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Evaluation of blood group diversity and Rhesus factor with blood virus infection: A cohort of HCV, HBV, and COVID-19 Egyptian patients

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Abstract

Blood groups and Rhesus factor have been discovered to be connected to the risk of several viral diseases. This study examined the relationship between blood group diversity, the Rhesus factor, and blood virus infection and the impact of viral infections on patients from April 2021 and December 2022. This was a study conducted in the governorate of Assiut. All blood samples were tested for HBV, HCV, and COVID-19 infections. To detect blood type, forward and reverse blood grouping were utilized. The results were run via the SPSS program to establish the statistical difference between the values in different categories. In all, 1250 serum samples were obtained from people visiting Assiut governorate hospitals, blood banks, and health centers. Out of 1250 blood samples, 115 people tested positive for HBV, HCV, and COVID-19, 65 (5.2%) were positive for COVID-19, 14 (1.12%) were positive for HBV, and 36 (2.88%) were positive for HCV. The occurrence of B+ blood type was more frequent among individuals with COVID-19, whereas individuals with HBV and HCV infections exhibited a higher prevalence of A+ and O+ blood types, and the results revealed statistically significant differences in the extent of the impact of infection between the positive and negative groups of viruses. Rh-positive was significantly increased in infection with all tested viruses. Fatigue, cough, and dysphagia were the most prevalent symptoms in patients infected with SARS-CoV-2. We advise that future research on the association between blood groups and blood-borne viruses be performed with solid evidence and large sample sizes.

Keywords: HBV, HCV, SARS-CoV-2, ABO group, Rh factor.

Introduction

Three of the world's most pressing public health issues contain hepatitis B and C viruses (HBV and HCV), as well as SARS-CoV2 (COVID-19). Various acute and chronic infections, cirrhosis, and liver cancer can result from viral hepatitis, which is a worldwide health crisis that affects millions of individuals and kills thousands of lives [1]. COVID-19 has had a devastating impact on the world, killing almost 6 million people. The spread of viral diseases is a serious public health issue for the World Health Organization (WHO) [2].

SARS-CoV2 has created epidemics with death rates of roughly 9.5%. As of October 29, 2020, the overall number of COVID-19 cases globally had surpassed 44 million, with over 1.17 million verified fatalities. At the beginning of the pandemic, understanding of COVID-19 and its treatment was limited, which

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made it urgent to use pharmaceutical repurposing and experimental drugs to diminish this unique viral infection. Since then, a great deal has been accomplished because of the dedicated efforts of clinical researchers everywhere, leading to the rapid development of innovative drugs and vaccinations and a greater understanding of COVID-19 and its therapy [3–5].

Chronic HCV and HBV infections are clinically severe because they cause a chronic inflammation process in the liver that can progress to fibrosis, cirrhosis, hepatocellular carcinoma, and death. Despite the advent of universal immunization programs, chronic HBV and HCV infections remain a severe public health concern throughout the world, with HCV infecting more individuals than other viruses. HBV continues to infect 350 million people, causing viral hepatitis morbidity and mortality. Hepatotropic viruses, for example, cause chronic liver infection by gradually altering virally infected hepatocytes [6–8].

Red blood cells (RBCs) surface contains specific antigens that determine an individual's ABO blood group, which is established at birth [9]. Blood type A has anti-B antibodies, blood type B has anti-A antibodies, blood type AB has no antibodies, and blood type O has both anti-A and anti-B antibodies. Immunoglobulins (IgG) and (IgM) antibodies against these antigens are found in blood plasma [9]. RBCs' surface also carries the Rhesus (Rh) factor, which is formed from D-antigens [10]. When D-antigens are present, a person is "Rh-positive," but when they are not, they are "Rh-negative" and produce anti-D IgG antibodies [10]. Since they were first discovered in 1900 [11], there are approximately thirty-three systems of blood groups in humans known to exist, with the ABO and Rh blood groups continuing to be crucial in the field of transfusion medicine [12].

Blood groups are divided into two categories: the sub-blood group Rh (antigen Rhesus) and the primary blood group ABO [13]. Antigens are polysaccharides, or proteins, that identify blood types [14]. The attachment stage of viral infection is where these antigens play a crucial role in viral pathogenesis [15]. Blood type A has been classified as having a high risk of developing COVID-19, despite blood groups other than A, including blood group O, being less likely to become infected with the virus [16]. Nevertheless, there is currently a lack of information in this area because the virulence of the virus may also be impacted by the Rhesus antigen present in certain blood types [16]. The ABO and Rh blood group systems are the most significant in modern medicine among the several blood group systems. The presence or lack of one or both of the A and B antigens on the membrane of the host red blood cell determines the phenotypes of the ABO blood group system, which includes B, A, O, and AB. The Rh blood group system, which is the second most significant system after the ABO, is extremely polymorphic, comprising around forty-four distinct antigens [1]. The presence or absence of the Rh D antigen on the host's red blood cell is the most important feature common to all pleomorphorphic forms [17]. most important characteristic of all the pleomorphic forms is the presence or lack of the Rh D antigen on the host's red blood cell [17]. Genetic factors play an essential part in deciding if an individual's red blood cells have Rh and ABO antigens. [18, 19]. The first proposition of a relationship between clinical COVID-19 symptoms and the ABO blood group was reported by Zhao et al. [20]. From then on, many studies tackled this association [21]. Furthermore, it is believed that the Rhesus (RH) factor, another RBC surface protein, plays a part in the COVID-19 infection [22].

In our study, we aimed to evaluate the relationship between the diversity of blood groups and Rh groups and blood-borne viruses such as HCV, HBV, and COVID-19 among the Assiut Government Population, which would enhance and increase the diagnostic rate and thus be useful in determining recommendations for national policy.

Material and methods

Study Population and Data Collection

Samples of blood were collected from a total of 1250 patients from Assiut governorate between April 2021 and December 2022 to explore the relationship between ABO and Rh blood groups and the rate of viral infections. HBV, HCV, and COVID-19 are the viruses in question. Out of 1250 patients, only 115 patients were seropositive for viruses. To collect data on the participants' demographics, structured questionnaires were used. Blood samples were taken from people visiting various hospitals, blood banks, private labs, and health centers and stored at -20 °C after being centrifuged for 10 minutes at 4000 rpm. Every serum sample was brought to the virology section at Assiut Central Hospital, where it was processed. We performed biochemical tests on infected people and an equal number of healthy people. We inquired about the patient's symptoms. To compare positive and negative persons, the findings of biochemical tests such as CBC, CRP, ferritin, AST, ALT, and D-dimer for COVID-19 patients [23], and CBC, AST, ALT, and albumin for HBV and HCV patients were employed [24].

ABO and Rh blood group detection

Venous blood was drawn from each participant and placed into a 4 ml EDTA vacutainer bottle. The bottle was then gently inverted to allow the anticoagulant to adequately mix with the blood. The blood was processed as soon as possible by being quickly delivered in a cold chain to the serology unit of the chosen health facilities' laboratory. Following collection, the blood sample was extracted in 50 microliters using a sterile Pasteur pipette, which was then mixed with the appropriate conventional blood grouping antisera. Each participant's blood group was then determined by the visible agglutination of the blood grouping antisera/blood complex.

The ABO and Rh blood types were determined by the slide agglutination technique, as previously mentioned [25]. In brief, each participant had a drop of blood deposited in three different spots on a spotless white surface. A drop of commercially prepared antisera A, B, and D were combined with each drop of blood, and the results were recorded for agglutination. To verify agglutination, each mixture (blood plus antisera) was examined under a microscope.

Serological detection

Serum markers for HCV infection

Third-generation ELISA tests (Ortho Diagnostics, Raritan, NJ, USA; and Abbott Diagnostics, North Chicago, IL, USA) were used to study anti-HCV. The EL x 800 universal micro-plate reader (Biotek Instruments Inc.) was used to read the results. Double ELISA was the same method used for all positive samples' retests. with confirmed HCV-positive cases via real-time reverse transcription-polymerase chain reaction [24]

Serum markers for HBV infection

Using a third-generation enzyme immunoassay (Murex HBsAg Version 3, AbbottMurex, South Africa), the HBsAg markers were serologically evaluated. The EL x 800 universal microplate reader (Bitek Instruments Inc.) was used to read the results. Every positive sample was retested with the identical technique (double ELISA), with confirmed HBV-positive cases via real-time reverse transcription-polymerase chain reaction [24].

Serum markers for SARS-CoV-2 infection

Different dilutions (1:50, 1:100, and 1:200) have been applied on an Immunoplate 96-well flat-bottom plate (Extra Gene, USA). Using our previously developed ELISA methods, 100 µL/well of mouse SARS-CoV-2 NCP-specific monoclonal antibody was coated using coating buffer (sodium bicarbonate, pH > 9) and incubated either overnight at 4°C or 37°C for an hour [26]. After decanting the unbound antibody, blocking buffer (150 µl/well; phosphate buffer saline (PBS), 0.1% Tween-20, and 2% bovine serum antigen (BSA)) was added, and the mixture was incubated for one hour at 37 °C to prevent nonspecific interaction. To prepare for the test process, wells were washed using 1x wash buffer (50 mm Tris, 0.05% Tween 20, 0.1% SDS,

0.8% NaCl, and distilled water), with confirmed COVID-19 positive cases via real-time reverse transcription-polymerase chain reaction [23].

Ethical Clearance

Approval was consulted and granted by the Faculty of Science's Ethical Research Committee, Al-Azhar University, with ethics references (AZHAR 13/2021). Before starting the process of collecting blood samples.

Statistical Analysis

The SPSS program (SPSS, 2017) was used to statistically analyze the data. The chi-squared test for categorical data was used to evaluate the data. A probability (p) value ≤ 0.05 has been considered statistically significant [27].

Results

Out of the 1250 participants evaluated, 65 (5.2%) were positive for COVID-19, 14 (1.12%) were positive for HBV, and 36 (2.88%) were positive for HCV. Age group (41–50) years was the highest percentage among COVID-19, HCV, and HBV patients, as shown in Table 1 and Figure 1. The results of age distribution among patients infected with COVID-19, HCV, and HBV revealed a significant difference between the different ages of each virus (Table 1 and Figure 1).

The highest seropositive of HBsAg, anti-HCV, and anti-COVID-19 were recorded in males (64%, 69%, and 62%, respectively) compared to females (36%, 31%, and 38%, respectively). The results of gender distribution among patients infected with COVID-19, HCV, and HBV infection revealed a significant difference between the different genders in each virus (Table 2 and Figure 2).

Patients with COVID-19 were recorded as having a highly infectious 24 (36%) with participants having B+ blood type, while patients with HBV and HCV infections had a higher prevalence with participants having A+ 7 (50%) and O+ 10 (28%) blood types, respectively, as shown in Table 3 and Figure 3. The results of the frequency of COVID-19 and HCV among different blood and Rh groups of infection revealed a significant difference, while the results of the frequency of HBV revealed a non-significant difference ($P > 0.05$) (Table 3 and Figure 3).

Fatigue, cough, and dysphagia were the most common symptoms among the positive COVID-19 participants, at 22 (95%), 21 (91%), and 21 (91%), respectively, as shown in Table 4. The results of the distribution of symptoms among positive COVID-19 patients revealed a significant difference between the different symptoms of the COVID-19 virus (Table 4 & Figure 4).

The following tests indicated statistically significant variations in their results: D-dimer, CRP, SGPT (ALT), and ferritin between negative and positive COVID-19 participants; SGOT (AST), albumin, neutrophils, and lymphocytes between positive and negative HCV participants; and SGOT (AST) and albumin between positive and negative HBV participants (P value < 0.05). In contrast, no statistically significant differences were demonstrated regarding other biochemical parameters (P value >0.05), as shown in Table 5. Findings showed a statistically significant difference in the severity of COVID-19 between diabetic and non-diabetic patients (P value= 0.005) and between patients with hypertension and patients with no hypertension history (P value=0.001); However, the results showed no significant difference between patients who have cardiac diseases and other who haven't (P Value=0.59)

Table 1. Age distribution among patients infected with COVID-19, HCV, and HBV.

Virus infection	Age (years)					Total patients	Chi-square value
	11Y - 20Y	21Y - 30Y	31Y - 40Y	41Y - 50Y	> 50Y		
COVID-19	1 (2%)	13 (20%)	17 (26%)	21 (32%)	13 (20%)	65	Calculate = 21.538 P = 0.000
HCV	2 (6%)	2 (6%)	11 (30%)	11 (30%)	10 (28%)	36	Calculate = 15.764 P = 0.003
HBV	0	1 (7%)	3 (21%)	7 (50%)	3 (21%)	14	Calculate = 12.857 P = 0.012

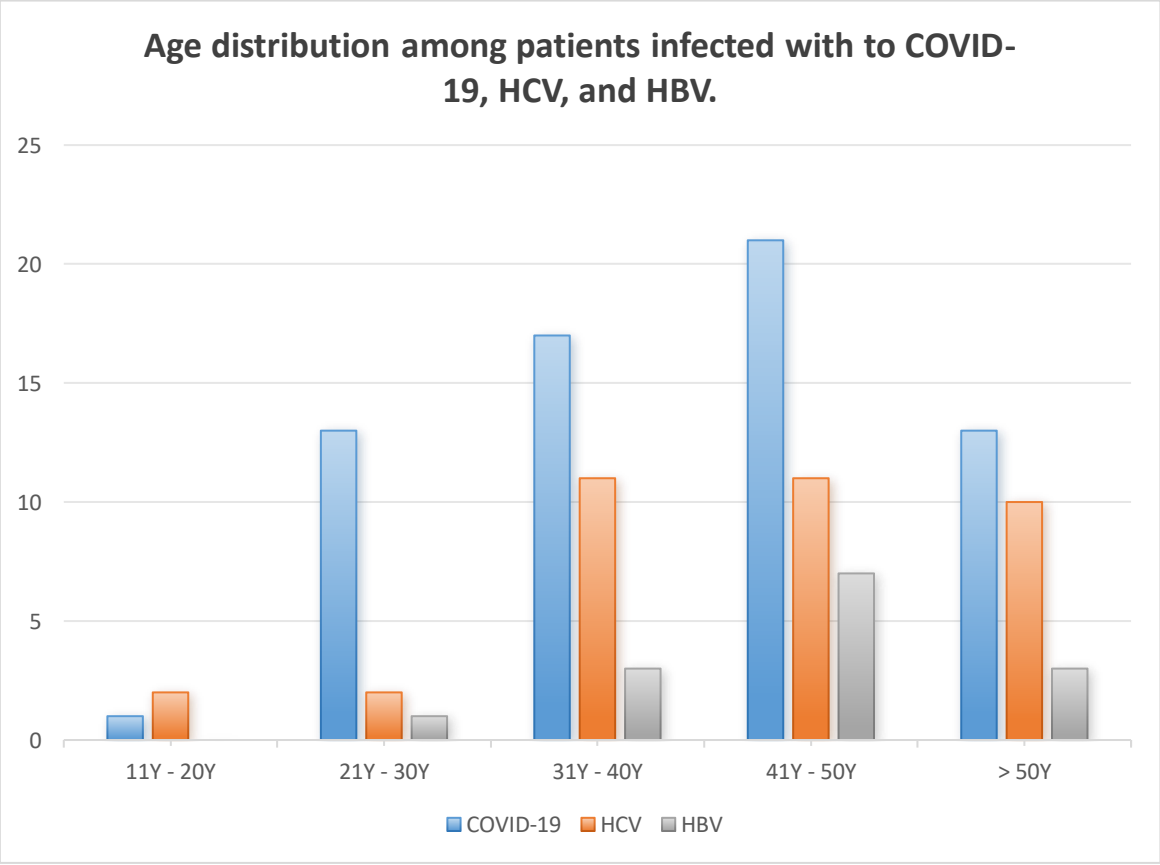


Figure 1. Age distribution among patients infected with to COVID-19, HCV, and HBV.

Table 2. Gender distribution among patients infected with COVID-19, HCV and HBV infection.

Gender	Virus infection			Total patients
	COVID-19	HCV	HBV	
Male	40 (62%)	25 (69%)	9 (64%)	74
Female	25 (38%)	11 (31%)	5 (36%)	41
Total	65 (100%)	36 (100%)	14 (100%)	115
Chi-square value	Calculate = 23.269 P = 0.000	Calculate = 21.125 P = 0.000	Calculate = 6.036 P = 0.014	

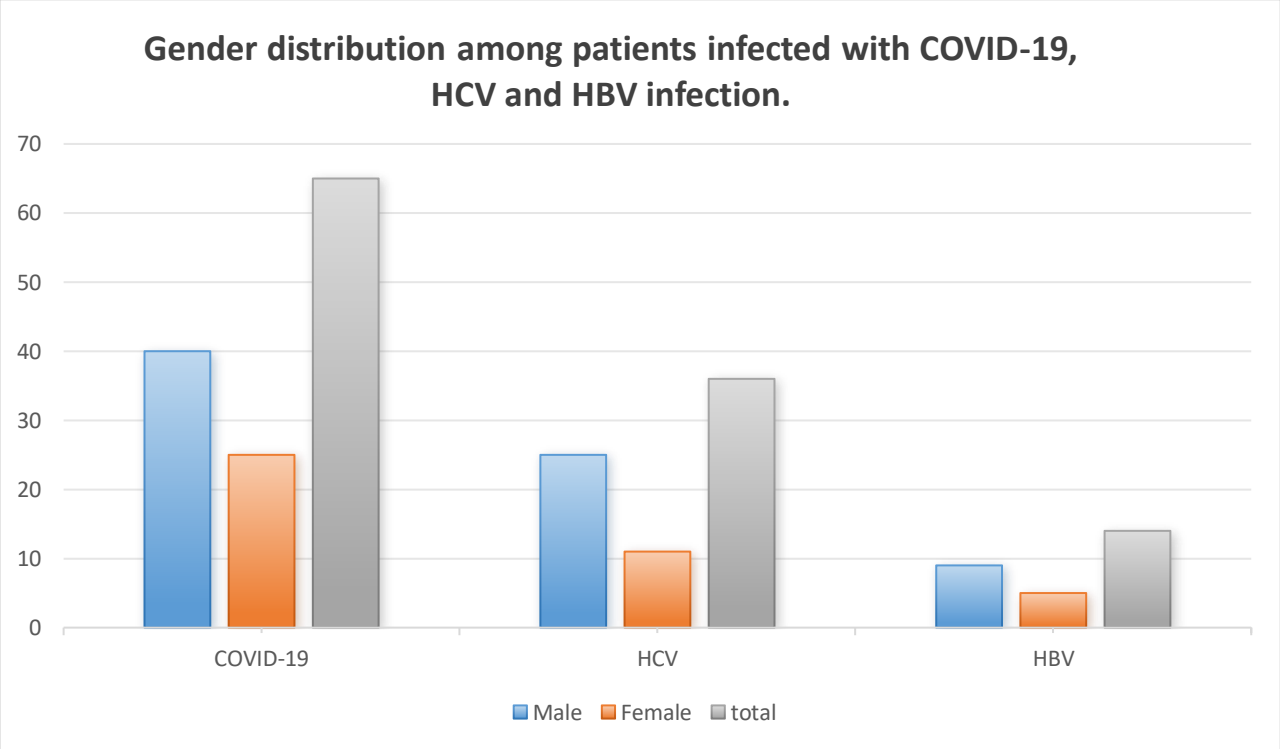
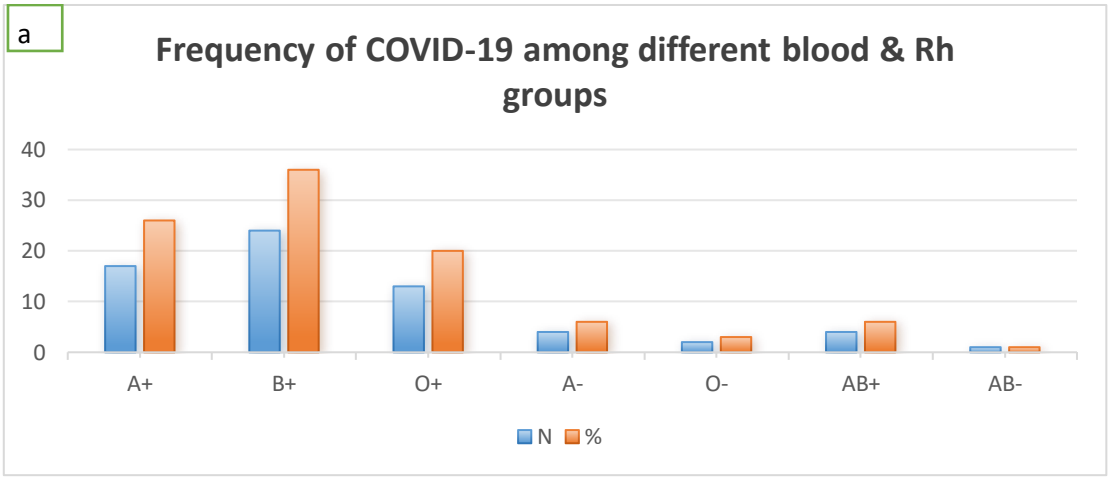
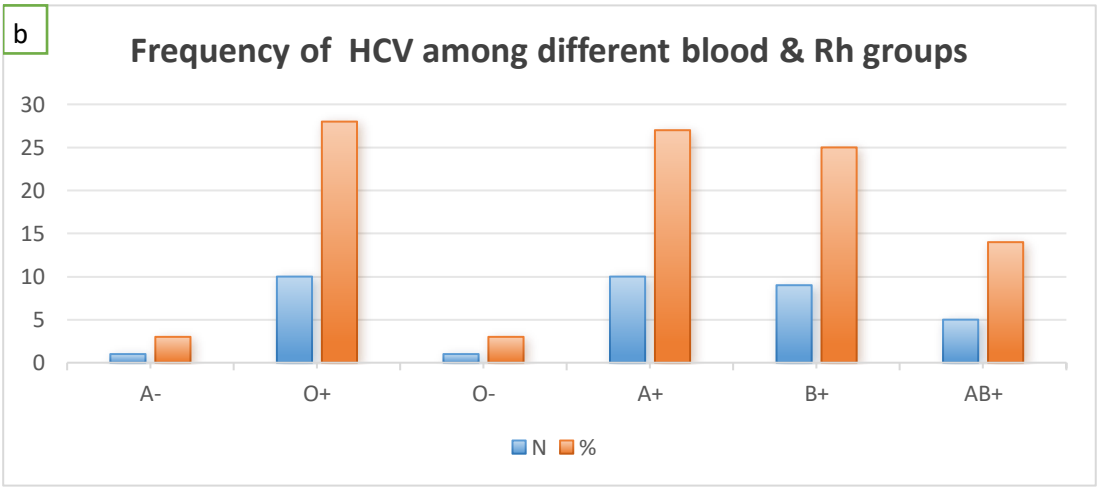


Figure 2. Gender distribution among patients infected with COVID-19, HCV, and HBV infection.

Table 3. Frequency of COVID-19, HCV, and HBV among different blood & Rh groups

		COVID-19 (n=65)		**Chi-square value**
		N	%	
Blood Group &RH	A+	17	26	Calculate = 58.728 P = 0.000
	B+	24	36	
	O+	13	20	
	A-	4	6	
	O-	2	3	
	AB+	4	6	
	AB-	1	1	
		HCV (n=36)		
		N	**%**	
Blood group& Rh	A-	1	3	Calculate = 18.400 P = 0.002
	O+	10	28	
	O-	1	3	
	A+	10	27	
	B+	9	25	
	AB+	5	14	
		HBV (n=14)		
		N	**%**	
Blood group& Rh	AB+	2	14	Calculate = 6.476 P = 0.091
	O+	3	21	
	A+	7	50	
	B+	2	14	





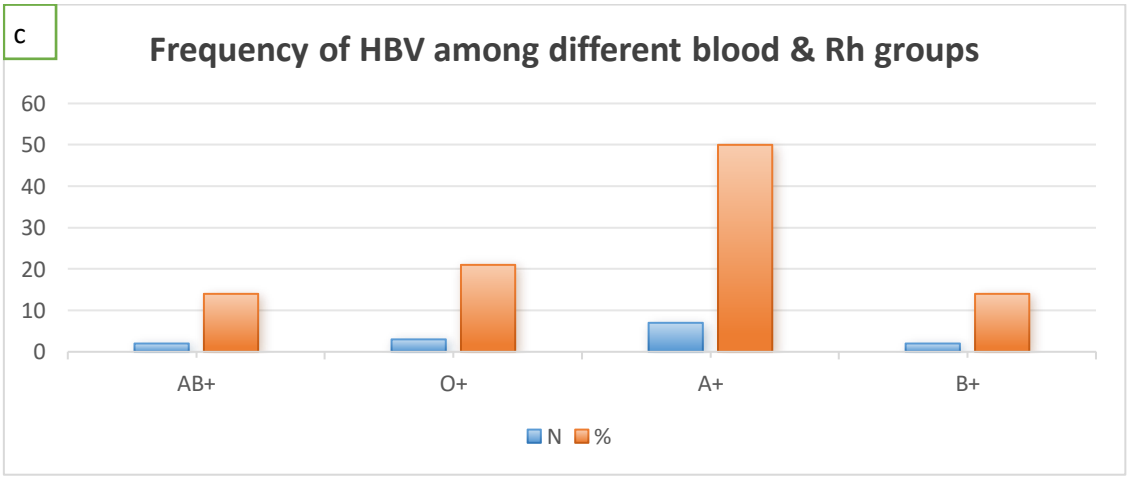


Figure 3 (a,b,c). Frequency of COVID-19, HCV, and HBV among different blood & Rh groups

Table 4. Distribution of symptoms among positive COVID-19 patients

COVID-19 symptoms		N=23	%	Chi-square value
Diarrhea	No	16	69.6	Calculate = 83.760 P = 0.000
	YES	7	30.4	
Cough	NO	2	8.7	
	YES	21	91.3	
Fatigue	NO	1	4.3	
	YES	22	95.7	
Dysphagia	No	2	8.7	
	YES	21	91.3	
Headache	NO	6	26.1	
	YES	17	73.9	
Fever	No	4	17.4	
	YES	19	82.6	
O2 Mask	NO	23	100	
	YES	0	0	

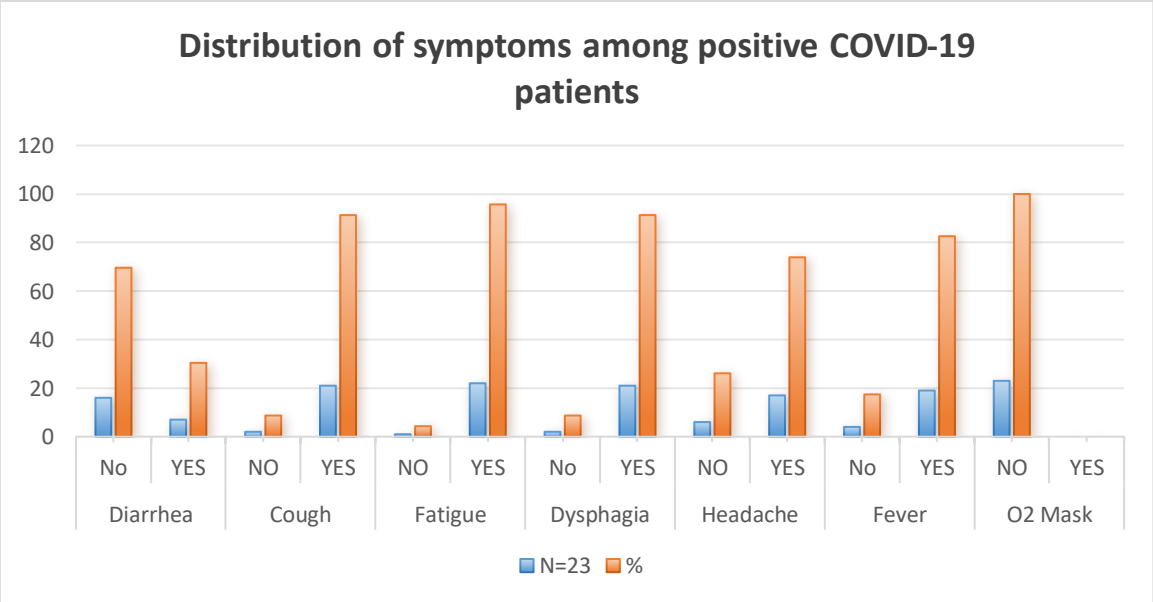


Figure 4. Distribution of symptoms among positive COVID-19 patients

Table 5 Association between Biochemical analysis and qualitative results of HBV, HCV, and COVID-19 of the population in Assiut governorate, Egypt.

COVID-19	**Positive (23)**	**Negative (23) (Control Group)**	
	Mean (SD)	**Mean (SD)**	**P Value**
D.dimer	0.59(0.35)	16(0.082)	0
Ferritin	263(250)	111(86)	0.009
CRP	25.9(46.2)	2.8(2.7)	0.021
ALT	33.6(19)	19.6(4.1)	0.001
MCH (pg)	28(2.5)	27.6(2.5)	0.605
MCV (FI)	80.1(16.8)	81.8(5.7)	0.646
RBCS(Millions/cmm)	4.8(0.68)	5.1(0.64)	0.274
Platelets (Thousands/cmm)	238.7(54)	254(47)	0.309
Haematocrit(pcv) %	40.6(4.3)	42.7(5.6)	0.166
Haemoglobin (g/dl)	13.5(1.4)	13.9(1.2)	0.333
Monocyte %	9.8(3.2)	9.1(1.8)	0.365
Lymphocyte %	32.8(17.2)	38.2(9.7)	0.204
WBCs (Thousands/cmm)	6.1(2.9)	7.2(1.7)	0.125
HCV	**Positive (13)**	**Negative (13) (Control Group)**	
AST	33(23.6)	18(3.7)	0.039
ALT	31(36.5)	21(8.01)	0.331
Albumin	4.5(0.24)	4.1(0.31)	0.002
MCH (pg)	27(2.6)	28(1.9)	0.523
MCV (FI)	84.5(6.3)	84.1(3.33)	0.857
RBCS(Millions/cmm)	4.9(0.64)	4.8(0.69)	0.766
Platelets (Thousands/cmm)	254(101)	256(34)	930
Haematocrit(pcv) %	41.4(4.6)	40.4(5.02)	0.587
Haemoglobin (g/dl)	13.7(1.8)	13.8(2)	0.84
Monocyte %	8.6(3.4)	7.6(1.9)	0.381
Neutrophils %	38.2(17.4)	56.5(12)	0.005
Lymphocyte %	84(17.2)	32(10.5)	0.009
WBCs (Thousands/cmm)	5.4(2)	6.7(1.4)	0.087
HBV	**Positive (14)**	**Negative (14) (Control Group)**	
AST	26.6(14.3)	18.3(3.7)	0.046
ALT	23.5(14.1)	20.7(7.9)	0.526
Albumin	3.9(0.27)	4.1(0.3)	0.049
MCH (pg)	27.5(3.3)	28.5(1.8)	0.364
MCV (FI)	83.2(8.7)	84(3.2)	0.766
RBCS(Millions/cmm)	5(0.5)	4.7(0.69)	0.271
Platelets (Thousands/cmm)	241(89)	255(33)	0.578
Haematocrit(pcv) %	39.2(11)	39.9(5.3)	0.835
Haemoglobin (g/dl)	13.8(1.7)	13.7(1.9)	0.889
Monocyte %	6.8(2.4)	7.7(1.8)	0.303
Neutrophils %	49.5(17.9)	55.1(12.7)	0.347
Lymphocyte %	40.6(18.3)	33.6(11.4)	0.239
WBCs (Thousands/cmm)	7.2(4)	6.6(1.37)	0.598

Discussion

Antigens of blood groups can be used to predict prognosis in a variety of diseases. The influence of blood types on the probability of transmitting viruses has been the subject of several studies. Additionally, several studies of research on the effect of blood groups on the COVID-19 virus have been conducted globally. Data on the severity of infections and the function of ABO blood groups in contamination with these pathogens can be obtained [28–29]. In CHC patients, a relationship between HCC risk and ABO blood group has been found. Their hospital's case-control study's findings showed that Chinese CHC patients with blood type A had a significantly higher risk of developing HCC than Chinese CHC patients with blood type O, as these were documented for cases of gastric, ovarian, and pancreatic malignancies [30]. In India, 20,000 random blood donors were examined for HCV status, and their results were associated with personal variables including age, sex, and blood group. In healthy blood donors, the study revealed 0.34% anti-HCV seropositivity, which matched previous findings from North India (ranging from 0.3% to 5.1%) [31].

In this study, we examined the possible impacts of ABO blood groups on vulnerability, disease severity, and symptom development in individuals who contracted blood-borne viruses, namely HBV, HCV, and COVID-19. Out of the 1250 participants evaluated, 5.2% were positive for COVID-19, 1.12% were positive for HBV, and 2.88% were positive for HCV. This study's seroprevalence of the hepatitis B surface antigen is consistent with studies conducted in India 0.61 % [32], Libya 1.5 % [33], Afghanistan 1.53 % [34] and Mexico 1.65 % [35]. Nonetheless, the study's HBsAg prevalence is lower than the 3.9% in Tanzania [36], the 4.0% in Egypt [37], and the 4.3% in Port Harcourt, South Nigeria [38]. In the current study, the seroprevalence of anti-HCV antibodies was determined to be 2.88 %. This result was more than that of a study that was carried out in Nigeria by 0.6% [39]. On the other hand, the results of this study were much lower than the anti-HCV antibody seroprevalence rates of 3.6 and 8.5% that were reported in Nigeria [40] and Yemen [41], respectively. Age group (41-50) years had the highest percentage among COVID-19, HCV, and HBV patients. On the other hand, Nascimento *et al*. (2008) conducted a study on Brazilian blood donors and discovered that HBV and HCV seroprevalence were low among Brazilian blood donors, with exposure increasing with age in both genders [42].

The highest seropositives of HBsAg, anti-HCV, and anti-COVID-19 were recorded in males (64%, 69%, and 62% respectively) compared to females (36%, 31%, and 38% respectively). According to these and related observations, which suggest that male sex is a risk factor for the prevalence of HBV and HCV as well as the development of HCC following HBV and/or HCV infection, female-to-male ratios of 1:4 to 1:7 indicate that males are more susceptible than females to developing HBV-associated HCC. [43]. Furthermore, it has been observed that female carriers of HBV have lower virus loads compared to male carriers [44, 45], and serum HBV surface antigen (HBsAg) prevalence is higher in men than in women [46]. Certain research findings indicate that elevated serum testosterone levels may be linked to a higher chance of HCC development in male HBV carriers [47].

Patients with COVID-19 in our study were recorded highly infection 36% with participants containing B^+ blood type, while patients with HBV and HCV infections had a higher prevalence with participants containing A^+ (50%) and O^+ (28%) blood types respectively. In line with these findings, Batool *et al*.(2017) found that B positive was the most common blood type, followed by O positive 2.30% of the donors tested positive for Hepatitis B infection. In comparison, 1.30% tested positive for Hepatitis C. Hepatitis B is associated with blood type A. There was no discernible relationship between blood group O donors and any blood-borne illnesses [48]. Furthermore, Iavarone et al. (2016)

found a statistically significant increase in the incidence of HCC in individuals with blood types other than O in research involving 90,731 participants [49]. Identical case-control studies with 1538 Korean patients who recently were given a diagnosis of HCC revealed that blood type A, and specifically the AA gene, was linked to the highest risk of HCC development in comparison to other blood types [50].

In the present study, we found that fatigue, cough, and dysphagia were the most common symptoms among the positive COVID-19 participants (95%), 91%, and 91%, respectively. Dysphagia was observed to co-occur with delirium, exhaustion, and trouble attaining efficient breath/swallow coordination by Dawson et al. (2020). It is well recognized that COVID-19 has a substantial emotional impact since it is primarily associated with double stressors, such as hospitalization or quarantine [51]. Patients may suffer from post-acute consequences, such as significant muscle weakness and exhaustion, joint stiffness, dysphagia, neurological psychological issues, and reduced functioning with regard to mobility and activities of daily living, according to Brooks et al. (2020) [52].

Conclusion

This study compared between the infections of hepatitis B & C and COVID-19 with some risk factors in blood donors, regarding Rh factor and blood grouping. We observed that the proportion of Rh-positive was considerably higher in COVID-19 cases, HBV cases, and HCV cases than in healthy individuals in all tested virus cases. However, no correlation with ABO typing was found. The risk of infection should not be taken lightly by those who are Rh-negative. Further research is advised to corroborate these results and discover other influencing factors and responsible molecular pathways. B+, A+, and O+ were the most frequent types of blood groups among infected patients. We recommend that future studies with strong evidence and large sample sizes are required to further investigate the association between the type of blood group and the blood-borne viruses.

Authors' Contribution:

Each author participated in every stage of the manuscript's development and agreed with the content.

Declaration of Conflicting Interests

Regarding the research, writing, and/or publication of this article, the author(s) have declared that they have no potential conflicts of interest concerning the research.

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