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Impact of Age and Clinico-Biochemical Parameters on Clinical Severity of SARS-CoV-2 Infection

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Abstract

Introduction: The surge in novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection leading to coronavirus disease-2019 (COVID-19) has overwhelmed the health system. To help health-care workers and policy makers prioritize treatment and to decrease the burden on health systems caused by COVID-19, clinical severity along with various clinico-biochemical parameters was evaluated by designing a cross-sectional study comprising 236 SARS-CoV-2-infected individuals from Kashmir Valley, India. ***Methods:*** Briefly, real-time polymerase chain reaction (RT-PCR) was used for the confirmation of SARS-CoV-2 infection. The principles of spectrophotometry and chemiluminescent microparticle immunoassay (CMIA) were employed to estimate the levels of glucose, TSH, and 25-hydroxy vitamin D levels in serum of infected patients. ***Results:*** A total of 236 patients infected with SARS-CoV-2 were taken for this cross-sectional study. Patients with COVID-19 had a male predominance (72.9 vs. 27.1%) and a higher prevalence of 25-hydroxy vitamin D deficiency (72.0 vs. 28.0%) with a mean 25-hydroxy vitamin D levels of 24.0 ± 13.9 in ng/mL. We observed a varied clinical spectrum of SARS-CoV-2 infection with 36.4%, 23.7%, and 29.7% patients having mild, moderate, and severe disease, respectively. We observed that severity of SARS-CoV-2 infection was significantly associated with older age group, hypertension, low TSH levels, and 25-hydroxy vitamin D deficiency. ***Conclusion:*** We conclude that not only old age but also hypertension and low levels of TSH and 25-hydroxy vitamin D levels could significantly lead to clinical severity of SARS-CoV-2 infection.

Introduction

Novel SARS-CoV-2 is an enveloped positive-sense RNA virus that typically gains entry to the cell via the angiotensin-converting enzyme 2 (ACE2) receptor [1],

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whereby it predominantly infects the lower respiratory tract, binding to ACE2 on alveolar epithelial cells [1, 2]. According to the latest data of January 2022 from Johns Hopkins University of Medicine Coronavirus Resource Center, a total number of confirmed coronavirus disease-2019 (COVID-19) cases have exceeded 280 million mark toward the beginning of January 2022, which means that one person on the globe has been infected every 4 s since the documented start of COVID-19 and around 797,017 cases have been reported everyday over the same period, with the global fatality rate standing at 2.1% [3].

As the spectrum of signs and symptoms among COVID-19-infected individuals is broad, ranging from mild to severe illness, requiring intensive care, identifying risk factors that may predict the patients' outcome is imperative for a better resource allocation in mitigating this current COVID-19 outbreak [4, 5]. COVID-19 shows an increased number of cases and a greater risk of severe disease with increasing age and understanding the role of age in transmission and disease severity is critical for determining the likely impact of social-distancing interventions on SARS-CoV-2 transmission [6]. On the one hand, diabetes and its associated comorbidities increase the risk of a more severe course of COVID-19 and increased mortality [7]. Several recent clinical studies have shown that hypertension is one of the most prevalent comorbidities of COVID-19 and a risk factor for severe COVID-19 infection [8]. Abnormal release of thyroid hormone affects the robustness of the immune response that is integral in maintaining COVID-19 outcomes [9]. Moreover, previous studies have linked decreased FT3, TSH, and FT4 with clinical deterioration of COVID-19 patients [10]. Nonetheless, the correlation between thyroid parameters and COVID-19 severity remains elusive as indicated by the variability of reported study results [11].

One of the most overlooked factors that could influence outcome of COVID-19 is the relative vitamin D status of populations [12] as this vitamin exerts well-known immunomodulatory functions spanning from the innate to the adaptive arms of the immune system and including the downregulation of proinflammatory cytokines, such as interleukin-6 [13]. Such a possibility is further supported by recent studies, suggesting a likely relationship between COVID-19 severity and the prevalence of vitamin D deficiency [14, 15], as well as from small reports in patients with or without COVID-19 [16, 17].

Jammu & Kashmir (North India), the place of our study, is also one of the worst-hit states of India with the total number of cases exceeding 3.4 lac among the total population of 12.5 million people with case-fatality rate of 1.4% [17]. Since in Kashmir (North India), India due to its unique geographical location and lifestyle, individuals are more prone to metabolic disorder and vitamin D deficiency; therefore, this cross-sectional study aimed to disentangle the relative contribution of demographic, clinical, and biochemical parameters on the outcome of patients with SARS-CoV-2 infection.

Materials and Methods

Study Design

This was a cross-sectional study conducted by the Department of Medicine in collaboration with the Department of Biochemistry, Government Medical College Srinagar and Associated Shri Maharaja Hari Singh (SMHS) Hospital, J&K, India. The study was approved by the Institutional Ethical Committee, Government Medical College Srinagar as per Helsinki declaration. Informed consent was taken from each participant after following proper procedure. Written informed consent in vernacular (Urdu/Kashmiri) and working language (English) with questionnaire response from SARS-CoV-2-infected patients or their caretakers was taken on record.

Study Subjects, Inclusion, and Exclusion Criteria

Two hundred thirty-six (n = 236) patients infected with SARS-CoV-2 attending the Medical Emergency were included in the study. The mean age of patients was 49.2 ± 15.4 years with a male: female ratio of 2.7:1. All the study subjects were of Kashmiri ethnicity. Patients with any sort of inflammatory/autoimmune disorders, genetic disorder, and cancer were excluded from the study. Pregnant women with SARS-CoV-2 infection were also excluded from the study.

Sample Size

Keeping the power of study as 80% and the effect size of 0.4 at type 1 error of 50%, the sample size came out to be 230. The sample size was calculated using the statistical software "G Power v. 16.2.3."

Real-Time Polymerase Chain Reaction for Detection of SARS-CoV-2

A positive real-time polymerase chain reaction (RT-PCR) test was considered as a gold standard for identification of SARS-CoV-2-infected patients who developed COVID-19. Nasopharyngeal swab was taken from individuals attending the Medical Emergency of SMHS Hospital with COVID-19 symptoms and immediately immersed in viral transport medium. The high-quality viral RNA was isolated from viral transport medium using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) following manufacturers' protocol. The multiplex RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 was carried out using TaqPath™ COVID-19 Combo Kit (Applied Biosystems, USA). The RT-PCR was performed using *Applied Biosystems™ 7,500 Fast Dx Real-Time PCR instrument* in the Department of Microbiology, associated C.D. Hospital, Srinagar, J&K [18].

Sample Collection

Blood (03 mL) was collected from each patient with COVID-19 on the next day when he/she came RT-PCR positive for SARS-CoV-2 infection. The blood sample was collected through venipuncture after an overnight fast of at least 8–10 h. Collected blood was immediately transferred into clot activator vials and centrifuged at 4,000 rpm for 2 min and serum was aliquoted into 2 mL microfuge tubes. The serum samples were stored at −20 °C until further analysis.

Quantitative Estimation of Glucose in Serum

Quantitative estimation of fasting serum glucose (by hexokinase G-6-PDH method) was performed using standard commercially available kits (Abbott, USA). Serum samples were processed and analyzed on an *ARCHITECT-C-4000* fully automated biochemistry analyzer (Abbott, USA) in the Biochemistry Diagnostic Laboratory, SMHS Hospital Srinagar, employing the principle of spectrophotometry and following reagent kit instructions. The reference range of fasting serum glucose was taken as 100–126 mg/dL [19].

Quantitative Estimation of Serum TSH and Vitamin D Levels

Levels of serum TSH and 25-hydroxy vitamin D levels of COVID-19 patients were estimated using chemiluminescent microparticle immunoassay technology with flexible assay protocols, referred to as Chemiflex. Serum samples were quantitatively analyzed on an *ARCHITECT i2000* fully automatic immunoassay analyzer (Abbott, USA) in the Biochemistry Diagnostic Laboratory, SMHS Hospital Srinagar, following the ARCHITECT reagent kit (Abbott, USA) instructions. The reference range of analytes was taken as: TSH: 0.35–5.2 μIU/mL and 25-hydroxy vitamin D: 28–53 ng/mL [20, 21].

Statistical Analysis

Statistical analysis was done using SPSS 23.0 statistical package (SPSS Inc., Chicago, IL, USA). Binary logistic regression analysis was used to untangle the association of various demographic and clinico-biochemical parameters with disease severity. Analysis was done by the *F*-test (ANOVA). The relative risk was estimated by odds ratios (OR) and 95% confidence intervals. $p \leq 0.05$ was considered as significant.

Results

Patient Characteristics

In our study, a total of two hundred thirty-six ($n = 236$) patients infected with SARS-CoV-2 were enrolled. Study included 172 male and 64 female patients. There were equal number of COVID-19 patients in both age groups (<50 years; 50.0%, ≥50 years; 50.0%). 19.5% (46/236) of patients infected with SARS-CoV-2 had T2DM, while 39.0% (92/236) had hypertension. COPD was present in 4.2% (10/236) of patients infected with SARS-CoV-2. TSH levels were high in 12.7% (30/236) of patients infected with SARS-CoV-2. Interestingly, 25-hydroxy vitamin D levels were reduced in 72.0% (170/236) of patients infected with SARS-CoV-2. Table 1 contains the demographic and clinico-biochemical characteristics of patients infected with SARS-CoV-2.

Clinical Severity of SARS-CoV-2 Infection

As per COVID-19 treatment guidelines, National Institutes of Health, SARS-CoV-2 infection is grouped into following categories as per the "severity of illness": *Asymptomatic or Presymptomatic Infection:* individuals who test positive for SARS-CoV-2 using a RT-PCR test but have no symptoms that are consistent with COVID-19. *Mild Illness:* individuals who have various signs and symptoms of COVID-19 but who do not have shortness of breath, dyspnea, or abnormal chest imaging. *Moderate Illness:* individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation (SpO_2) ≥94% on room air at the sea level. *Severe Illness:* individuals who have SpO2 <94% on room air at sea level, a respiratory rate >30 breaths/min, or lung infiltrates >50%. *Critical Illness:* individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.

In our study, 10.2% (24/236) were asymptomatic, 36.4% (86/236) were having mild illness, and 23.7% (56/236) and 29.7% (70/236) were having moderate and severe illness, respectively. Interestingly, none of the enrolled patients had critical illness. Table 2 includes the severity of SARS-CoV-2 infection in enrolled patients.

Association of Demographic and Clinico-Biochemical Parameters with Clinical Spectrum of SARS-CoV-2 Infection

Table 3 depicts the association of various demographic and clinico-biochemical parameters with clinical spectrum of SARS-CoV-2 infection. Almost 45.0% (16/36) of asymptomatic individuals belonged to older age group (≥50 years) compared to a higher percentage of older age group (≥50 years) patients having moderate (74.0%; OR = 3.5; $p = 0.008$) and severe diseases (75.0%; OR = 3.7; $p = 0.004$). More than 60.0% of patients among the moderate and severe disease groups were found to be hypertensive, which is statistically significant ($p < 0.0001$). Among asymptomatic SARS-CoV-2-infected patients, only 6.0% (02/36) were having decreased TSH levels as compared to 26.0% (12/46) of moderately ill patients having decreased TSH levels and the difference was statistically significant (OR = 6.0; $p = 0.02$).

We observed a significantly higher percentage of moderately ill patients having decreased 25-hydroxy vitamin D levels compared to relatively lower percentage of asymptomatic individuals with decreased 25-hydroxy vitamin D levels (78.3 vs. 56.0%; OR = 2.8; $p = 0.03$). Similarly, a significantly higher percentage of severely ill

Table 1. Demographic and clinico-biochemical characteristics of COVID-19 patients taken for the study

Demographic and clinico-biochemical characteristics	Cases (n = 236)
	n (%)
Gender	
Male	172 (72.9)
Female	64 (27.1)
Age group	
<50 years	118 (50.0)
≥50 years	50.0
T2DM	
No	190 (80.5)
Yes	46 (19.5)
Hypertension	
No	144 (61.0)
Yes	92 (39.0)
COPD	
No	226 (95.8)
Yes	10 (4.2)
TSH levels	
Normal	206 (87.3)
Low	30 (12.7)
25-Hydroxy vitamin D levels	
Normal	66 (28.0)
Low	170 (72.0)

COVID-19, coronavirus disease-2019; T2DM, type 2 diabetes mellitus; COPD, chronic obstructive pulmonary disease.

Table 2. Clinical spectrum of SARS-CoV-2 infection

Severity of SARS-CoV-2 infection	Cases (n = 236)
	n (%)
Asymptomatic	24 (10.2)
Mild	86 (36.4)
Moderate	56 (23.7)
Severe	70 (29.7)
Critical	00 (0.0)

patients were having decreased 25-hydroxy vitamin D levels as compared to asymptomatic individuals having decreased 25-hydroxy vitamin D levels (82.2 vs. 56.0%; OR = 3.6; p = 0.007). We did not observe association of any other parameter with severity of SARS-CoV-2 infection.

Discussion

Our study involved two hundred thirty-six (n = 236) SARS-CoV-2-infected individuals. A higher percentage of males were observed compared to females signifying that the risk of getting SARS-CoV-2 infection was more in case of males compared to females. Consistent feature of the ongoing COVID-19 pandemic, caused by the SARS-CoV-2, is the male bias toward the transmission [22]. Sex differences in both the innate and adaptive immune systems have been previously reported and may account for the female advantage in COVID-19 as females have higher numbers of CD4+ T cells [23], more robust CD8+ T-cell cytotoxic activity [24], increased B-cell production of immunoglobulin compared to males [25], and decreased expression of ACE2 receptors [26]. Females replete with estradiol have greater number and functionality of ACE2, likely a factor in their greater ability to handle SARS-CoV-2 infections. Additionally, estradiol-mediated RAAS modulatory actions provide further cardiovascular protection to females [27]. Gender-based sociocultural and behavioral differences could contribute to the sex difference seen in COVID-19 transmission. Men are less likely to wash their hands with soap after entering a restroom, and in many cultures, men may be more likely to leave the house and enter crowded areas. In addition, unequal access to healthcare and testing between sexes may skew toward a male bias in infection rates [28].

We observed quite a good number of SARS-CoV-2-infected individuals having hypertension (39%). Growing data show a higher risk of SARS-CoV-2 infections in hypertensive patients. As per previous multicenter study, 30% of COVID-19 patients had previously coexisting hypertension [29]. Another large study reported a prevalence of hypertension as 15% among COVID-19 patients [30]. A weaker immune system is one of the reasons hypertensive individuals are at higher risk for SARS-CoV-2 infections [8]. We observed that more than two-third of the SARS-CoV-2-infected individuals had vitamin D deficiency. Vitamin D deficiency has been shown to potentially increase the risk of severe respiratory infections [31]. According to a previous study, patients with vitamin D deficiency were 4.6 times more likely to be positive for COVID-19 than patients without vitamin D deficiency [32].

Assessing the severity of COVID-19 is crucial to determine the appropriateness of mitigation strategies and to enable planning for health-care needs as epidemics unfold. In our study, most of the SARS-CoV-2-infected patients had mild disease (36.4%), followed by individuals with severe (29.7%) and moderate disease (23.7%), respectively. A small proportion of study population was asymptomatic (10.2%). As of January 2022, more than 270 million cases of COVID-19 have been reported globally, including more than 5.3 million deaths

Table 3. Association of various demographic and clinico-biochemical parameters with clinical spectrum of SARS-CoV-2 infection

Severity of SARS-CoV-2 infection	*N* = 236 (%)	Characteristics		Or (95% CI)	*p* value
		Gender, *n* (%)			
		Male	Female		
		172 (72.9)	64 (27.1)		
Asymptomatic	36 (15.3)	26 (72.0)	10 (28.0)	Ref. (1.00)	0.9
Mild	98 (41.5)	70 (71.4)	28 (28.6)	1.04 (0.4–2.4)	0.5
Moderate	46 (19.5)	30 (65.0)	16 (35.0)	1.3 (0.5–3.5)	0.2
Severe	56 (23.7)	46 (82.0)	10 (18.0)	0.56 (0.2–1.5)	
		Age group, *n* (%)			
		<50 years	≥50 years		
		118 (50.0)	118 (50.0)		
Asymptomatic	36 (15.3)	20 (55.0)	16 (45.0)	Ref. (1.00)	0.06
Mild	98 (41.5)	72 (73.0)	26 (27.0)	0.45 (0.2–1.01)	0.008
Moderate	46 (19.5)	12 (26.0)	34 (74.0)	3.5 (1.4–8.9)	0.004
Severe	56 (23.7)	14 (25.0)	42 (75.0)	3.7 (1.5–9.1)	
		T2DM, *n* (%)			
		No	Yes		
		190 (80.5)	46 (19.5)		
Asymptomatic	36 (15.3)	30 (83.0)	06 (17.0)	Ref. (1.00)	0.5
Mild	98 (41.5)	86 (88.0)	12 (12.0)	0.7 (0.24–2.0)	0.5
Moderate	46 (19.5)	36 (78.0)	10 (22.0)	1.4 (0.4–4.2)	0.1
Severe	56 (23.7)	38 (68.0)	18 (32.0)	2.4 (0.8–6.7)	
		Hypertension, *n* (%)			
		No	Yes		
		144 (61.0)	92 (39.0)		
Asymptomatic	36 (15.3)	32 (89.0)	04 (11.0)	Ref. (1.00)	0.06
Mild	98 (41.5)	72 (73.0)	26 (27.0)	2.9 (0.9–8.9)	<0.0001
Moderate	46 (19.5)	18 (39.0)	28 (61.0)	12.4 (3.7–41.1)	<0.0001
Severe	56 (23.7)	22 (39.0)	34 (61.0)	12.3 (3.8–39.8)	
		COPD, *n* (%)			
		No	Yes		
		226 (95.8)	10 (4.2)		
Asymptomatic	36 (15.3)	34 (94.0)	02 (6.0)	Ref. (1.00)	0.9
Mild	98 (41.5)	98 (100.0)	00 (0.0)	0.1 (0.01–1.1)	0.8
Moderate	46 (19.5)	44 (96.0)	02 (4.0)	0.7 (0.15–4.0)	0.4
Severe	56 (23.7)	50 (89.0)	06 (9.0)	2.0 (0.3–10.7)	
		TSH levels, *n* (%)			
		Normal	Low		
		206 (87.3)	30 (12.7)		
Asymptomatic	36 (15.3)	34 (94.0)	02 (6.0)	Ref. (1.00)	0.6
Mild	98 (41.5)	90 (92.0)	08 (8.0)	1.5 (0.3–7.4)	0.02
Moderate	46 (19.5)	34 (74.0)	12 (26.0)	6.0 (1.2–28.8)	0.2
Severe	56 (23.7)	48 (86.0)	08 (14.0)	2.8 (0.5–14.1)	
		25-Hydroxy vitamin D levels, *n* (%)			
		Normal	Low		
		66 (28.0)	170 (72.0)		
Asymptomatic	36 (15.3)	16 (44.0)	20 (56.0)	Ref. (1.00)	0.1
Mild	98 (41.5)	30 (30.6)	68 (69.4)	1.8 (0.8–3.9)	0.03
Moderate	46 (19.5)	10 (21.7)	36 (78.3)	2.8 (1.1–7.5)	0.007
Severe	56 (23.7)	10 (17.8)	46 (82.2)	3.6 (1.4–9.5)	

COVID-19, coronavirus disease-2019; T2DM, type 2 diabetes mellitus; COPD, chronic obstructive pulmonary disease.

amounting to a global fatality rate of almost 2.1% [33]. According to a previous study from the USA, including 1.3 million COVID-19 cases, 14% of patients required hospitalization, 2% were admitted to the intensive care unit, and 5% died [34]. According to the report from the World Health Organization (WHO) – China Joint Mission on COVID-19, 80% of COVID-19 cases had mild-to-moderate disease, while 13.8% developed severe disease, and 6.1% required intensive care [35]. According to the latest report of Ministry of Health and Family Welfare, India reported total cases of around 40 million with a fatality rate of around 1.3% [17].

When stratified, several factors shape the clinical severity of SARS-CoV-2 infection and outcome of COVID-19 patients. Our findings, together with early evidence, suggest that there is age dependence in susceptibility and clinical severity of COVID-19 [36]. According to the latest report from Centers for Disease Control and Prevention (CDC), the patients above 30 years of age had 2 to 10 times higher rate of hospitalization and a whopping 4 to 370 times higher rate of mortality compared to reference age group of 18–29 years [37]. The hypothesis for age-related difference in the severity of COVID-19 is multifactorial. It has been maintained that elderly people have weak immunity, organ dysfunction apart from the higher frequency of comorbidities, which makes them more susceptible to SARS-CoV-2 severity [38]. Previous study investigating the isolated or direct effect of age on COVID-19 disease severity, after accounting for important age-related risk factors such as diabetes, cardiovascular disease, and chronic pulmonary disease, has observed rather smaller effect of age on disease severity [39]. Moreover, younger individuals have stronger mucosal innate immune response, which helps clear the virus [40]; lower tendency to develop a cytokine storm due to low levels of cytokines [41]; higher lymphocyte counts with a higher proportion of naïve T cells, T regulatory cells, and T follicular helper cells [42]; and less prone to endothelial damage and abnormal clotting [43].

In our study, hypertension was an independent predictor of worst outcome in SARS-CoV-2-infected patients. Hypertension is the single largest global contributor to disability-adjusted life year's lost and major risk factor for COVID-19 severity [44]. A large case series from China observed an increase in case-fatality rate to 6.0% from 2.3% in COVID-19 patients with hypertension [45]. End organ damage and cardiovascular events are associated with poorer control of high blood pressure and it seems plausible that hypertension leads to more severe SARS-CoV-2 infection [46]. In addition, in hypertensive patients with COVID-19, due to the imbalance in the renin-angiotensin system (RAS), the NADH/NADPH oxidase system may be activated by excessive inflammatory reactions [45], resulting in cell damage and vasoconstriction [47], aggravating lung damage in COVID-19 and leading to a poor prognosis. On the one hand, the use of angiotensin-converting enzyme inhibitors can result in a decrease in ACE and an increase in the expression of ACE2 in the lungs [48]. More ACE2 may mediate the invasion and infection of SARS-CoV-2 into lung cells, resulting in viral spread and aggravation of symptoms [44].

In our study, low TSH levels were significantly associated with moderate disease severity. A previous study on Chinese population showed that TT3 and TSH levels decreased with the severity of COVID-19 [49]. As per a previous report, 56% COVID-19 patients had significantly lower-than-normal TSH levels in the severe and critical group compared with non-COVID-19 pneumonia patients [49]. Additionally, Wei et al. showed that the decreased TSH concentration might be associated with the changes in TSH-secreting cells in the pituitary [50], which might be due to direct viral effect on the pituitary cells or an indirect effect wherein various systemic changes such as the activation of various proinflammatory cytokines caused by the virus infection or its treatment led to hormonal changes in the pituitary-endocrine axis feedback loops [49]. It has been emphasized that thyroid hormone dysregulates RAS, which contributes to increased ACE2 expression [51], thus leading to more severe disease. Several studies along with some meta-analysis have found that thyroid hormones play a role in modulating the cellular level of both innate and adaptive immune responses and abnormal thyroid hormone levels were significantly associated with a higher risk of COVID-19 severity [9, 52].

We observed a significantly strong association of decreased serum 25-hydroxy vitamin D levels with COVID-19 severity. Such a likelihood is more held by recent epidemiology data and small reports, suggesting a likely relationship between the risk of severe respiratory dysfunction/mortality during the course of hospitalization in patients affected by COVID-19 and the prevalence of vitamin D deficiency independently of inflammatory markers, age, or presence of major comorbidities such as obesity, diabetes, and hypertension [16, 53]. Vitamin D supports production of antimicrobial peptides in the respiratory epithelium in response to both viral and bacterial stimuli. Second, vitamin D might help to reduce the inflammatory response to infection, thus reducing the chances of SARS-CoV-2 severity [54, 55]. In a wide variety of animal studies and in in vitro cell models, 25-hydroxy vitamin D has been demonstrated to downregulate the

production of inflammatory cytokines, such as TNF-α and IL6, while increasing inhibitory cytokines. These studies promote the chance that sufficient levels of 25-hydroxy vitamin D may reduce the incidence of cytokine storm in COVID-19 [56]. Moreover, vitamin D protected the pulmonary vascular barrier from acute inflammatory injury in mice by locally targeting the RAS [57], whose dysregulation has been implicated in favoring the SARS-CoV-2 entry into alveolar cells with massive cytokine activation and development of ARDS [58]. Added affirmative effects of 25-hydroxy vitamin D include the stimulation of surfactant synthesis by alveolar type-II cells, thus improving alveolar surface tension and exerting a protective role against inflammation and oxidative stress, thereby refining the pulmonary vascular barrier and limiting the cytokine storm [59, 60]. Prevalence of vitamin D deficiency is reported to be high in chronic obstructive pulmonary disease (COPD) patients and increases with the severity of COPD, which, in turn, can influence the prognosis of many COVID-19 patients having COPD as a collateral disease [61]. In fact, as per many previous studies, a high-dose, oral vitamin D supplementation to augment 25-hydroxy vitamin D >50 ng/mL helped to achieve SARS-CoV-2 RNA negativity in greater proportion of asymptomatic vitamin D-deficient individuals with SARS-CoV-2 infection along with a significant decrease in inflammatory marker, while, as per other studies, Vitamin D supplementation showed protective effects against mortality and ICU admission in COVID-19 patients [62, 63].

To our knowledge, this is the first study from this part of the world (Kashmir Valley, North India) to comprehensively summarize the effect of age and clinico-biochemical parameters on clinical severity of SARS-CoV-2 infection. Since the population is ethnic with conserved gene pool, the effect of various factors on severity of COVID-19 disease is more profound. The number of SARS-CoV-2-infected patients enrolled in our study is modest; we need a larger sample size of clinical data to support our conclusions. In addition, we did not analyze complications such as acute renal injury, etc. However, these limitations will not affect the reliability of our overall results.

Conclusion

We observed an increased clinical severity of SARS-CoV-2 infection in elderly patients: patients having hypertension, decreased TSH, and 25-hydroxy vitamin D deficiency. Identifying the risk factors for clinical severity of SARS-CoV-2 infection will not only attract early intervention and appropriate medical decisions but also assist to design intervention trials aimed at exploring whether vitamin D replacement therapy might prevent the risk of respiratory failure in patients with SARS-CoV-2 infection, in current global pandemic.

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Statement of Ethics

The research work was in compliance with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The written informed consent and the study protocol were approved by Institutional Review Board of Government Medical College Srinagar vide no. 1010/ETH/GMC dated 12-06-2020. All the samples were collected after taking written informed consent from the patients and proper ethical procedures were followed.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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Author Contributions

Conceptualization, formal analysis, investigation, methodology, and visualization: S.A.W., B.G., and M.S.K.; data curation and writing – original draft: M.S.K.; funding acquisition, project administration, and supervision: S.M. and I.A.B.; Resources: S.M; Software: M.S.K; validation: M.S.K., S.M., and I.A.B; writing – review and editing: M.S.K. and I.A.B.; and approval of final manuscript: all authors.

Data Availability Statement

Detailed data are not publicly available due to ethical reasons. The data will be made available upon request. Further inquiries can be directed to the corresponding author.

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