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Research Article

Seroprevalence of *Toxoplasma gondii*, Rubella, Group A *Streptococcus*, CMV and HSV-1 in COVID-19 Patients with Vitamin D Deficiency

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Abstract

Background and Objective: In recent years, respiratory tract viral infections have caused many pandemics that impact the whole world. To investigate the seropositivity of *Toxoplasma gondii*, rubella, CMV, HSV-1 and group A *Streptococcus* in recovered COVID-19 patients and correlate these findings with vitamin D levels. **Materials and Methods:** A total of 417 COVID-19 patients with diarrhoea were enrolled in this study. Vitamin D and seroprevalence for *Toxoplasma gondii*, rubella, CMV, HSV-1 and group A *Streptococcus* were evaluated and correlated. **Results:** It was found that recent infection in COVID-19 patients with HSV-1, rubella, *Toxoplasma* and CMV, respectively. IgG was detected indicating the development of adaptive immunity with all microbes. **Conclusion:** Current study detected a correlation between vitamin D levels and HSV-1 and no correlation between this infection and vitamin D deficiency with the other microbes.

Key words: Co-infection, diarrhoea, COVID-19 patients, vitamin D deficiency, HSV-1

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In recent years COVID-19 pandemic has had an impact all over the world. Countries have suffered in many aspects, economically and socially and the education system struggled during the lockdown, but health services were the most affected. Mortality and morbidity have been increasing since the emergence of Wuhan-Hu1, which caused about 150,000 deaths only six months after its first report¹⁻³. Zoonotic origin viruses were the key players in pandemics in the last two decades, such as the Middle East respiratory syndrome (MERS) and SARS, which are both from the same genus as SARS-COV-2³. Reports have documented virus transmissions that have occurred between humans and other mammals, which open the door for mutations and the emergence of new genotypes that can cause many problems and disrupt mass vaccination strategies. Furthermore, challenges of new microbial emergence are increasing, accompanied by the threat of antimicrobial resistance and lack of vaccination, thus countries with dense populations and a poor economy will suffer the most⁴. Many microbes have a pandemic potential: whenever a new mutation occurs accompanied by poor management at the site of a pandemic, it can spread to impact the world. SARS-COV-2 is an RNA virus, which increases the risk of rapid mutations that affect the immune system's coping with this infection with every new mutation.

Non-skeletal functions of vitamin D are common, especially those related to the immune system and fighting infection. *Toxoplasma gondii* prevalence is common in many countries and depends on several factors. Toxoplasmosis has been correlated with many diseases⁵. A study detected vitamin D deficiency during acute toxoplasmosis episodes⁶. Another study found a correlation between the rising titer of *Toxoplasma* IgG and levels of vitamin D⁶. Regarding rubella, one study detected no correlation between vitamin D and rubella⁷. Cytomegalovirus (CMV) has been studied in the past years regarding its pathological effect during many diseases, past studies have found a strong correlation between CMV and expansion of many immune cells^{8,9}. A low level of vitamin D was associated with late-CMV infection and the risk of kidney transplant rejection¹⁰. Herpes simplex virus type 1 (HSV-1) and vitamin D deficiency were associated with recurrent activation in seropositive patients¹⁰.

The objective of the current study was to evaluate the prevalence of *Toxoplasma*, CMV, rubella, group A

Streptococcus and HSV-1 in COVID-19 patients and the possible correlation between those microbes and vitamin D deficiency.

MATERIALS AND METHODS

Study area: This study was performed between January-May, 2021 in Jazan University, Jazan city and Taif University, Taif city.

Ethics statement: This study was approved by the Directorate of Health Affairs in Taif (IRB number HAP-02-T-067, approval number 427, dated 20/09/2020). Patients were provided with a consent form with details of the study. Samples were only used to measure vitamin D levels and serological analysis of *Toxoplasma gondii*, rubella, CMV, group A *Streptococcus* and HSV-1.

Study group: A total of 417 participants were included in this study. The patients included in this study were from Taif city and diagnosed with COVID-19. The targeted study group included those who had diarrhoea episodes as part of their COVID-19 clinical symptoms.

Vitamin D evaluation: Serum levels of 25-hydroxycholecalciferol has been classified into severely deficient, deficient, insufficient and normal groups. 3 mL of venous blood was collected into a plain tube and analyzed by ROCHE COBAS® platform e501. When vitamin D serum level ≥ 50 ng mL⁻¹, it was considered normal, 30-49 ng mL⁻¹ was insufficient, 20-29 ng mL⁻¹ was deficient and <20 ng mL⁻¹ was severely deficient.

Serological analysis: For group A *Streptococcus*, a Siemens N Latex ASL (lot 110779) was used following the manufacturer's manual, agglutination of the sample was considered positive results. The Siemens BEP® III System was used to test *Toxoplasma gondii* IgM (lot EK0150) positive value ratio >1.2 IU mL⁻¹, *Toxoplasma gondii* IgG (lot ab108776) positive value ratio >1.3 IU mL⁻¹, rubella IgG (D91030054T) positive value ratio >1.2 IU mL⁻¹, rubella IgM (91031) positive value ratio >1.3 IU mL⁻¹, CMV IgM (ESR109M) positive value ratio >1.2 IU mL⁻¹, CMV IgG (D91010043T) positive value ratio >1.3 IU mL⁻¹, HSV-1 IgG (0720/D) positive when antibody index >11 and HSV-1 IgM (1120/D) positive when antibody index >11.

Statistical analysis: GNU PSPP (1.2.0-g0fb4db) software was used for statistical analysis. Data of this study were assessed for normality and it has shown that data do not follow the normal distribution. Pearson correlation coefficients were applied to determine the degree of correlation between independent variables such as vitamin D levels and with other dependent variables and the seroprevalence results. Furthermore, as current data were categorical, a binary regression test was applied to estimate the correlation between vitamin D levels and the seroprevalence statuses of the patients. Moreover, $p \leq 0.05$ was treated as a significant difference.

RESULTS

Demographic and vitamin D evaluation: The age group 30-50 years was the most common, followed by <30 and >50. Females comprised 64% of the current study group. When Pearson's Chi-squared test was applied to compare the data, all demographic results were statistically significant, as vitamin D severe deficiency and deficiency were common in age and sex groups in Table 1.

Detection of targeted microbes by immunoassay and vitamin D deficiency: *Toxoplasma*, rubella, HSV-1 and CMV

prevalence were diagnosed by immunoassay via the detection of IgG and IgM and via Antistreptolysin O test for group A *Streptococcus* shown in Table 2. A total of 268 (64.2%) patients were *Toxoplasma* IgG seropositive and two (0.4%) IgM seropositive. A total of 382 (91.6%) patients were seropositive for rubella and seven (1.6%) were IgM seropositive. A total of 333 (80%) patients were seropositive for CMV and two (0.4%) were IgM seropositive. Only three patients (0.7%) were group A *Streptococcus* seropositive and 123 patients (30%) were HSV-1 seropositive. Severe vitamin D deficiency was common and statistically significant in most patient groups: 58.5% of *Toxoplasma* IgG, rubella IgG 53% and IgM 57%, CMV IgG 54.3% and IgM 100%, HSV-1 IgG 46% and 60% IgM and 66.7% of group A *Streptococcus*. IgG seropositivity for *Toxoplasma*, rubella, HSV-1 and CMV indicate immunity against these infections immunity can be by vaccination, in the case of rubella (91.6%), or due to natural infection, for the three microbes. Only a small percentage who have IgM for *Toxoplasma*, rubella and CMV, indicating recent infection, also have vitamin D deficiency. Of the 417 tested patients, only 3 (0.7%) were Antistreptolysin O positive and have vitamin D deficiency. The 30% of all patients were HSV-1 IgM seropositive, 60% of those have severe deficiency and 26% deficiency. To compare between the sex groups, regarding infection and vitamin D level shown

Table 1: Demographic and clinical characteristics of all patients

Characteristics	Severe deficiency	Deficiency	Insufficiency	Normal	Total
Age					
<30	91 (60%)*	42 (27%)	17 (11%)	3 (2%)	153
30-50	115 (49%)*	61 (26%)	45 (25%)	13 (5%)	234
>50	14 (46%)*	10 (34%)	4 (13%)	2 (7%)	30
Male	50 (33%)	51 (34%)*	39 (26%)	10 (7%)	150
Female	170 (63%)*	62 (23%)	27 (11%)	8 (3%)	267
Total number of patients	220 (52%)	113 (27.5%)	66 (16%)	18 (4.5%)	417

*p-value was statistically significant as calculated by Pearson's Chi-squared test

Table 2: Association between the infection type and levels of vitamin D

Serological test	Severe deficiency	Deficiency	Insufficiency	Normal	Chi-sq (p-value)	Total
<i>Toxoplasma</i>	IgG	157 (58.5%)	60 (22.7%)	38 (14.2%)	13 (4.6%)	268
	IgM	0	2 (100%)	0	0	2
Rubella	IgG	203 (53%)	101 (26.4%)	63 (16.5%)	15 (4.1%)	382
	IgM	4 (57%)	1 (14.3%)	2 (28.7%)	0	7
CMV	IgG	181 (54.3%)	88 (26.4%)	50 (15%)	14 (4.3%)	333
	IgM	2 (100%)	0	0	0	2
HSV-1	IgG	94 (46%)	62 (30.5%)	37 (18.2%)	10 (5%)	203
	IgM	74 (60%)	32 (26%)	14 (11.4%)	3 (2.6%)	123
Group A <i>Streptococcus</i>	2 (66.7%)	1 (33.3%)	0	0	405.09 (.001)	3

Pearson's Chi-squared test was applied, positive values were considered as follows: Toxo-IgM positive >1.2 IU mL⁻¹, Toxo-IgG positive >1.3 IU mL⁻¹, Rubella-IgG positive >1.2 IU mL⁻¹, Rubella-IgM positive >1.3 IU mL⁻¹, CMV-IgM positive >1.2 IU mL⁻¹, CMV-IgG positive >1.3 IU mL⁻¹, HSV-1 IgM and IgG positive when antibody index >11 and Group-A-strep ASL agglutination is positive

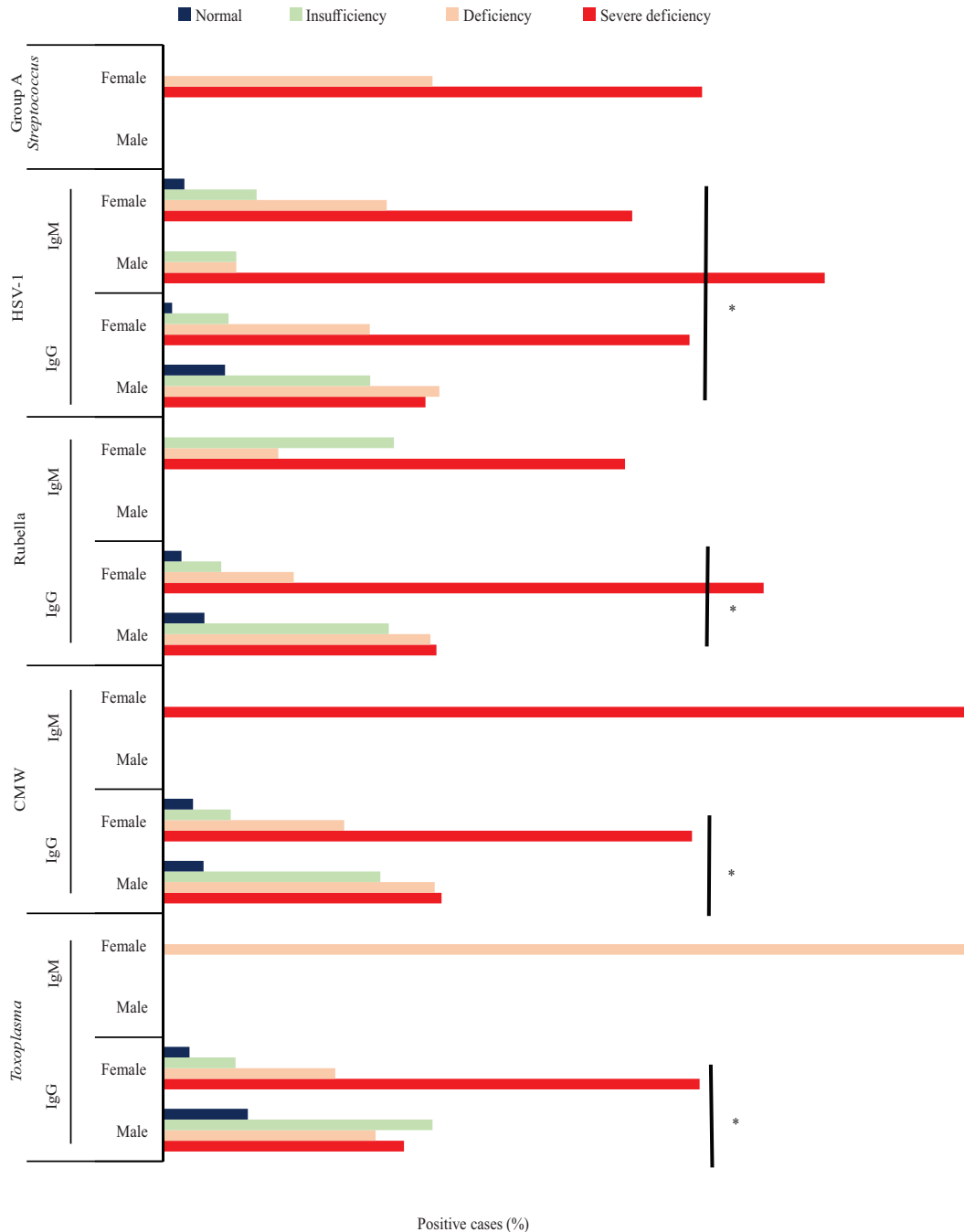


Fig. 1: Comparison between sex groups regarding serological test results and vitamin D levels

Pearson's Chi-squared test was applied, positive values were considered as follows: Toxo-IgM positive > 1.2 IU mL⁻¹, Toxo-IgG positive > 1.3 IU mL⁻¹, rubella-IgG positive > 1.2 IU mL⁻¹, rubella-IgM positive > 1.3 IU mL⁻¹, CMV-IgM positive > 1.2 IU mL⁻¹, CMV-IgG positive > 1.3 IU mL⁻¹, HSV-1 IgM and IgG positive when antibody index > 11 and Group-A-strep ASL agglutination is positive

in Fig. 1, female patients were significantly higher than males in terms of seropositive for IgG of *Toxoplasma*, rubella, HSV-1 and CMV along with severe vitamin D deficiency.

Correlation between vitamin D and the infection type: For correlation between vitamin D levels and those infected,

binary regression was applied. Overall, even though many of the current study groups have shown a deficiency in vitamin D and most of them suffer from severe deficiency, only HSV-1 IgM seropositive male patients have a significant correlation between vitamin D levels and infection in Table 3.

Table 3: Correlation between the infection type and vitamin D

Serological test		B	Standard error	Exp (B)	Wald X ²	p-value	95% CI	
							Lower	Upper
<i>Toxoplasma</i> IgG	Male	-0.35	0.18	0.70	3.63	0.057	0.49	1.01
	Female	0.22	0.18	1.25	1.56	0.212	0.88	1.77
<i>Toxoplasma</i> IgM	Male	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Female	-0.57	0.7	0.56	0.67	0.412	0.14	2.21
CMV IgG	Male	0.19	0.01	1.21	0.81	0.37	0.98	1.04
	Female	0.15	0.18	1.17	0.66	0.417	0.81	1.67
CMV IgM	Male	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Female	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Rubella IgG	Male	0.19	0.3	1.21	0.43	0.514	0.68	2.18
	Female	-0.09	0.3	0.92	0.08	0.993	0.51	1.65
Rubella IgM	Male	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Female	-0.27	0.42	0.76	0.4	0.525	0.33	1.75
HSV-1 IgG	Male	-0.14	0.22	0.87	0.4	0.87	0.57	1.33
	Female	0.17	0.17	1.18	0.95	0.33	0.84	1.66
HSV-1 IgM	Male	1.51	0.59	5.54	6.63	0.01	1.44	14.37
	Female	-0.18	0.16	0.83	1.28	0.258	0.62	1.14
Group A <i>Streptococcus</i>	Male	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Female	0.38	0.91	1.45	0.17	0.670	0.24	8.2

A binary regression test was applied, N/A: Not applicable due to zero results

DISCUSSION

This study is the first that focused on the seroprevalence of *Toxoplasma*, rubella, CMV, group A *Streptococcus* and HSV-1 in COVID-19 patients who have diarrhoea episodes and correlated the findings with vitamin D levels. It was hypothesized that the effect that was exerted by SARS-CoV-2 on the body of the infected patients can lead to several pathological effects, including the co-infection with several microbes and that levels of vitamin D can affect the immune system and weakens its defences against SARS-COV-2 and other microbes. Overall, respiratory infections are common and affect all age groups and all countries. When respiratory infection disseminates to reach the lower respiratory tract, it can lead to pneumonia. Many types of viruses, bacteria and fungi can cause respiratory illness, nonetheless, recent decades have proven that influenza and coronaviruses are assuming the role for causing pandemics and global panics and this can increase when the origin of the microbe is zoonotic. The COVID-19 pandemic has been expanding since 2019 and vaccination seems to be the possible solution for this virus. The high-risk groups for respiratory infections are those with chronic diseases, young children, elderly people and pregnant women, however, studies have detected less prevalence of COVID-19 in children¹¹⁻¹³.

In the current study, HSV-1 reactivation in male patients was found to correlate with COVID-19 with diarrhoea episodes and vitamin D deficiency. HSV-1 reactivation was detected in a COVID-19 case with vitamin D deficiency with conjunctivitis,

which is consistent with the current study¹⁴. Regarding *Toxoplasma*, the current study has found no correlation between this parasite and vitamin D levels, which is consistent with another study¹⁵ that reported a negative correlation between COVID-19 and *Toxoplasma* and this negative correlation was reported due to *Toxoplasma* antiviral effect exerted by the parasite. Only two cases of female patients have shown recent infection by this parasite based on the presence of IgM. Rubella IgM was detected in seven female patients indicating recent infection, with four of them having severe vitamin D deficiency, IgG levels were high, indicating immunity against rubella, which can be due to natural infection or vaccination. A study has claimed that the booster measles, mumps and rubella measles-mumps-rubella vaccine can protect against COVID-19¹³. CMV latent infection inside the host has been the focus of much research, due to its many effects, especially in immunocompromised patients. Only two female patients in the current study have IgM seropositive indicating recent infection. CMV reactivation in COVID-19 was reported in two cases recently¹¹.

CONCLUSION

COVID-19 patients' diarrhoea can be due to other factors, including this disorder. The current study has detected that there is no correlation between this infection with *Toxoplasma*, rubella, CMV and group A *Streptococcus* infection and with vitamin deficiency. Moreover, correlate with vitamin D levels and HSV-1.

SIGNIFICANCE STATEMENT

This study has discovered a correlation between vitamin D deficiency and the coinfection of HSV-1 and SARS-CoV2. HSV-1 is a lifelong infection and known for causing cold sores or oral herpes. Also, it is transmitted through the oral-to-oral route. Ventilated COVID-19 patients could be at high risk of being infected with HSV-1 or the reactivation of this virus. Also, this study can assume that the imbalance in the immune system during COVID19 can lead to the reactivation of HSV-1 infection.

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