European Journal of Biomedical Research
www.ej-biomed.org

Seasonal Respiratory Virus Circulation in a Tertiary Care Hospital in Greece

Antonia Mourtzikou, Marilena Stamouli, ElpidaToka, Panagiotis Koumpouros, Georgia Kalliora, Christina Seitopoulou, and Maria Kimouli

ABSTRACT

Background: **The COVID-19 pandemic caused by the novel SARS-CoV-2 virus affected health care systems and public health worldwide dramatically. Several measures were applied in order to prevent or stop the rapid transmission of the virus and the subsequent disease, such as lockdowns, physical distancing, strictly hygiene, along with travel restrictions. Global population after vaccination programs against COVID-19 were carried out, is facing a "tripledemic" situation threat, with the co-existance of SARS-CoV-2, influenza and RSV. The aim of the present study was to evaluate the co-existence of SARS-CoV-2, influenza and RSV, as well as the correlation with gender, age, Cts and vaccination doses.**

Methods: **A total of 302 patients were included in the study. All patients were admitted to the emergency department of General Hospital Nikea, Piraeus with common upper respiratory tract symptoms and were suspected for COVID-19 disease, between March to July 2022. Patients' age, gender, vaccination doses, and results from RT-PCR detection for SARS-CoV-2, RSV and Influenza viruses were recorded.**

Results: **139 were male and 163 female, aged between 18-94 years. Out of the patients included in the study, 206 were vaccinated and 96 were not vaccinated. Among vaccinated patients 97 were male and 109 were female. A percentage of 3.3% had received one vaccination dose, 16.9% two and 47.7% three. Moreover, 88 patients presented infection symptoms; 81 patients had a positive rapid test result. We detected 15 cases of co-infection of SaRS-CoV-2 and RSV and only one case, with co-infection of SaRS-CoV-2 with influenza virus.**

Conclusions: **The majority of patients admitted to the emergency department of GHNP with common upper respiratory tract clinical manifestations were female. A significant lower rate on co-infection with SARS-CoV-2 and RSV was detected in patients having received 2 vaccination doses, compared to patients having received 3 out of 3 vaccination doses or up to 1 vaccination dose. Ct values for SARS-CoV-2 and RSV pathogens were between 10-17. Co-infection with SARS-CoV-2 and Influenza was detected in only 1 patient.**

Keywords: COVID-19, co-infections, Ct, Influenza viruses, RSV, SARS-CoV-2, vaccines.

Published Online: July 02, 2023

ISSN: 2796-0056

DOI : 10.24018/ejbiomed.2023.2.3.65

A. Mourtzikou*
Laboratory of molecular diagnostics, GHNP "Agios Panteleimon," Greece.
(e-mail: antoniamour@med.uoa.gr)
M. Stamouli
Biochemistry laboratory, Naval and Veterans Hospital of Athens "NNA," Greece.
(e-mail: marilena_stamouli@yahoo.com)
E. Toka
Application specialist in Molecular Biology, Greece.
(e-mail: etoka@emedgroup.gr)
P. Koumpouros
Laboratory of molecular diagnostics, GHNP "Agios Panteleimon" and Department of Biochemistry, GHNP "Agios Panteleimon," Greece.
(e-mail: pkoump78@gmail.com)
G. Kalliora
Faculty of Biology, National and Kapodistrian University of Athens, Greece.
(e-mail: georginakall3@gmail.com)
C. Seitopoulou
Laboratory of molecular diagnostics, GHNP "Agios Panteleimon," Greeece.
(e-mail: xseitopoulou@yahoo.gr)
M. Kimouli
Laboratory of Microbioloy, GHNP "Agios Panteleimon," Greeece.
(e-mail: kimoulimaria@gmail.com)

Corresponding Author

I. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in December 2019 in Wuhan, China and rapidly spread worldwide leading to a pandemic level in early March 2020 [1], [2]. Therefore, there was a high need to diagnose suspected cases rapidly and accurately, in order to prevent or control the spread of SARS-CoV-2 infection. The major technique to achieve that purpose was using real-time reverse transcription polymerase chain reaction (rRT-PCR) [3], [4]. Moreover, wearing masks in all daily human activities, handwashing, physical distancing, travel restrictions and several lockdowns were applied, to stop or partially block the highly infectious virus transmission among the population. Those measures critically affected the human-virus relations, thus preventing the occurrence of other types of respiratory diseases from circulating among worldwide population [5]–[9].

There are several respiratory diseases, caused mainly by influenza A and B viruses (IAV and IBV) and respiratory syncytial virus (RSV). These viral infections cause and share mild to severe symptoms such as cough, fever, headache, muscle ache and pneumonia, similar to COVID-19 disease [10], [11]. During December 2019 until mid-2022, the highly increased existence of COVID-19 disease and the measures taken to prevent spreading among human population, led to a parallel dramatic decrease of IAV, IBV and RSV presence

[12]–[14]. As vaccination against COVID-19 increased over the last 2 years of pandemic status, those measures were partially lifted off or stopped, resulting the increased presence of those respiratory viruses in addition to SARS-CoV-2 virus. The simultaneously presence of SARS-CoV-2, influenza and RSV viruses described as a "tripledemic" situation [15], [16].

This surge of "tripledemic" situation have risen the need of rapidly and accurately diagnose the respiratory virus infection and treat disease appropriately. The objective of the study was to examine the presence of influenza, RSV and SARS-CoV-2 viruses on mid-March 2022- mid-July 2022 (prior winter surge of "tripledemic") on 302 patients that were admitted to Nikaia General Hospital "Agios Panteleimon", Piraeus, Greece, a tertiary care hospital and possibly predict the winter burst of hospitalized patients.

II. Materials and Methods

Our study was performed following the guidelines of the Helsinki Declarationof ethical principles for medical research that include human subjects.The study included 302 symptomatic patients admitted to tertiary General Hospital of Nikaia"Agios Penteleimon", between March and July 2022.Clinical specimens were collected by hospital professional medical staff. Nasopharyngeal sampling method and storage was in accordance with the guidelines of the Disposable Virus Sampling Tube Kit. Collected specimens were randomly numbered by the Director of the Emergency Department and then stored at 4°C, for no more than 48 hours, before they were tested. All samples were tested with Bosphore SARS-CoV-2/ Respiratory Pathogens Panel kit v1. using Bosphore SARS-CoV-2/ Respiratory Pathogens Panel kit v1 and Unio Viral DNA-RNA Extraction Kit 600, manufactured by Anatolia Geneworks, as presentedinTable 1. The PCR testing of the specimens took place at the Molecular Department of General Hospital of Nikaia "Agios Penteleimon". Statistical analysis was performed with MINITAB 17 statistical package.

TABLE I: Materials and Analytical Methods Applied for the Study

Reagent Name	Bosphore SARS-CoV-2/ Respiratory Pathogens Panel kit v1	Unio Viral DNA-RNA Extraction Kit 600	Disposable Virus Sampling Tube
REF No	ABSCR3	UVDR600	30 TUBE/KIT
Storage conditions	-20 °C	+25 °C *	2 °C ~35 °C
Company	Anatolia Geneworks	Anatolia Geneworks	BiobaseBiodustry (Shandong) Co., Ltd

*After resuspension PK and Carrier RNA were stored at +4°C and -20°C respectively.

A. Testing Procedure

After specimen collection, the RNA extraction from the samples was performed. For the RNA extraction and purification, we used Unio B2448 Extraction System and Unio Viral DNA-RNA Extraction Kit 600, both manufactured by Anatolia Geneworks, which is based on magnetic bead method. The kit consists of cartridges prefilled with lysis and wash buffers, as well as the magnetic beads. Disposable tips and rods are included in the cartridge.According to the manual of the kit, Proteinase K (PK), Carrier RNA and sample need to be inserted into the lysis well of each cartridge as presented in Table II.

TABLE II: Cartridge Preparation

Reagent	Amount (μL)
Proteinase K	20 μL
Carrier RNA	10μL
Sample	600μL

All samples were handled and tested separately (no batch testing was performed) and the preparation of the cartridge was completed in the laminar of our lab. For more accurate results, all samples were vortexed for 30 seconds, before their addition into the lysis well of the cartridge. After all cartridges were placed into Unio B24 Extraction System, the following settings were selected according to manufacturer's guidelines (Table III):

TABLE III: Extraction Program Settings

Kit Selection	VDR600
Kit Control and Sample Number	1-24 Samples
Sample Volume and Position	600μL - Directly in well
Elution Volume and Position	60μL - Directly in well

Until the extraction and purification of the sample was completed, PCR Master Mix preparation was performed. For the SARS CoV-2 detection we used Bosphore SARS-CoV-2/ Respiratory Pathogens Panel kit v1. The PCR Master Mix was prepared according to the manufacturer's instructions, by mixing PCR Master Mix 1 and RT Mix. The final volume of the PCR Master Mix was calculated in accordance with the number of samples tested in each run+ 10% (Table IV).

TABLE IV: Master mix Preparation Volumes per Sample

PCR Master Mix 1	25,6 μl
RT Mix	0,4 μl
Sample	14 μl
Test Master Mix volume	26 μl

After mixing and smoothly shaking the PCR Master Mix we divided into the 0,2 mL PCR 8strips by inserting 26 μl of master mix into each strip. As soon as the extraction step was completed, 14μl of the purified sample RNA was added into each strip well containing the PCR Master Mix. Finally, the PCR 8 strips were sealed and placed into Montania 4896 thermocycler manufactured by Anatolia Geneworks. For each PCR run, a positive and negative control sample were tested. Positive control tube contains synthetic DNA for SARS-CoV-2, Influenza A/B, RSV A/B and Human RNase P gene region.

Bosphore SARS-CoV-2/ Respiratory Pathogens Panel kit v1 detect target genes ORF1ab and N for SARS-CoV-2 at FAM channel, mp2 gene for FluA and NS1 gene for FluB at Cy5 channel and Np gene for RSV A&B at HEX channel. Human endogenous nucleic acid sequence RNase P is used as an endogenous internal control (IC). The analytical sensitivity of the kit for nasopharyngeal swab samples is as 100 copies/ml for SARS-CoV-2, 504 copies/ml for Influenza A, 672 copies/ml for Influenza B, 504 copies/ml for the RSV A, 1176 copies/ml for RSV B.The applied thermal protocol is presented in Table V:

TABLE V: THERMAL PROTOCOL FOR BOSPHORE SARS-COV-2/ RESPIRATORY PATHOGENS PANEL KIT V1

Stage	T °C	Time (min)	Cycles
Reverse Transcription	50	17:00 min	1
Initial denaturation	95	06:00 min	1
Denaturation	97	00:30 min	
Annealing (Data Collection)	62	00:40 min	40
Hold	32	02:00 min	1

B. Result Interpretation

As the thermal protocol is completed, SLAN 8.3.2 software automatically calculates the baseline cycles and the threshold to export the interpretation of the results. The Threshold Value Ct for the positive control is determined ≤30 and ≤32 for the internal control (IC) (Table VI).

TABLE VI: RESULT INTERPRETATION

Positive control	IC (Texas Red)	Result
+	+/-	Positive
-	+	Negative
-	-	Invalid

III. RESULTS

The study included 302 patients, 139 (46.0%) were male and 163 (54.0%) were female. Patient ages ranged from 14 to 99 years. Age of male patients ranged from 18 to 94 years (mean value 51.35 years). Age of female patients ranged from 14 to 99 years (mean value 50.45 years). The median value was equal to 49.00 years in both gender groups. There is not a statistically significant difference of the mean age value between the two groups (p-value = 0,707; 95% CI for difference: (-5,60; 3,80). Gender distribution is presented in Fig. 1.

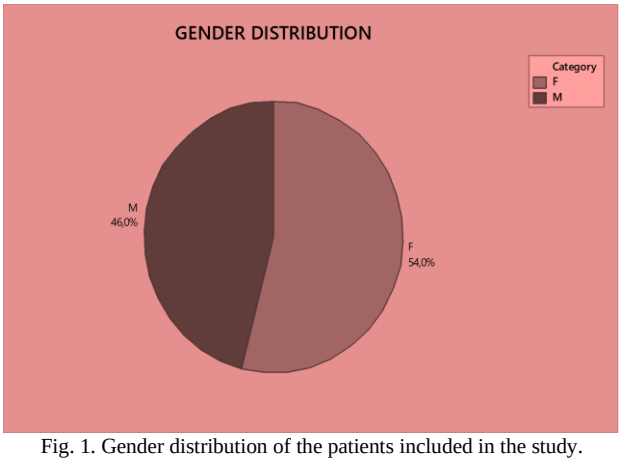

Fig. 1. Gender distribution of the patients included in the study.

Out of the patients included in the study 206 (68.2%) were vaccinated against COVID-19 and 96 (31.8%) were not vaccinated. Among vaccinated patients 97 were male and 109 were female. A percentage of 3.3% (5 female and 5 male) of the patients had received one vaccination dose, 16.9% (33 female and 18 male) had received two vaccination doses, 47.7% (70 female and 74 male) had received three vaccination doses and 0.3% (1 female patient) had received four vaccination doses. Vaccination rates and vaccination doses are presented in Fig. 2 and 3.

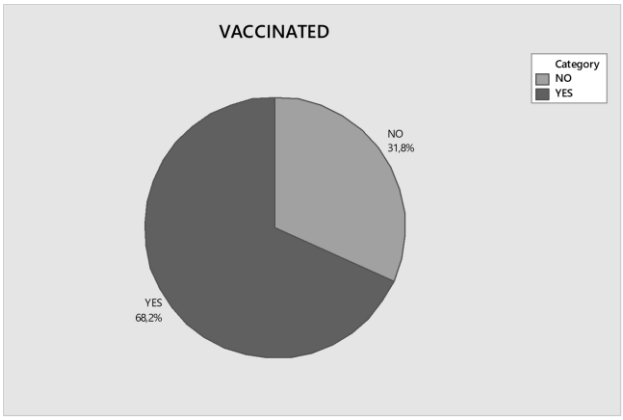

Fig. 2. Vaccination rates of the patients included in the study.

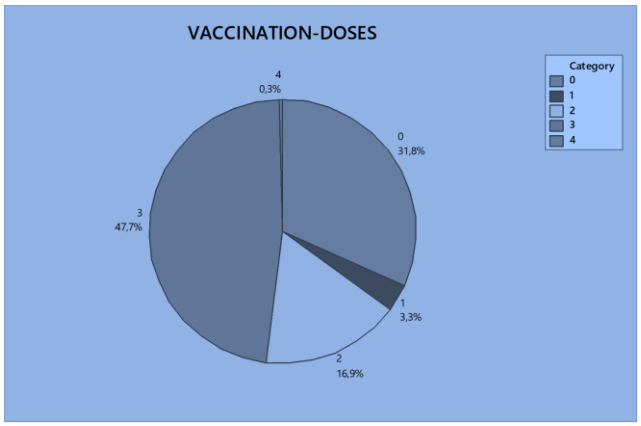

Fig. 3. Vaccination doses received by the patients included in the study.

Moreover, 88 patients (36 male and 52 female) presented infection symptoms, while 214 patients (103 male and 111 female) did not present any infection symptoms. Infection rates in the patient group is presented in Fig. 4.

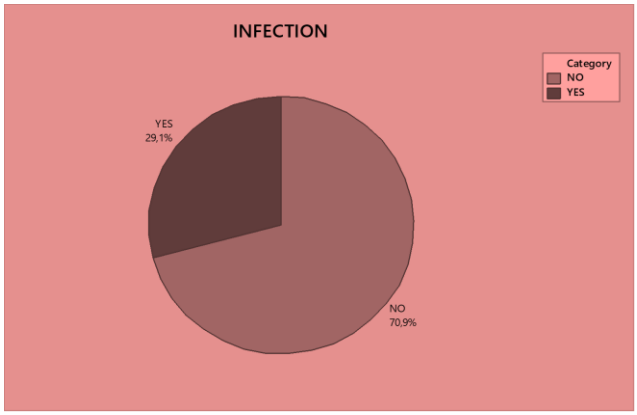

Fig. 4. Infection rates among the patients included in the study.

We performed a rapid test for Sars-CoV-2 in all patients included in the study. 221 patients (73.2%; 105 males and 116 females) had a negative rapid test result, while 81 (26.8%; 34 males and 47 females) had a positive rapid test result. Rapid test results are presented in Fig. 5.

We performed RCR tests for the detection of Sars-CoV-2 virus, Influenza virus and Respiratory Syncytial Virus (RSV) to all patients included in the study. 213 patients (70.5%; 100 males and 113 females) had a Sars-CoV-2 negative result and 89 (29.5%; 39 males and 50 females) had a Sars-CoV-2 positive result. PCR results for Sars-CoV-2 are presented in Fig. 6.

European Journal of Biomedical Research
www.ej-biomed.org

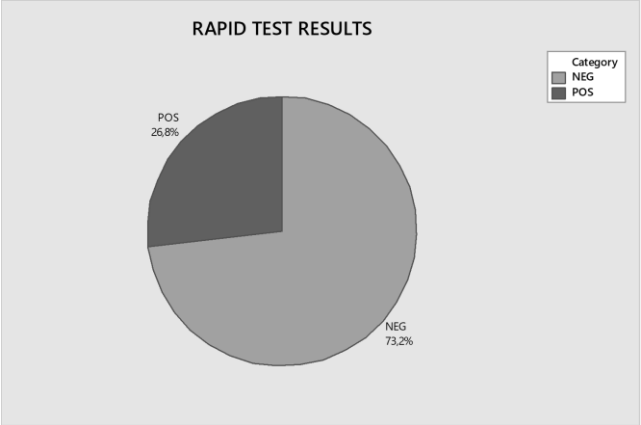

Fig. 5. Rapid test results among the patients included in the study.

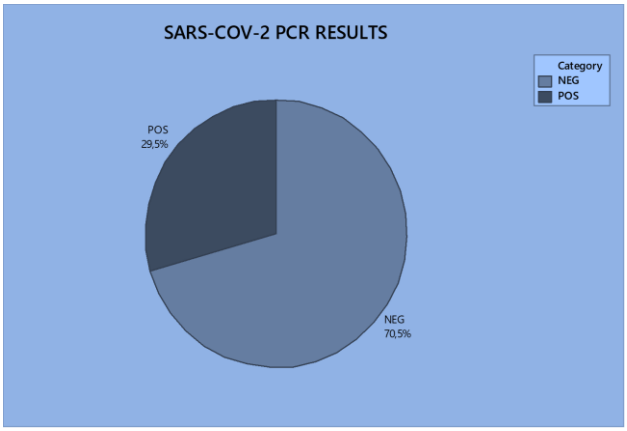

Fig. 6. PCR results for Sars-CoV-2 among the patients included in the study.

288 patients (95.4%; 133 males and 155 females) had an Influenza negative result and 14 patients (4.6%; 6 males and 8 females) had an Influenza positive result. PCR results for influenza are presented in Fig. 7.

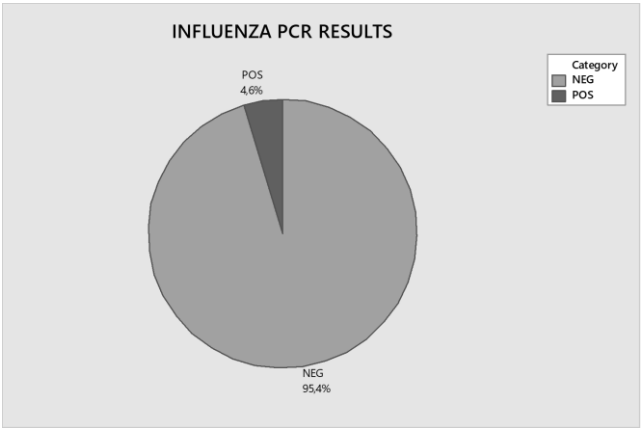

Fig. 7. Influenza results among the patients included in the study.

288 patients (94.4%; 132 males and 153 females) had an RSV negative result and 17 patients (5.6%; 7 males and 10 females) had an RSV positive result. PCR results for RSV are presented in Fig. 8.

We detected 15 cases of co-infection (7 males and 8 females) of SARS-CoV-2 and RSV (4,97%) and only one case, a male patient, with co-infection of SARS-CoV-2 with influenza (0,33%) (Fig. 9).

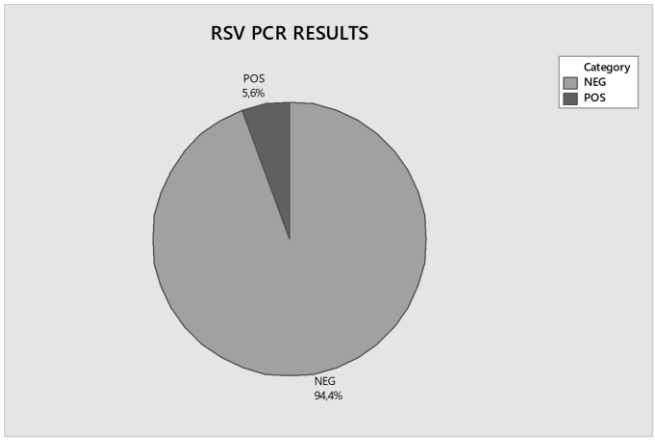

Fig. 8. RSV results among the patients included in the study.

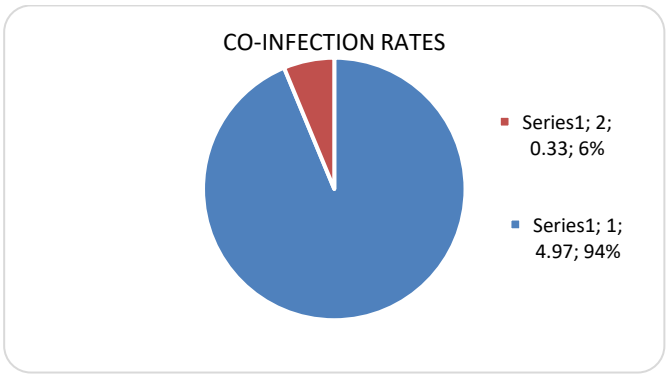

Fig. 9. Co-infection rates among the patients included in the study.

We performed a chi-square test of independence to observe all the possible associations between the following parameters: gender, vaccination, infection, rapid antigen test result, SARS-CoV-2 result, Influenza result and RSV result. The results and the respective p-values are presented in Table VIII.

We observed significant associations between infection and rapid test results, between infection and PCR result for SaRS-CoV-2, between rapid test results and PCR results for all viruses and between SARS-CoV-2, Influenza and RSV (p-value <0.05). The p-value for the association between Influenza virus and rapid test was marginal.

A logistic regression model was fitted, with response variable the infection (yes, no) and explanatory variables: gender, age, vaccination, vaccination doses, rapid test result, SARS-CoV-2, Influenza and RSV, (Table IX). The estimated model was the following:

Regression Equation

P(YES) = exp(Y')/(1 + exp(Y'))

Y' = -0,087 - 0,01067 AGE + 1,605 VACCINATION DOSES + 0,0 GENDER_F + 0,004 GENDER_M + 0,0 VACCINATION_NO - 4,86 VACCINATION_YES + 0,0 RAPID TEST_NEG + 0,010 RAPID TEST_POS + 0,0 SARS-COV_NEG + 0,493 SARS-COV_POS + 0,0 INFLUENZA_NEG - 1,72 INFLUENZA_POS + 0,0 RSV_NEG - 0,647 RSV_POS

TABLE VIII: CHI-SQUARE TEST RESULTS AND P-VALUES

	Gender	Vaccination	Infection	Rapid Test	SaRS-CoV-2	Influenza	RSV
Gender		0.588	0.251	0.392	0.619	0.807	0.680
Vaccination			0.068	0.147	0.125	0.123	0.751
Infection				0.000	0.000	0.093	0.593
Rapid test					0.000	0.055	0.000
SaRS-CoV-2						0.034	0.000
Influenza							0.876
RSV							

TABLE IX: REGRESSION ANALYSIS RESULTS WITH P-VALUES

Logistic regression model statistics			
Coefficient	Estimate	Std error	P-value
Constant	-0.087	0.466	0.002
Age	-0.01067	0.00747	0.150
Vaccination doses	1.605	0.500	0.000
Gender	0.004	0.290	0.998
Vaccination	-4.860	1.460	0.000
Rapid test	0.010	0.573	0.986
Sars-Cov	0.493	0.570	0.390
Influenza	-1.720	1.070	0.052
RSV	-0.647	0.657	0.312

IV. DISCUSSION AND CONCLUSION

The results of this study revealed a higher admission to the Emergency Department of Nikaia hospital for female patients (54%) with common upper respiratory tract clinical manifestations, than male (46%). Similar results are described in the literature [17]–[20], [28]. However, there are studies conducted in India,which conclude the opposite results about hospital admission regarding gender [21], [22]. This fact arises the question whether females inWestern countries are more susceptible to upper respiratory symptoms appearance or they have a different health awareness compared to men and Eastern populations [23].

Most of the patients in our study were vaccinated (68,2%); 47,7% had received 3 vaccination doses and 16,9% only 2 vaccination doses. According to the study of Heftdal *et al*., 2022 patients who received 2 out of 3 doses of a COVID-19 vaccine have a lower risk of breakthrough infections with SARS-CoV-2 [24]. In agreement with that, we noticed a significant lower rate on co-infection with SARS-CoV-2 and RSV in patients having received 2 vaccination doses (5,9%), compared to patients having received 3 vaccination doses (52,9%) or up to 1 vaccination dose (41,2%). Co-infection with SARS-CoV-2 and Influenza was found only in 1 patient (0,33%), which is in absolute accordance with the findings of Acuña-Zegarra, *et al*., 2021 [25,27]. These findings are based on real-time PCR testing of nasopharyngeal samples from symptomatic patients, where Ct values for both pathogens (SARS-CoV-2 and RSV) were between 10 and 17 [25]. Moreover, our findings indicate a significant relation between 2 out of 3 vaccination doses and PCR positivity in men for SARS-CoV-2. In detail, only 10% of PCR positive men have received 2doses of vaccination while 42,2% and 47,5% have received 0, 1 and 3 doses of vaccination respectively. Additionally, 2 doses vaccination seems to be more effective in men than women, with a total 10% PCR positivity in men and 28,6% PCR positivity in women. Veerapu *et al*. [21] studying vaccination effectiveness of Indian vaccines found also a higher vaccination effectiveness in males than females [21]. In the present study, Ct values range was between 10 and 19, indicating that symptomatic patients have a high viral load [26]. The limitations of our study lay on the small number of patient samples, absence of data about illness stage and severity.

CONFLICT OF INTEREST

The authors declared no conflicts of interest.

REFERENCES

[1] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al*. China novel coronavirus investigating and research team. A novel Coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*., 2020;382(8):727-733doi: 10.1056/NEJMoa2001017.

[2] WHO Director-General's opening remarks at the media briefing on COVID-19-11 March 2020 n.d. https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020.

[3] Hong KH, Lee SW, Kim TS, Huh HJ, Lee J, Kim SY, *et al*. Guidelines for Laboratory Diagnosis of Coronavirus Disease 2019 (COVID-19) in Korea. *Ann Lab Med*., 2020; 40(5):351-360. doi: 10.3343/alm.2020.40.5.351.

[4] Hong KH, Kim GJ, Roh KH, Sung H, Lee J, Kim SY, *et al*. COVID-19 Task Force, the Korean Society for Laboratory Medicine and the Bureau of Infectious Disease Diagnosis Control, the Korea Disease Control and Prevention Agency. Update of Guidelines for Laboratory Diagnosis of COVID-19 in Korea. *Ann Lab Med*., 2022;42(4):391-397. doi: 10.3343/alm.2022.42.4.391.

[5] Pletz MW, Dürrwald R, Reiche J, Rose N, Scherag A, Weis S; CoNAN study group. Impact of the COVID-19 pandemic on influenza and respiratory syncytial virus antibody titres in the community: a prospective cohort study in Neustadt, Thuringia, Germany. *Eur Respir J*., 2022;60(5):2200947. doi:10.1183/13993003.00947-2022.

[6] Groves HE, Piché-Renaud PP, Peci A, Farrar DS, Buckrell S, Bancej C, *et al*. The impact of the COVID-19 pandemic on influenza, respiratory syncytial virus, and other seasonal respiratory virus circulation in Canada: A population-based study. *Lancet Reg Health Am*., 2021;1:100015. doi: 10.1016/j.lana.2021.100015.

[7] Dähne T, Bauer W, Essig A, Schaaf B, Spinner CD, Pletz MW, *et al*. Members of the CAPNETZ study group. The impact of the SARS-CoV-2 pandemic on the prevalence of respiratory tract pathogens in patients with community-acquired pneumonia in Germany. *Emerg Microbes Infect.*, 2021; 10(1):1515-1518. doi: 10.1080/22221751.2021.1957402.

[8] Koutsakos M, Wheatley AK, Laurie K, Kent SJ, Rockman S. Influenza lineage extinction during the COVID-19 pandemic? *Nat Rev Microbiol*., 2021;19(12):741-742. doi: 10.1038/s41579-021-00642-4.

[9] Olsen SJ, Winn AK, Budd AP, Prill MM, Steel J, Midgley CM, *et al*. Changes in Influenza and other respiratory virus activity during the COVID-19 pandemic – United States, 2020-2021. *MMWR Morb Mortal Wkly Rep*., 2021;70(29):1013-1019. doi: 10.15585/mmwr.mm7029a1.

[10] Fratty IS, Reznik-Balter S, Nemet I, Atari N, Kliker L, Sherbany H, *et al*. Outbreak of influenza and other respiratory viruses in hospitalized patients alongside the SARS-CoV-2 pandemic. *Front Microbiol.*, 2022;13:902476. doi: 10.3389/fmicb.2022.902476.

[11] Agca H, Akalin H, Saglik I, Hacimustafaoglu M, Celebi S, Ener B. Changing epidemiology of influenza and other respiratory viruses in the first year of COVID-19 pandemic. *J Infect Public Health*, 2021;14(9):1186-1190. doi: 10.1016/j.jiph.2021.08.004.

[12] Nairz M, Todorovic T, Gehrer CM, Grubwieser P, Burkert F, Zimmermann M, *et al*. Single-center experience in detecting influenza virus, RSV and SARS-CoV-2 at the emergency department. *Viruses*, 2023; 15(2):470. doi: 10.3390/v15020470.

[13] Papachristou E, Rokka C, Sotiriadou T, Maneka L, Vassilakis A, Sapounas S, *et al* Low circulation of respiratory syncytial and influenza viruses during autumn-winter 2021 in the industrial workplace and long-term healthcare facilities in Athens, Greece. *Front Med* (*Lausanne*), 2022;9:1025147. doi: 10.3389/fmed.2022.1025147.
[14] Razanajatovo NH, Randriambolamanantsoa TH, Rabarison JH, Randrianasolo L, Ankasitrahana MF, Ratsimbazafy A, *et al*. Epidemiological patterns of seasonal respiratory viruses during the COVID-19 pandemic in Madagascar, March 2020-May 2022. *Viruses*, 2022;15(1):12. doi: 10.3390/v15010012.
[15] Tanne JH. US faces triple epidemic of flu, RSV, and covid. *BMJ*, 2022; 379:o2681. doi: 10.1136/bmj.o2681.
[16] Piret J, Boivin G. Viral Interference between respiratory viruses. *Emerg Infect Dis*., 2022; 28(2):273-281. doi: 10.3201/eid2802.211727.
[17] Almeida A, Boattini M, Christaki E, Moreira Marques T, Moreira I, Cruz L, *et al*. Comparative virulence of seasonal viruses responsible for lower respiratory tract infections: a southern European multi-centre cohort study of hospital admissions. *Infection*, 2021; 49(3):483-490. doi: 10.1007/s15010-020-01569-3.
[18] Boattini M, Almeida A, Christaki E, Cruz L, Antão D, Moreira MI, *et al*. Influenza and respiratory syncytial virus infections in the oldest-old continent. *Eur J Clin Microbiol Infect Dis*., 2020; 39(11):2085-2090. doi: 10.1007/s10096-020-03959-9.
[19] Kim JH, Roh YH, Ahn JG, Kim MY, Huh K, Jung J, Kang JM. Respiratory syncytial virus and influenza epidemics disappearance in Korea during the 2020-2021 season of COVID-19. *Int J Infect Dis*., 2021; 110:29-35. doi: 10.1016/j.ijid.2021.07.005.
[20] Costa VGD, Gomes AJC, Bittar C, Geraldini DB, Previdelli da Conceição PJ, *et al*. Burden of Influenza and Respiratory Syncytial Viruses in Suspected COVID-19 Patients: A Cross-Sectional and Meta-Analysis Study. *Viruses*, 2023; 15(3):665. doi: 10.3390/v15030665.
[21] Veerapu N, Inamdar DP, Kumar BPR, Anuradha B, Guddanti P, Issapuri SD, *et al*. Effectiveness of COVID-19 Vaccines against SARS-CoV-2 Infection among Persons Attending the RT-PCR center at a Medical College Hospital in Telangana: A Case- Control Study. *Indian J Community Med*., 2022;47(4):587-590. doi: 10.4103/ijcm.ijcm_273_22.
[22] Mahallawi WH, Alsamiri AD, Dabbour AF, Alsaeedi H and Al-Zalabani AH. Association of viral load in SARS-CoV-2 patients with age and gender. *Front. Med*., 2021; 8:608215. doi: 10.3389/fmed.2021.608215.
[23] Irizar P, Pan D, Kapadia D, Bécares L, Sze S, Taylor H, *et al*. Ethnic inequalities in COVID-19 infection, hospitalisation, intensive care admission, and death: a global systematic review and meta-analysis of over 200 million study participants. *EClinicalMedicine*, 2023; 57:101877. doi: 10.1016/j.eclinm.2023.101877.
[24] Heftdal LD, Schultz M, Lange T, Knudsen AD, Fogh K, Hasselbalch RB, *et al*. Incidence of Positive Severe Acute Respiratory Syndrome Coronavirus Polymerase Chain Reaction After Coronavirus Disease 2019 Vaccination With up to 8 Months of Follow-up: Real-life Data From the Capital Region of Denmark. *Clin Infect Dis*., 2022; 75(1):e675-e682. doi: 10.1093/cid/ciac012.
[25] Acuña-Zegarra M.A., Díaz-Infante S.,Baca-Carrasco D., Olmos-Liceaga D.COVID-19 optimal vaccination policies: A modeling study on efficacy, natural and vaccine-induced immunity responses. *Mathematical Biosciences*, 2021; 337. Article 108614, 10.1016/j.mbs.2021.108614.
[26] Mourtzikou, A., Korre, A., Stamouli, M., Seitopoulou, C., Petraki, I., Kalliora, G., *et al*. Suspected COVID-19 cases admitted in a tertiary care hospital. correlation of demographic and clinical characteristics with viral load results and hospitalization. *European Journal of Biomedical Research*, 2022; 1(2):1. https://doi.org/10.24018/ejbiomed.2022.1.2.6.
[27] World Health Organization, "Influenza Update 380," Tech. Rep. November, World Health Organization, 2020.
[28] Si, Y., Zhao, Z., Chen, R., Zhong, H., Liu, T., Wang, M., *et al*. Epidemiological surveillance of common respiratory viruses in patients with suspected COVID-19 in Southwest China. *BMC Infect Dis*, 2020; 20(1):688.