Estimation the Role of CD4 and CD8 in patients infected with Varicella-Zoster Virus in Al-Najaf Al-Ashraf City

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Abstract. About 60 samples were collected from patients with varicella zoster, including 24 males and 36 females, as well as from healthy people, which included 29 samples (15 males and 14 females), aged between 15 and 60 years. Data were collected in the holy city of Najaf between November, 2022 and July, 2023. VZV patients were selected from Sadr Medical City and private laboratories. The research used BD FACS Canto II Flow Cytometry technology to measure the CD4 and CD8 cells/ml in the blood of patients and healthy controls. The majority of patients fell within the age groups of 26–34 and 36–45, constituting approximately 25%, with a mean standard deviation of 37.17 ± 12.38. Conversely, the control group mostly belonged to the age group of 25 years and above, representing about 34.48%, with a mean and standard deviation of 26.86 ± 4.74. The results revealed that patients with VZV exhibited a significantly decrease of CD4 count (303.7833 ± 64.276) compared to the control group (626.103 ± 122.07) (p-value < 0.0001). Patients with Varicella-Zoster Virus (VZV) showed a significant increase in CD8 count (1197.717 ± 201.369) compared to the control group (580.379 ± 98.391) with a p-value ≤ 0.0001.

1 Introduction

The Varicella-Zoster Virus (VZV), also known as Human Herpes Virus 3 (HHV-3), is responsible for causing both chickenpox (varicella) and shingles (herpes zoster). The reactivation of VZV in the body leads to shingles, a condition with diverse clinical manifestations that are crucial for distinguishing between diseases. Additionally, shingles can lead to severe complications that may be life-threatening [1]. This virus is a pathogen belonging to the herpesvirus family and it is responsible for causing both chickenpox and shingles and this virus has the ability to establish latency in nerve ganglia after the initial infection, potentially leading to shingles later and this virus is mainly spread through respiratory droplets, presenting a significant challenge to public health, especially in crowded environments with susceptible individuals to understanding the virology, epidemiology, and clinical characteristics of VZV is crucial for effectively preventing, diagnosing, and treating these diseases [2].

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Proteins located on cell surfaces are categorized under the Cluster of Differentiation (CD) classification and each molecule is assigned a distinct number to distinguish the cell phenotype effectively [3]. Cell surface molecules are examined and analyzed using a technique that offers valuable information for immunophenotyping and the activity of cells is influenced by CD antigens in several ways. These antigens often serve as ligands or receptors, initiating signal cascades that alter cell behavior, in addition to signaling functions, many CD antigens play essential roles in cell adhesion, activation, or inhibition [4]. CD antigens are frequently utilized as cell markers in immunophenotyping, enabling the classification of cells according to the surface molecules they possess [5]. Within the immune system, a CD4 cell serves as a type of white blood cell that acts as a primary defense mechanism. Monitoring the CD4 count of individuals infected with HIV is crucial to assessing their overall health status and understanding the impact of the infection on their immunity [6]. CD4 T cells mature in the thymus after originating from common lymphoid progenitors in the bone marrow and they primarily function in peripheral tissues and various lymphoid organs. The capability of CD4 T cells to carefully manage their growth allows them to recognize a wide array of external antigens efficiently, all while avoiding self-reactivity [7]. When stimulated by antigen-presenting cells like dendritic cells, naive CD4 T cells undergo a process of differentiation, leading to the development of different T effector populations and these T effector populations play a critical role in the host's defense mechanisms against pathogens [8]. Effector CD4 T cells release specific molecules known as effector cytokines to eliminate infected cells and support B cells in generating antibodies and these effector cytokines also trigger the activation of other immune cells such as macrophages and CD8 T cells and this coordinated action initiates humoral immune responses, contributing to the body's defense against pathogens [9]. The CD8 T cell surface antigen, a type I membrane protein, is a heterodimer composed of alpha and beta chains linked by two disulfide bonds and the maturation of CD8 T cells relies on the specific presence of this protein on a particular group of T cells [10]. CD8 plays a significant role in T-cell mediated death by recognizing cytotoxic/suppressor T cells that interact with targets carrying MHC class I molecules and the activation process of naïve CD8+ T lymphocytes involves their identification of the specific peptide presented by MHC class I on antigen presenting cells (APCs) in secondary lymphoid organs and naïve CD8+ T cells detect this peptide, they become activated, undergo proliferation, and transform into effector CD8+ T cells and this transformation is triggered by costimulatory signals and cytokine stimulation [11].

2 Material and Method

2.1 Sample Collection

Sixty samples were collected from a person diagnosed with varicella zoster, while 29 samples were collected from healthy individuals in Najaf City between November 2022 and July 2023. Samples were collected from patients with varicella zoster in Sadr Medical City and private laboratories. The samples included individuals of all genders and ages ranging from 15 to 60 years. Samples were drawn and analyzed by using flow cytometry technique.

2.2 Flow-cytometry Method

Flow cytometry is a rapid technology utilized for analyzing individual cells or particles in motion through lasers while immersed in a salt-based solution. Each particle is examined for visible light scatter and fluorescence properties. The measurements of forward scatter

(FSC) and side scatter (SSC) evaluate cell size and internal complexity, respectively. It is essential to differentiate between light scatter evaluations and fluorescence analysis [12].

2.3 Estimation of human CD4

The CD4 was measured by immunoassay technique and the kit is used was achieved by Spark Violet™ 423 anti-human CD4 Biolegend /USA and the CD4 was estimation in the sample of whole blood.

2.4 Estimation of human CD8

the CD8 was measured by immunoassay technique and the kit is used was achieved by APC anti-human CD8 Biolegend /USA. and the CD8 was estimation in the sample of whole blood.

2.5 Statistical Analysis

All values are expressed as mean ± standard deviation of mean and the study results were statistically analyzed using the software statistical package for the social sciences (SPSS) version 26. The Chi-square test (χ^2) and unpaired T-test was employed for categorical data analysis A $P \leq 0.05$ was considered significant [13].

3 Results

The data presented in Table 1 shows that the majority of patients fall within the age ranges of 26-34 and 36-45, each accounting for approximately 25% of the total population. The average age of the patients is 37.17 years with a standard deviation of 12.38 years. In contrast, the control group primarily consists of individuals aged 25 years or younger, who make up about 34.48% of the total. The average age of the control group is 26.86 years with a standard deviation of 4.74 years. A notable proportion of patients (25%) are in the age group of 26-35 years. On the other hand, the control group is skewed towards younger age categories, with more than a third (34.48%) being 25 years old or younger. Furthermore, females constitute a slight majority in both the patient group (60%) and the control group (48.28%).

Table 1. Summarize the frequency distribution of Demographic Data for the studied groups.

Demographic Data		Study Groups			
		Patients		Control	
		No.	%	No.	%
Age groups (Years)	≤ 25	12	20	10	34.48
	26 - 35	15	25	9	31.03
	36 - 45	15	25	2	6.9
	46 - 55	12	20	3	10.34
	56 ≥	6	10	5	17.24
	Mean ± SD	37.17 ± 12.38		26.86 ± 4.74	
Gender	Males	24	40	15	51.72
	Females	36	60	14	48.28
Total		60		29	

The patients with VZV exhibited a significantly decreased (P≤0.0001) in CD4+ count (303.7833 ± 64.276) cell/ml compared to the control group (626.103 ± 122.07) (p-value < 0.0001) see figure (1).

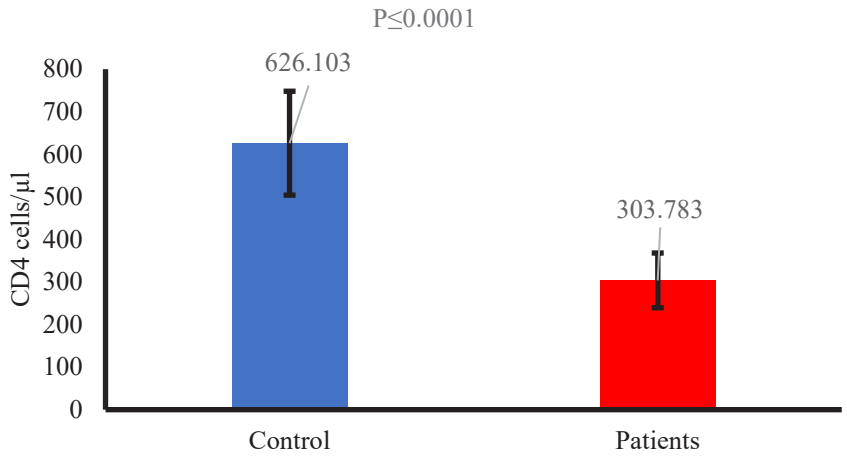

Fig. 1. Mean account of CD4 in patients with VZV and control group.

The diagram in Figure 2 compares the average CD4 counts between male and female Varicella-Zoster Virus (VZV) infected patients and a control group. The results show that there were no significant differences between males and females. In male patients with VZV, the average CD4 count is 320.625 ± 60.336, while female patients have an average CD4 count of 292.556 ± 65.177. These differences were not found to be statistically significant. The p-value suggests that the observed variances are more likely due to chance rather than genuine disparities between genders see (figure 2).

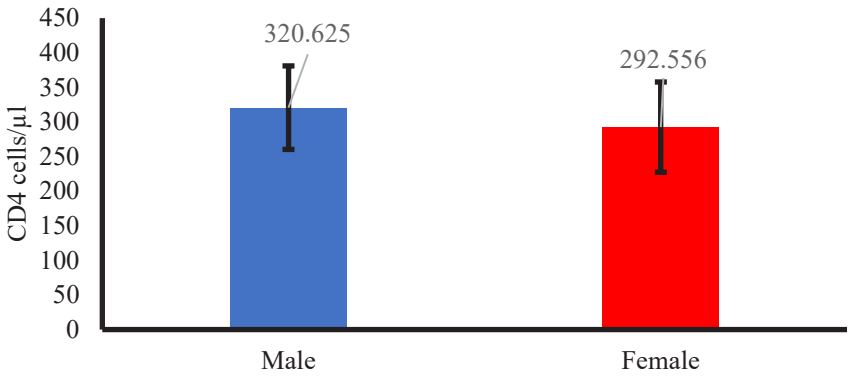

Fig. 2. Mean account of CD4 in patients with VZV and control group according to the gender.

In a recent study, patients with Varicella-Zoster Virus (VZV) showed a significant increase (P≤0.0001) in CD8+ count (1197.717 ± 201.369) compared to the control group (580.379 ± 98.391) see figure (3).

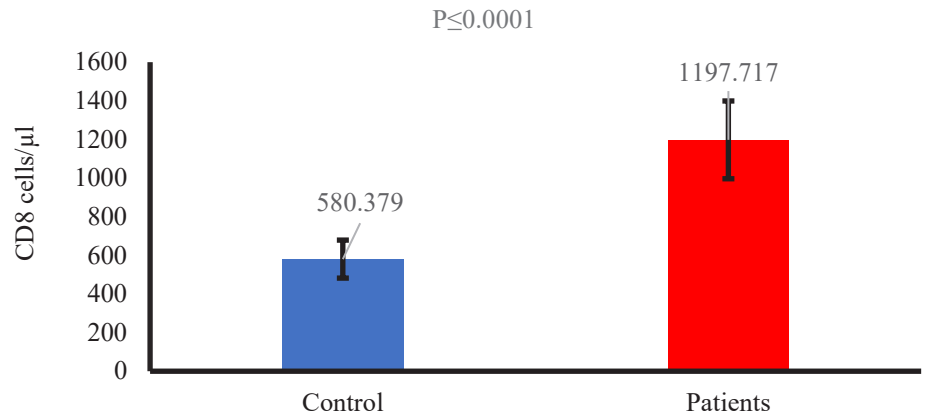

Fig. 3. Mean account of CD8 in patients with VZV and control group.

In Figure 4, the average CD8 count for male patients with VZV is (1189.958 ± 190.143), while female patients show an average CD8 count of (1202.889 ± 211.015). Analysis of the CD8 count comparison between males and females indicates no significant differences.

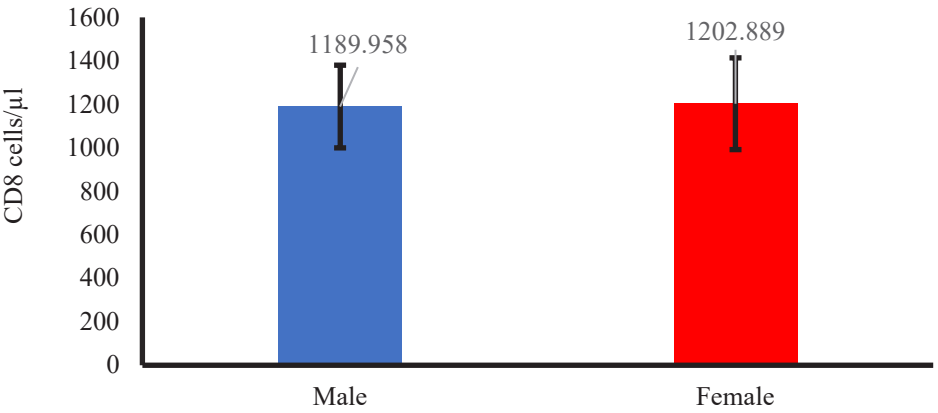

Fig. 4. Mean account of CD8 in patients with VZV and control group according to the gender.

A notable negative correlation exists between CD4 and CD8. This indicates that as the levels of CD8 rise, the levels of CD4 generally decrease, and vice versa as illustrated in figure (5).

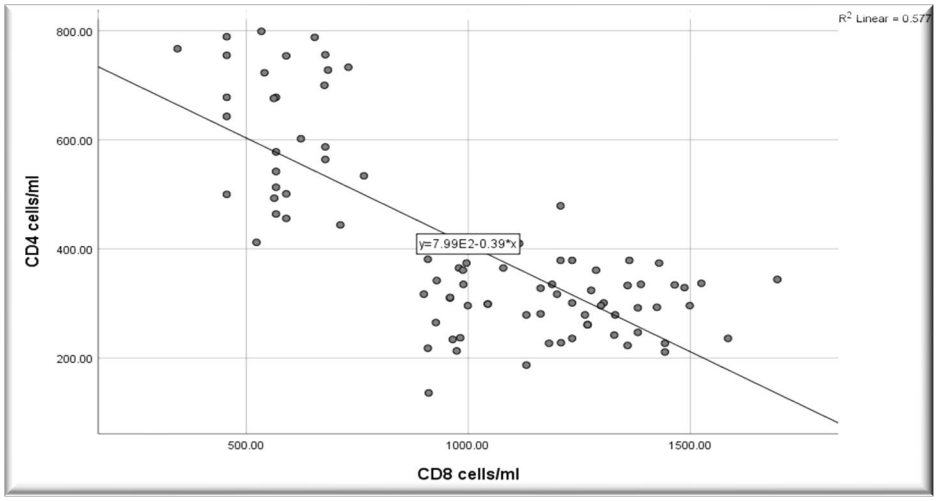

Fig. 5. Scatter diagram between number of CD4 and CD8 in patients with VZV

4 Discussion

The Varicella Zoster virus (VZV) is mainly responsible for causing varicella, known for its high level of contagiousness. While the disease is typically mild in healthy children with strong immune systems, it can be life-threatening for adults and people with weakened immune systems and the infection rate can exceed 85% following exposure and this virus has a single identified serotype, and it solely infects humans [14]. Both skin lesions, droplets, and aerosols from the nasopharynx can shed viruses and the characteristic rash usually appears 1 or 2 days before the incubation phase, which lasts between 10- and 21-days also infection continues until all lesions have cleared the crust, which usually occurs within 5 to 7 days, Varicella infection in temperate climates ranges from 13 to 16 days [15]. The elevated average age of patients at 37.17 years, in contrast to controls at 26.86 years, hints at a potential link between age and VZV infection. Additionally, there was a slight female majority among both patients (60%) and controls (48.28%). Nevertheless, the absence of a noteworthy variance in gender distribution between patients and controls indicates that gender might not significantly impact susceptibility to VZV infection within the study population [16]. Moreover, Stratification of patients across different age groups can affect disease severity and clinical outcomes. This study found that older patients, especially those aged 56 years and older, have a greater risk of complications associated with VZV infection, such as herpes zoster (shingles), and conversely, the greater number of younger individuals in the control group showed less severe symptoms of the disease or a decreased chance of infection with VZV [17]. Differences in the age distribution between patients and controls may suggest differences in the strength of the immune system and previous encounters with VZV. Older individuals may have experienced the primary VZV infection at a young age, which aided in developing their immunity. On the other hand, younger individuals might be more susceptible to either contracting the primary infection or experiencing reactivation of the virus [18]. On the other hand, the even distribution of genders between patients and controls suggests that gender might not have a major impact on the likelihood of contracting VZV in this specific group. Nonetheless, other factors like hormonal influences or behavioral variations could be influential and would benefit from additional research [19].

In the same table, with respect to gender distribution, most patients were female (60%), while males made up 51.72% in the control study group. The gender distribution observed emphasizes a significant difference between the patient and control groups. Within the patient cohort, females made up the majority, accounting for 60% of all participants. On the other hand, the control group showed an overrepresentation of males, making up 51.72% of all participants [20].

The gender difference between the two groups has major repercussions on the results and conclusions of the study, and it is important to recognize the effect of gender on the condition studied. Its results may be that some medical conditions affect males and females differently, which leads to differences in treatment responses that are affected by hormonal differences or societal roles [21]. The analysis of T cell markers revealed that male patients infected with the VZV virus had an average CD4 count of (320.625 ± 60.336), while female patients had an average CD4 count of (292.556 ± 65.177). These differences in CD4 counts between genders were not found to be statistically significant. The p value suggests that the observed variances are likely due to random chance rather than true disparities in baseline CD4 counts between male and female patients. The data illustrates the mean CD4 cell counts in VZV patients categorized by gender. Interestingly, male patients exhibited a slightly higher mean CD4 cell count than female patients. It is crucial to acknowledge that the standard deviation reflects the variability within each group, underscoring the individual variances in CD4 counts among patients [22].

Exploring additional factors that affect the differences in CD4 counts between genders, like hormonal effects or genetic vulnerabilities, could offer valuable understandings into the underlying mechanisms of VZV infection. Moreover, considering the significance of CD4 counts in immune response and disease advancement, these discoveries emphasize the necessity for individualized medical treatment customized to each patient's distinctive qualities, encompassing gender [23]. In a recent investigation, individuals afflicted with Varicella-Zoster Virus (VZV) demonstrated a notable elevation in CD8+ count (1197.717 ± 201.369) in comparison to the control group (580.379 ± 98.391), yielding a p-value of less than 0.0001. This surge in CD8+ levels among VZV patients is indicative of an immune reaction characterized by the activation and multiplication of cytotoxic T cells essential for combatting virus-infected cells. The increased CD8+ count observed in VZV patients signals an active immune response, with CD8+ T lymphocytes playing a crucial role in eliminating viruses by detecting and eradicating infected cells. This uptick in CD8+ count reflects the body's endeavor to confront and eradicate the VZV infection [24]. The significant difference in CD8+ counts between patients and the control group emphasizes the specific immune response to VZV infection and the notable increase in average CD8+ counts demonstrate the robustness of the immune reaction to VZV and this indicates the seriousness of the infection and corresponds with the typical clinical signs of VZV, including inflamed skin sores and systemic symptoms [25]. The discovery highlights the important function of CD8+ T lymphocytes in fighting VZV and provides useful information about the immune system's response to this infection and studying the changes in CD8+ response over time could help in creating specific treatments to strengthen antiviral defenses and reduce the effects of VZV infection on individuals [26]. Male patients with VZV have an average CD8 count of 1189.958 ± 190.143, whereas female patients have an average CD8 count of 1202.889 ± 211.015. The comparison of CD8 counts reveals a minor difference in mean values, with females showing a slightly higher average CD8 count than males [27]. Considerable variability is evident within each gender category based on the standard deviations observed. The divergence in average CD8 counts between males and females might suggest potential differences in immune responses linked to gender. Various factors such as hormones and genetics could potentially influence these disparities [28]. The variation in CD8 counts within gender groups, as indicated by the

standard deviations, highlights the distinctive diversity in immune function across individuals. While there may be a slight difference in average CD8 counts between genders, the wide range within each group suggests that factors beyond gender might influence these counts. To gain a better understanding of CD8 counts in male and female participants, it is crucial to investigate the distribution of CD8 counts within each gender group. This process involves analyzing the range of CD8 counts, detecting any outliers or trends in the data, and obtaining insights into the clinical characteristics or demographics of the participants, which can offer valuable information for further study [29]. Studying the correlations between CD8 counts and variables like age, disease severity, and duration of VZV infection can help advance our understanding of the immune response in individuals infected with VZV. Additionally, examining possible linkages between CD8 counts and other immune markers such as CD4 counts or cytokine levels can provide a more comprehensive understanding of immune dysregulation in VZV infection [30]. Conducting longitudinal studies to monitor CD8 counts in VZV patients over time can unveil the changes in the immune response from the beginning of infection to its resolution. This method of continuous observation can provide important insights into the predictive significance of CD8 counts and their potential as indicators for disease progression or treatment effectiveness [31]. In individuals with Varicella-Zoster Virus (VZV), there exists a notable negative correlation between CD4 and CD8 counts. This signifies an opposite connection between these two immune factors. The correlation coefficient (R) of (-0.577) indicates a moderate to strong negative relationship. Essentially, as the CD8 count rises, the CD4 count tends to decline, and vice versa [32]. In Figure 5, a scatter diagram visually illustrates a negative correlation. The diagram depicts the connection between the quantities of CD4 and CD8 cells in VZV patients. Data points on the scatter diagram show a downward trend, suggesting that higher CD8 counts are generally linked with lower CD4 counts, and vice versa. This negative correlation highlights the intricate balance of immune responses during VZV infection. CD4-positive T cells (helper T cells) and CD8-positive T cells (cytotoxic T cells) work together in a complementary manner within the immune system [33]. While CD4 cells assist in coordinating immune responses, CD8 cells actively target and eliminate infected cells. The negative correlation observed indicates the dynamic interactions between these two cell populations during VZV infection. This emphasizes the sophisticated nature of the immunological processes involved in host defense and viral clearance [34].

5 Conclusion

Varicella-Zoster Virus (VZV) infection involves intricate immunological interactions that impact disease progression. Factors related to age can affect susceptibility to and severity of VZV infection, potentially placing older individuals at greater risk of complications like herpes zoster. The study revealed notable variances in CD4 and CD8 levels among patients with VZV infection, but no significant distinctions were found between males and females in these levels. Additionally, a negative correlation was observed between CD4 and CD8 counts.

References

1. A. Patil, M. Goldust, & U. Wollina, Viruses, **14(2)**, 192 (2022)
2. Hatami, Hossein, et al. Biomedicines, **11.3**, 957 (2023).
3. Tarozzi, Marco, et al. Biomedicines **12.2**, 436 (2024)
4. Amin Rani, Nurul, et al., Frontiers in Microbiology, **14**, 1291868 (2023).

5. Joanna von Hofsten, *Herpes virus retinitis-clinical and virological characteristics* (2023)
6. Oripelaye, Mufutau Muphy, et al. Journal of Clinical Sciences, **21.1**, 14-19 (2024)
7. A.A.J. Aljanaby, Q.M.H. Al-Faham, I.A.J. Aljanaby, and T.H. Hasan, Gene Reports, 101514 (2022) doi.org/10.1016/j.genrep.2022.1015
8. Zhuang, Zhen, et al. Vaccines, **12.5**, 478 (2024)
9. Perciani, Catia Taniela, *Setting the Stage for a Potential Varicella-Zoster Virus (VZV)-based HIV Vaccine: Characterization of the Mucosal Immune Response Induced by VZV Vaccination in Humans* (University of Toronto, Canada, 2019)
10. Goyal, Falak, et al. Advanced Therapeutics, **7.2**, 2300255 (2024)
11. Jain, Abhinav, et al. Frontiers in immunology, **14**, 1250916 (2023)
12. Allen, Caroline Patricia, *The role of natural killer cells in control of varicella zoster virus infection* Diss. (2024)
13. M.A. Ali and A.A.J. Aljanaby, IOP Conf. Ser.: Earth Environ. Sci., **1215**, 012066 (2023) https://doi.org/10.1088/1755-1315/1215/1/012066.
14. Kumar, Abhinendra, et al. Annals of Medicine, **55.2,** 2253733 (2023)
15. Wreghitt, Tim, and Goura Kudesia, *Clinical and diagnostic virology* (Cambridge university press, 2024)
16. Najafi, Saeideh, et al. Jundishapur Journal of Microbiology, **9.3** (2016).
17. R. H. Elhalag, K. R. Motawea, N. E. Talat, S. S. Rouzan, S. M. Reyad, S. M. Elsayed, ... & J. Shah, Medicine, **102(43)**, e34503 (2023)
18. Shin, Linda, et al. " *Vaccine*, **41.32**, 4679-4684 (2023)
19. Thapa, Sangharsha, et al. Health Science Reports, **7.3**, e1941 (2024)
20. Gabutti, Giovanni, et al. ImmunoTargets and therapy, 15-28 (2019)
21. Alameedy, Fadyia Mahdi Muslim, Migration Letters, **20.S2**, 283-288 (2023)
22. M. Hentzien, F. Bonnet, E. Bernasconi, E. Biver, D. L. Braun, A. Munting,... & A. Calmy, BMC infectious diseases, **24(1),** 329 (2024)
23. Hussain, Md Sadique, et al. Reviews in Medical Virology, **34.1**, e2491 (2024)
24. Al Moussawy, Mouhamad, and Hossam A. Abdelsamed, Frontiers in Immunology, **13**, 1001129 (2022)
25. Jin, Wenjie, et al. Pathogens and Immunity, **7.2,** 171 (2022)
26. V. Marrella, A. Facoetti, & B. Cassani, International Journal of Molecular Sciences, **23(19),** 11845 (2022)
27. C. K. Kang, E. Chang, J. Jung, E. Lee, K. H. Song, P. G. Choe ... & M. D. Oh, Journal of Infection and Public Health, **15(7),** 734-738 (2022)
28. M. Hentzien, F. Bonnet, E. Bernasconi, E. Biver, D. L. Braun, A. Munting, ... & A. Calmy, BMC infectious diseases, **24(1),** 329 (2024)
29. Qi, Qian, et al. PLoS pathogens, **12.10**, e1005892 (2016)
30. Malavige, Gathsaurie Neelika, et al., PloS one, **3.11**, e3789 (2008)
31. Kang, Hao, et al. BMC ophthalmology, **21.1**, 193 (2021)
32. Liu, Peng-Cheng, et al. Annals of Dermatology, **36** (2024)
33. Kolakowska, Agnieszka, et al., *Cross sectional survey of Varicella-Zoster virus and measles seropositivity in people living with HIV in a Parisian suburb and a review of current immunization guidelines*, Vaccine (2023)

34. Purohit, Shivam K., et al., Frontiers in Immunology, **14**, 1121714 (2023)