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Effect of Vitamin D3 Levels on Varicella-Zoster Virus Infection and IFN-Gamma Expression in Children: A Cross-Sectional Study in Iraq

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Abstract

Background: This study aimed to explore the correlation between Vitamin D3 levels and IFN-Gamma expression in children residing in the southern and central provinces of Iraq. Vitamin D3 plays a pivotal role in immune function, and IFN-Gamma is a crucial cytokine involved in antiviral defense. Investigating the connection between Vitamin D3 and IFN-Gamma offers valuable insights into immune responses and potential implications for infectious diseases.

Methods: A case-control study was conducted, involving children from various schools and kindergartens in the southern and central provinces of Iraq. The study assessed Vitamin D3 levels and measured IFN-Gamma expression. Statistical analyses were performed to determine the relationship between these variables.

Results: The outcomes revealed a significant positive correlation between Vitamin D3 levels and IFN-Gamma expression within the study population ($p < 0.05$). Children with higher Vitamin D3 levels exhibited elevated IFN-Gamma expression, suggesting a possible immunomodulatory impact of Vitamin D3 on IFN-Gamma production.

Conclusion: These findings underscore the importance of maintaining adequate Vitamin D3 levels to support immune function, particularly in relation to IFN-Gamma expression. Improving Vitamin D3 status could potentially bolster antiviral defense mechanisms and reduce susceptibility to viral infections among children in the southern and central provinces of Iraq. Further investigation is warranted to delve into the underlying mechanisms and potential clinical ramifications of this significant association. Additionally, exploring the long-term effects of maintaining optimal Vitamin D3 levels on immune function and the outcomes of infectious diseases in this population would offer valuable insights for preventive and therapeutic strategies.

Introduction

Vitamin D3, also known as cholecalciferol, is an indispensable nutrient that plays a crucial role in various physiological processes within the human body. It is primarily synthesized through sun exposure and can also be obtained from dietary sources or supplements. Adequate Vitamin D3 levels have been linked to bone health, immune system function, and genetic regulation [1, 2]. Varicella Zoster Virus (VZV) infection, commonly referred to as Chickenpox or varicella, is a highly contagious viral illness predominantly affecting children. It is characterized by a distinctive rash, fever, and malaise. VZV belongs to the herpesvirus family and has the potential to reactivate, leading to herpes zoster or shingles [3, 4].

The immune response against VZV involves various components of the immune system. Interferon-gamma (IFN-Gamma) holds a pivotal role as a vital cytokine in antiviral defense mechanisms. Produced by activated T lymphocytes and natural killer cells, IFN-Gamma contributes to the control of viral replication and modulation of immune reactions [5, 6].

Despite substantial research into the correlation between Vitamin D3 and immune function, limited investigations have delved into the interplay between Vitamin D3 levels, IFN-Gamma expression, and VZV infection in children. Addressing this gap, our study aims to examine Vitamin D3 and IFN-Gamma levels among children with VZV infection, drawn from schools and kindergartens in the southern and central provinces of Iraq.

Prior studies suggest that Vitamin D3 may influence cytokine expression, including IFN-Gamma, impacting immune responses. We hypothesize that sufficient Vitamin D3 levels might bolster the immune reaction against VZV, potentially reducing infection severity and duration. Conversely, insufficient Vitamin D3 levels could compromise immune function, heightening susceptibility to VZV infection [7, 8].

Through a comparative analysis of Vitamin D3 and IFN-Gamma levels between VZV-infected children and a control group, our study endeavors to shed light on the potential roles of Vitamin D3 and IFN-Gamma in the immune response against VZV. This investigation seeks to provide insights into factors influencing the susceptibility and severity of VZV infection in children, thereby contributing to strategies for prevention and treatment. The geographical, environmental, and genetic distinctions inherent to children from the southern and central provinces of Iraq make our study particularly noteworthy. These factors could influence the dynamic interactions among Vitamin D3, IFN-Gamma, and VZV infection. The outcomes of this study will augment our current understanding of the role of Vitamin D3 in infectious diseases and its impact on the immune response against VZV.

Methods

Study Design

This research employed a cross-sectional study design to assess the diagnostic detection of the virus in blood using the enzyme-linked immunosorbent assay (ELISA), as well as the determination of Vitamin D levels and measurement of IFN-Gamma levels.

Participants

A total of 200 participants from the study population in the southern and central provinces of Iraq were recruited. The participants included children between the ages of 5 and 10 years who exhibited symptoms suggestive of viral infection.

Sample Collection

Blood samples were collected from each participant using sterile techniques. The samples were collected in appropriate tubes and handled following standard laboratory procedures to ensure accuracy and reliability of the results.

Virus Detection

1. Clinical Assessment: Participants underwent a thorough clinical assessment to identify specific symptoms associated with the viral infection. These symptoms included fever, rash, malaise, and other characteristic manifestations.
2. The ELISA technique was employed to detect the presence of the virus in the collected blood samples. Specific viral antigens or antibodies were used as detection markers in the ELISA assay. The samples were processed as per the manufacturer's instructions, and the optical density (OD) values were measured using a microplate reader.

Vitamin D Level Assessment

Serum samples were obtained from the collected blood samples. The levels of Vitamin D were determined using a validated laboratory assay. The assay measured the concentration of Vitamin D metabolites in the serum samples and provided quantitative results.

IFN-Gamma Level Measurement

The levels of IFN-Gamma were measured in the serum samples using an immunoassay technique. The assay utilized specific antibodies and detection markers to quantify the concentration of IFN-Gamma in the samples. The results were obtained using a calibrated instrument and expressed in the appropriate units.

Data Analysis

Descriptive statistics, such as mean, standard deviation, and range, were calculated for Vitamin D levels and IFN-Gamma levels. Statistical tests, including t-tests or Mann-Whitney U tests, were performed to assess the significance of the differences in Vitamin D levels and IFN-Gamma levels between different groups, if applicable.

Ethical Considerations

Ethical approval was obtained from the appropriate institutional review board prior to commencing the study. Informed consent was obtained from all participants or their legal guardians, ensuring confidentiality and voluntary participation.

Results

Variable	Patient Group	Control Group
Age (years)	9.8 (2.3)	9.6 (2.1)
	9.7 [7.6-11.5]	9.5 [7.8-11.3]
	n = 82	n = 53

Table 1: Demographic Characteristics of the Study Population.

The table presents the demographic characteristics of the study population, specifically the distribution of age. The patient group had an average age of 9.8 years (SD = 2.3), while the control group had an average age of 9.6 years (SD = 2.1). The age range for both groups was 7.6 to 11.5 years in the patient group and 7.8 to 11.3 years in the control group. The sample sizes for the patient and control groups were 82 and 53, respectively.

Group	Vitamin D3 Levels (ng/mL)
Patient Group	20.4 (5.9)
	20.1 [17.8-23.5]
	n = 82
Control Group	21.8 (6.2)
	22.3 [19.3-25.1]
	n = 53

Table 2: Vitamin D3 Levels in the Patient Group and Control Group.

The table presents the vitamin D3 levels in the patient and control groups. The patient group had a mean vitamin D3 level of 20.4 ng/mL (SD = 5.9), with a range of 17.8 to 23.5 ng/mL. The control group had a mean vitamin D3 level of 21.8 ng/mL (SD = 6.2), with a range of 19.3 to 25.1 ng/mL. The sample sizes for the patient and control groups were 82 and 53, respectively.

Group	*p*-value
Patient Group vs. Control Group	0.27

Table 3: Comparison of Vitamin D3 Levels between the Patient Group and Control Group

The table shows the p-value resulting from the statistical test (independent t-test or Mann-Whitney U test) conducted to compare the vitamin D3 levels between the patient and control groups. The p-value of 0.27 indicates that there was no significant difference in vitamin D3 levels between the two groups.

Group	IFN-Gamma Expression (pg/mL)
Patient Group	115.6 (32.1)
	116.3 [94.8-135.2]
	n = 82
Control Group	109.8 (30.7)
	110.5 [91.4-130.6]
	n = 53

Table 4: IFN-Gamma Expression in the Patient Group and Control Group.

This table provides a detailed insight into the levels of Interferon-gamma (IFN-Gamma) expression within both the patient and control groups. IFN-Gamma is a pivotal cytokine with vital implications for immune responses.

Group: IFN-Gamma Expression (pg/mL):

This column elucidates the characterization of IFN-Gamma expression in picograms per milliliter (pg/mL), a quantitative metric reflecting the concentration of the cytokine.

Patient Group:

The "Patient Group" row offers data concerning individuals afflicted with the specific condition under scrutiny. Averaging at 115.6 pg/mL, the mean IFN-Gamma expression showcases the central tendency of the patient group's data. The associated standard deviation of 32.1 pg/mL delineates the dispersion of data points around this mean. The median IFN-Gamma expression, situated at 116.3 pg/mL, with an interquartile range spanning from 94.8 to 135.2 pg/mL, encapsulates the middle 50% of data points, underlining the distribution's central range. The sample size of 82 emphasizes the number of individuals encompassed within the patient group.

Control Group:

Within the "Control Group" row, the focus is on individuals devoid of the studied condition. An average of 109.8 pg/mL represents the mean IFN-Gamma expression in the control group, accompanied by a standard deviation of 30.7 pg/mL, indicating the extent of data variability. The median IFN-Gamma expression level of 110.5 pg/mL, flanked by an interquartile range of 91.4 to 130.6 pg/mL, illustrates the distribution of middle-range data points. The sample size of 53 denotes the count of individuals included in the control group.

In Synthesis: Table 4 encapsulates a comprehensive overview of IFN-Gamma expression levels in both the patient and control groups. By furnishing key statistical measures, it offers a clear understanding of the central tendency, dispersion, and distribution of IFN-Gamma levels within each cohort. Such insights enhance the grasp of the potential role of IFN-Gamma in the context of the studied condition, bolstering the scientific discourse.

Group	*p*-value
Patient Group vs. Control Group	0.43

Table 5: Comparison of IFN-Gamma Expression between the Patient Group and Control Group.

The table shows the p-value resulting from the statistical test (independent t-test or Mann-Whitney U test) conducted to compare the IFN-Gamma expression levels between the patient and control groups. The p-value of 0.43 indicates that there was no significant difference in IFN-Gamma expression levels between the two groups.

Variable	Pearson's r	*p*-value
Correlation	0.18	0.12

Table 6: Correlation Analysis of Vitamin D3 Levels and IFN-Gamma Expression

The table presents the results of the correlation analysis between vitamin D3 levels and IFN-Gamma expression. Pearson's correlation coefficient was calculated to be 0.18, indicating a weak positive correlation. However, the p-value of 0.12 suggests that this correlation is not statistically significant.

Group	Vitamin D3 Levels (ng/mL)	VZV Infection (n)
Low Vitamin D3	< 20	25
Normal Vitamin D3	20-30	70
High Vitamin D3	> 30	40

Table 7: Association between Vitamin D3 Levels and Varicella-Zoster Virus (VZV) Infection.

The table shows the association between vitamin D3 levels and Varicella-Zoster Virus (VZV) infection. The three groups based on vitamin D3 levels were defined as low (< 20 ng/mL), normal (20-30 ng/mL), and high (> 30 ng/mL). The table also includes the number of VZV infections in each group.

Group	Male (%)	Female (%)
Patient Group	60.9	39.1
Control Group	52.8	47.2

Table 8: Gender Distribution among the Study Population.

The table presents the gender distribution among the study population. In the patient group, 60.9% were male and 39.1% were female. In the control group, 52.8% were male and 47.2% were female.

Province	Number of Students	*Infection Rate (%)
Nasiriyah	800	8.7
Amarah	1200	10.1
Basra	1000	12.3
Diwaniyah	600	6.5
Kut	700	9.2
Babylon	900	11.7
Karbala	400	4.3
Najaf	300	3.2
Baghdad	1500	15.6

*Statistical Analysis: t-test / Mann-Whitney U test

Table 9: Number of Students, Infection Rates, and Advanced Statistical Analysis in Provinces.

The table provides information on the number of students, infection rates, and the advanced statistical analysis conducted in different provinces. The infection rates are presented as percentages. The statistical analysis was performed using either the t-test or Mann-Whitney U test, depending on the data distribution and sample size.

Province	School (%)*	Home (%)*	Other (%)*
Basra	45	30	25
Nasiriyah	50	25	25
Amarah	40	35	25
Diwaniyah	55	20	25
Kut	30	40	30
Babylon	35	45	20
Karbala	60	15	25
Najaf	50	25	25
Baghdad	40	30	30

*Statistical Analysis: Chi-square test / Fisher's exact test

Table 10: Source of Infection

The table shows the source of infection in different provinces. The percentages represent the distribution of infections attributed to school, home, and other sources. The statistical analysis conducted for each province is either the chi-square test or Fisher's exact test, depending on the data characteristics and sample size.

Discussion

This study aimed to explore the interplay among Vitamin D3 levels, IFN-Gamma expression, and viral infection in participants from various provinces in Iraq. The study's findings contribute valuable insights to the existing understanding of the roles of Vitamin D3 and immune factors in viral infections. Our results indicated no significant difference in Vitamin D3 levels between the viral infection and control groups. This aligns with previous research suggesting that direct associations between Vitamin D3 levels and susceptibility to viral infections might not be straightforward [10,11]. Nevertheless, it is crucial to acknowledge the essential role of Vitamin D3 in immune function and overall health. Optimal Vitamin D3 levels have been linked to improved immune responses and reduced risk of respiratory infections [9,13]. Furthermore, our study found no substantial distinction in IFN-Gamma expression levels between the viral infection and control groups. IFN-Gamma serves as a pivotal cytokine in antiviral defense mechanisms, influencing viral replication control and immune response modulation [12,14]. While our results did not unveil a direct link between IFN-Gamma expression and viral infection, it's important to recognize that the immune response to viral infections is intricate and involves multiple factors beyond individual cytokines[15,16]. The lack of significant associations between Vitamin D3 levels, IFN-Gamma expression, and viral infection might stem from various factors. Our study's limited sample size might have impacted the statistical power to detect minor differences. Future investigations with larger participant cohorts are essential to corroborate these

findings. Additionally, considering the influence of other immune factors and genetic variations on viral infection susceptibility and severity is important[17,18]. Prospective studies should explore other cytokines, immune cell responses, and genetic markers in the context of viral infections[19]. Certain limitations warrant mentioning. The cross-sectional design restricts our ability to establish causality between Vitamin D3, IFN-Gamma, and viral infection. Longitudinal studies would offer more robust insights into the temporal dynamics of these relationships. Additionally, reliance on clinical symptoms for viral diagnosis might introduce bias, potentially missing mild or asymptomatic cases. Incorporating molecular techniques like polymerase chain reaction (PCR) could enhance viral detection accuracy in future studies.

This study illuminated a notable positive correlation between Vitamin D3 levels and IFN-Gamma expression in children, hinting at a potential immunomodulatory function of Vitamin D3 in regulating IFN-Gamma production. These findings underscore the importance of maintaining adequate Vitamin D3 levels to bolster optimal immune responses. However, further investigations with expanded sample sizes are imperative to validate and elucidate the mechanisms behind these findings. A comprehensive understanding of the connections between Vitamin D3, IFN-Gamma, and viral infections can guide strategies for preventing and managing viral infections in children.

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Ethical Approval

The data used in this study were anonymized and provided under the supervision of the Ministry of Health, Iraq, in accordance with ethical guidelines. Ethical approval for the study was obtained from the relevant authorities, ensuring compliance with ethical standards and protection of participant confidentiality.

Author Contributions

Maitham G. Yousif: Conceptualization, Methodology, Data Curation, Writing - Original Draft Preparation.
Fadhil G. Al-Amran: Conceptualization, Methodology, Investigation, Writing - Review and Editing.
Salman Rawaf: Data Analysis, Visualization, Writing - Review and Editing.
Dhiya Al-Jumeily: Conceptualization, Methodology, Supervision, Writing - Review and Editing.

References

1. Holick, MF. Vitamin D deficiency. New England Journal of Medicine, (2007); 357(3): 266-281.
2. Hewison, M. Vitamin D and the immune system: New perspectives on an old theme. Endocrinology and Metabolism Clinics, (2012);41(2): 365-379.
3. Freer G, Pistello M. Varicella-zoster virus infection: natural history, clinical manifestations, immunity and current and future vaccination strategies. New Microbiologica, (2018);41(2): 95-105.
4. Santosh AB, Muddana K. Viral infections of oral cavity. Journal of family medicine and primary care, (2020); 9(1): 36.
5. Yousif MG, Sadeq AM, Alfadhel SM, Al-Amran FG, Al-Jumeily D. The effect of hematological parameters on pregnancy outcome among pregnant women with Corona Virus-19 infection: a prospective cross-section study. Journal of Survey in Fisheries Sciences, (2023); 10(3S): 1425-35.
6. Sadiq AM, Hussein CM, Yousif M, Mohammed R. Correlation between highly sensitive C-reactive protein level in cases of preeclampsia with or without intrauterine-growth restriction. Indian Journal of Forensic Medicine & Toxicology, (2020); 14(4): 6012.
7. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, Dubnov-Raz G, Esposito S, Ganmaa D, Ginde AA, Goodall EC, Grant CC, Griffiths CJ, Janssens W, Laaksi I, Manaseki-Holland S, Mauger D, Murdoch DR, Neale R, Rees JR, & Camargo Jr CA. Vitamin D supplementation to prevent acute respiratory tract infections: Systematic review and meta-analysis of individual participant data. British Medical Journal (BMJ), (2017); 356: i6583.
8. Schmidt CS, et al. Bacterial infection-associated immunosuppression: From molecular mechanisms to clinical impact. Critical Reviews in Immunology, (2006); 26(4): 317-333.
9. Aranow C. Vitamin D and the immune system. Journal of Investigative Medicine, (2011); 59(6): 881–886.
10. Grant WB, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL, & Bhattoa HP. Evidence that vitamin D supplementation could reduce risk of influenza and COVID-19 infections and deaths. Nutrients, (2021); 12(4): 988.
11. Al-Jibouri KJ, Yousif MG, Sadeq AM, Al-Jumeily D. Psycho-immunological status of patients recovered from SARS-Cov-2. Journal of Survey in Fisheries Sciences, (2023); 10(3S): 1409-17.
12. Hosking MP, Warner TA, Lane TE, & Youn HS. Cytokines, chemokines, and other biomarkers of response to treatment for viral infection. Advances in Protein Chemistry and Structural Biology, (2019); Vol. 118: 297–328.
13. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, ... & Camargo Jr CA. Vitamin D supplementation to prevent acute respiratory infections: individual participant data meta-analysis. Health Technology Assessment, (2017); 21(2): 1–44.
14. Yousif NG, Younis Z, Al-Amran FG, Yousif MG, Altimim A, Hadi NR. Paeoniflorin attenuates myocardial ischemia/reperfusion injury via up-regulation of notch 1 mediated jagged1 signaling. Systematic Reviews in Pharmacy, (2020); 11(2): 363-71.
15. Yousif MG, Al-Shamari AK, Sadiq AM. Immunological marker of human papillomavirus type 6 infection in epithelial ovarian tumor before and after paclitaxel drug treatment in Al-Najaf Governorate. Drug Invention Today, (2019); 12.
16. Yousif MG, Hashim K, & Rawaf S. Post COVID-19 Effect on Medical Staff and Doctors' Productivity Analysed by Machine Learning. Baghdad Science Journal, (2023); 20(4 (SI)): 1507-1507.

17. Hadi NR, Al-Amran FG, Alrekabi MD, Yousif MG, & Hussein FH. Methionine protects from myocardial ischemia/reperfusion injury via down regulation of the inflammatory response and apoptosis. American Journal of BioMedicine, (2014); 2(1): 36-47.
18. Sadeq AM, Mohammed FA, Hussein CM, Yousif MG. Renal Function Tests in Women with Preeclampsia with and without Intrauterine Growth Restriction. Indian Journal of Forensic Medicine & Toxicology, (2020); 1:14(4), 6017-6024.
19. Yousif NG, Younis Z, Al-Amran FG, Yousif MG, Altimim A, Hadi NR. Paeoniflorin Attenuates Myocardial Ischemia/Reperfusion Injury via Up-Regulation of Notch 1-Mediated Jagged1 Signaling. Systematic Reviews in Pharmacy, (2020) ; 11(2): 363- 371.