Seroprevalence of Herpes simplex virus type 1 and type 2 and coinfection with hiv and syphilis: The First National Seroprevalence Survey in Saudi Arabia. ***Sex Transm Dis*** 2015; 42: 526-532.
12. Taha AE, Ghazy A, Almaeen A, Taher I, El-Metwally T, Alayyaf MA, et al. Estimation of Seroprevalence of Anti-Herpes Simplex Virus Type-1 IgG Among Healthy Blood Donors in Sakaka City, Aljouf, Saudi Arabia. ***J Med Virol*** 2023; 16: 136606.
13. Leinweber B, Kerl H, Cerroni L. Histopathologic features of cutaneous herpes virus infections (herpes simplex, herpes varicella/zoster): A broad spectrum of presentations with common pseudolymphomatous aspects. ***Am J Surg Pathol*** 2006;30: 50–58.
14. Almehmadi M, Hammad A, Heyworth S, Moberly J, Middleton D, Hopkins MJ, et al. CD56+ T cells are increased in kidney transplant patients following cytomegalovirus infection. ***Transplant Infectious Disease*** 2015 1;17: 518–526.
15. Quatrini L, Della Chiesa M, Sivori S, Mingari MC, Pende D, Moretta L. Human NK cells, ptors and function. ***Eur J Immunol*** 2021; 51: 1566-1579.
16. Schneider CL, Hudson AW. The Human Herpesvirus-7 (HHV-7) U21 Immunoevasin Subverts NK-Mediated Cytoxicity through Modulation of MICA and MICB. ***PLoS Pathog*** 2011; 7: 1002362.
17. Campbell TM, McSharry BP, Steain M, Slobedman B, Abendroth A. Varicella-zoster virus and herpes simplex virus 1 differentially modulate NKG2D Ligand Expression during productive infection. ***J Virol*** 2015; 89: 7932.
18. Carter C, Savic S, Cole J, Wood P. Natural killer cell receptor expression in patients with severe and recurrent Herpes simplex virus-1 (HSV-1) infections. ***Cell Immunol*** 2007; 246: 65–74.
19. Lenart M, Kluczewska A, Szaflarska A, Rutkowska-Zapała M, Wąsik M, Ziemiańska-Pięta A, et al. Selective downregulation of natural killer activating receptors on NK cells and upregulation of PD-1 expression on T cells in children with severe and/or recurrent Herpes simplex virus infections. ***Immunobiology*** 2021; 226: 152097.
20. Li T, Yan GL, Luo Z, Xie Q, Lai MM, Chen ZG, et al. Chronic Pediatric Immune Thrombocytopenia Is Not Associated With Herpes Virus Infection Status. ***Front Pediatr*** 2021; 9: 641535.