Proceedings of the Pakistan Academy of Sciences: B Life and Environmental Sciences 61(S): 69-80 (2024)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(61-sp1)1011

Pakistan Academy of Sciences

Research Article

Molecular Epidemiology of Coronavirus Disease and its Effect on the Hematological Profile in District Kech, Balochistan

Niamatullah Kakar[1*], Talal Qadir[1], Zameera Wahid[1], Bakhtawar R. Baksh[1], S. Sameera Khan[1], Ghulam Nabi[1], Zalia Majeed[1], Irfan Shahzad Sheikh[2], and Habib Ur Rehman[2]

[1]Department of Natural and Basic Sciences, University of Turbat, Turbat, Balochistan, Pakistan
[2]Center of Advanced Studies in Vaccinology and Biotechnology, University of Balochistan Quetta, Pakistan

Abstract: The coronavirus disease (COVID-19) is a respiratory disease caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). This study aimed to investigate the occurrence of COVID-19 in district Kech, associated risk factors, clinical presentation, and its effect on blood cells. A structured questionnaire was designed to collect the data and the samples were investigated by RT-PCR. In total, 575 suspects were screened, among which 64 (11.16%) had COVID-19 infection, 40.6% were males, and 59.4% were females. An increased positivity of 53.12% was measured in the younger age group (15-30 years) and the lowest (18.75%) was noticed in elders > 50 years. 95.3% of the patients were newly infected, and 7.7% had a contact history with COVID-19-infected patients. The clinical symptoms, such as fever, cough, nausea, vomiting, and diarrhea, were identified in 89.1%, 67.2%, 51.6%, 46.9%, and 12.5%, respectively. The prominent clinical features, like fever, cough, nausea, diarrhea, chest distress, chest pain, and psychological stress were observed. Noticeably, 10.9% of obesity and 7.8% of asthma patients were co-infected with COVID-19 disease including 39.1% of cases showed psychological trauma. Leucocytosis and a decrease in hemoglobin concentration and platelets were determined in COVID-19-infected patients. A significant effect was observed on the hematological profile, however, the effect was severe in the older age group (>50 years). The results suggest that the large population of the study area is infected by SARS-CoV-2, appealing to the need for surveillance on a large scale and the implementation of preventive measures to control further dissemination of the disease.

Keywords: Blood, Coronavirus disease, Epidemiology, Risk factors, SARS-CoV-2

1. INTRODUCTION

The coronavirus disease (COVID-19) was first identified in December 2019 In China, and later on March 12, 2020, declared as a pandemic. The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was involved in causing COVID-19 disease identified in a seafood market in Wuhan, China [1]**.** A quick transmission from human to human was later reported [2] and updated the earlier findings of transmission between animals and humans [3]. Previous research has shown that the SARS-CoV epidemics in 2003 and Middle East Respiratory Syndrome (MERS) coronaviruses in 2015, belonged to the same virus family [4].

By December 2020, WHO [5] announced that most countries were affected by COVID-19 infection, and above 67.5 million confirmed cases and >1.5 million deaths occurred and spread rapidly and hit countries worldwide, including South Asia [6].

The first case in South Asia was reported in Nepal, having a traveling history from China, and the infection was spread to the neighboring countries of Sri Lanka and India, respectively [7]. In Pakistan, till May 10, 2023, the WHO has reported 1,580,631 confirmed cases, among which the mortality rate was 30,656 [6]. The first case in Balochistan was reported on March 10th, 2020, in

Received: October 2023; Revised: December 2023; Accepted: January 2024
*Corresponding Author: Niamatullah Kakar <niamatullah.kakar@uot.edu.pk>

Quetta city and the victim had a travel history from Iran [8].

The rural population is at greater risk of coronavirus disease infection than the urban population, as the people in urban areas are more educated and have basic knowledge about COVID-19 disease and the hygienic conditions are better than those in urban areas. However, combined sources of transport, and not following the proper preventive measures in urban areas are considered primary risk factors in the COVID-19 pandemic [9, 10].

Different studies have determined the epidemiology, clinical features, and transmission patterns of COVID-19 [11]. In general, COVID-19-infected individuals suffer from the symptoms of fever, cough, anosmia, loss of taste, and abdominal pain [12, 1]. In a previous study, it was shown that most of the participants were suffering from anxiety (71.0%) and depression (52.0%) during the COVID-19 pandemic, and about 32.4% of the participants had poor knowledge about the COVID-19 disease [10]. The severity of the clinical features depends on age, gender, and associated infections such as diabetes, obesity, high blood pressure, cardiac disease, and renal disease [13, 14].

Earlier, it was considered that the lungs were the target organs of SARS-CoV-2, but later, it was determined that it can also damage other body organs, such as the intestines, blood vessels, and kidneys [15]. Kidney infection has been seen in several cases, and the lungs were observed as the second most affected organ in COVID-19 infection [16]. Patients with cardiac and metabolic disorders like diabetes mellitus were severely affected by COVID-19 disease, and an increased mortality rate has been observed in diabetic patients [17]. In addition, hematological abnormalities, like leucocytosis, lymphopenia, and thrombocytopenia also reported during COVID-19 infection [18]. The COVID-19 pandemic harmed lives globally and people suffered from various social, physiological, economic, and psychological complications, and psychopathologies [19].

District Kech is situated in the Makran division of Balochistan province which consists of 909,116 individuals according to the 2017 census. To the best of our knowledge, a detailed study of the COVID-19 situation such as epidemiological characteristics, clinical traits, and related risk factors, is not well studied in Kech district. Therefore, we designed this study to evaluate the molecular epidemiology of COVID-19, identify risk factors, demographic characteristics, clinical features, comorbidities, and psychological trauma, and specifically effect on the blood physiology in COVID-19 infected patients.

2. MATERIALS AND METHODS

2.1. Study Design

The study was designed to determine the molecular epidemiology, risk factors, demographic characteristics, clinical features, comorbidities, and psychological trauma of the COVID-19 disease and its effect on the hematological profile of COVID-19-infected patients.

2.2. Study Area

This research was conducted in the areas of Dasht, Ginnah, Sharak, and Tijaban in district Kech. Dasht is located in the west of district Kech, around 50 km away from Turbat city, and has a population of about 65,000 individuals. Ginnah is located about 20 km far from Turbat City and has an estimated population of about 25,000. Sharak and Tijaban are about 60-70 kilometers away from Turbat. Both cities have a population of approximately 15,000 individuals each.

2.3. Study Population

All the participants were willingly allowed to participate in this study. There was no gender or age limitation. Individuals who were facing problems in the sampling, due to surgery, or had allergies from sampling swabs were excluded from the study.

2.4. Questionnaire Design

A pre-structured questionnaire was used to collect the demographic data, clinical features, risk factors, comorbidities, and psychological trauma such as stress, anxiety, and depression.

2.5. Sample Collection and Processing

Ethical approval was obtained from the Ethical Research Committee, University of Turbat. Oral informed consent was taken, and the nasopharyngeal samples were collected by inserting the swab through the nostril and placing it for a few seconds at the posterior wall of the nasopharynx to allow the swab to be well-saturated with the specimen. The samples were placed in a viral transport medium (VTM), and transported immediately to the laboratory for investigation. The blood samples were collected under aseptic conditions and analyzed for a hematological profile at the district headquarters (DHQ) hospital laboratory, Turbat Kech.

2.6. RNA Extraction

RNA was extracted from the SARS-CoV-2 by a nucleic acid extraction kit (Zybio Inc), following the manufacturer's instructions. Briefly, the extraction kit was equilibrated at room temperature for up to 5 minutes. The aluminum film of the 96-well plates was carefully opened, and 15µl Proteinase-K was added to positions A1H1 and A7H7 in order, followed by 200µl sample (B-100: 100µl) in order. The automatic nucleic acid extraction system was turned on, and the extraction process was set according to instructions. The extracted RNA was proceeded for RT-PCR.

2.7. RT-PCR

The COVID-19 RT-PCR kit was equilibrated to room temperature. The components were vortexed to mix properly and prepared according to the instructions of the manufacturer (Sansure Biotech Inc). Briefly, 10µl of the template RNA was added to the ready-to-use master mix to the PCR reaction tube. Two reaction tubes for positive and negative control respectively were included with the test samples, including internal control. A program was set in the RT-PCR system and the equipment was run according to the manufacturer's instructions.

2.8. Haematology Profile

The blood of a COVID-19-positive patient was collected under aseptic conditions, and the effect on the blood physiology was determined by measuring blood parameters with an automatic cell counter according to the instructions of the manufacturer. The blood physiological parameters such as haemoglobin (Hb%), erythrocytes (RBCs), leucocytes (WBCs), and thrombocytes (platelets), and indices such as PCV, MCV, MCH, and MCHC, were analyzed.

3. RESULTS

3.1. Epidemiological and Demographic Features

In total, 575 individuals were screened for COVID-19 infection. Results showed 64 (11.16%) were positive among the total screened individuals for COVID-19 infection. The data showed that the highest positivity rate (53.12%) was realized in the younger age group 15–30 years, followed by the age group 31–50 years, with a positivity rate of 28.12%. Interestingly, the lowest positivity rate (18.75%) was noticed in the age group > 50 years. Among total positive cases, 40.6% were male and 59.6% were female. The highest number of positive patients were from Ginnah (53.1%), followed by Dasht (23.4%), Tijaban (18.8%), and Sharak (4.7%), respectively. 95.3% of patients were newly infected and 7.7% of positive cases had a contact history with COVID-19-infected patients. The results further showed that 82.8% of the COVID-19-positive individuals were unemployed and 67.2% were uneducated. Moreover, 70.4% of individuals were married among the positive, and their socio-economic condition was low on average (Table 1).

3.2. Clinical Features

The common symptoms such as fever and cough were realized in 89.1% and 67.2%, respectively. Nausea and vomiting were observed in 51.6% and 46.9%, respectively. The diarrhea was realized in fewer cases (12.5%), and patients complaining of abdominal pain were 34.4%. The symptoms of chest distress were realized in 48.4%, and chest pain was observed in 43.8%; however, shortness of breath was seen in 28.1%. Other common symptoms like muscle pain, fatigue, runny nose, and sore throat were 56.2%, 78.1%, 25%, and 28.1%, respectively. The less common symptoms of loss of taste and smell were 23.4% and 17.2%, respectively. Noticeably, only 10.9% of the COVID-19 positive patients were involved in smoking (Table 2).

Table 1. Epidemiological and demographic characteristics.

Characteristics	Frequency (%)
Positive	64 (11.16)
Negative	511 (88.84)
Age (Years)	
15-30	34 (53.12)
31-50	18 (28.12)
>50	12 (18.75)
Gender	
Male	26 (40.6)
Female	38 (59.4)
Region	
Ginnah	34 (53.1)
Dasht	15 (23.4)
Tijaban	12 (18.8)
Sharak	3 (4.7)
New infected	59 (95.3)
Contact history	5 (7.7)
Employment status	
Employed	11 (17.2)
Unemployed	53 (82.8)
Education level	
Uneducated	43 (67.2)
Primary	12 (18.8)
Secondary	2 (3.1)
Higher-Secondary	7 (10.9)
Marital status	
Married	45 (70.4)
Unmarried	19 (29.6)
Socio-economic status	
High	3 (4.7)
Moderate	4 (6.2)
Low	57 (89.1)

3.3. Risk Factors Involve

More than half (53.1%) of the infected patients were not vaccinated against SARS-CoV-2. In 59.3% of cases, 3–4 people were living together in close contact. There was no trend of quarantine, and 100% of the patients either didn't isolate themselves or followed social distancing, and 90.6% of the positive individuals were visiting crowded places (Table 3).

Table 2. Clinical features of the COVID-19 positive patients.

Characteristics	Frequency (%)
Fever	
Yes	57 (89.1)
No	7 (10.9)
Cough	
Yes	43 (67.2)
No	21 (32.8)
Nausea	
Yes	33 (51.6)
No	31 (48.4)
Vomiting	
Yes	30 (46.9)
No	34 (53.1)
Diarrhea	
Yes	8 (12.5)
No	56 (87.5)
Abdominal pain	
Yes	22 (34.4)
No	42 (65.6)
Chest distress	
Yes	31 (48.4)
No	33 (51.6)
Chest pain	
Yes	28 (43.8)
No	36 (56.2)
Shortness of breath	
Yes	18 (28.1)
No	46 (71.9)
Muscle pain	
Yes	36 (56.2)
No	28 (43.8)
Bodyache	
Yes	36 (56.2)
No	28 (43.8)
Fatigue	
Yes	50 (78.1)
No	14 (21.9)
Runny nose	
Yes	16 (25.0)
No	48 (75.0)
Sore throat	
Yes	18 (28.1)
No	46 (71.9)

Characteristics	Frequency (%)
Loss of Smell	
Yes	11 (17.2)
No	53 (82.8)
Loss of Taste	
Yes	15 (23.4)
No	49 (76.6)
Smoking	
Yes	7 (10.9)
No	57 (89.1)

Table 3. Risk factors involved in causing COVID-19 infection.

Characteristics	Frequency (%)
Vaccination	
Yes	30 (46.9)
No	34 (53.1)
No individuals/room	
1-2/room	18 (28.2)
3-4/room	38 (59.3)
6-7/room	9 (9.3)
8-9/room	2 (3.2)
Social distancing	
Yes	00
No	64 (100)
Quarantining	
Yes	00
No	64 (100)
Close contact setting	
Yes	54 (84.4)
No	10 (15.6)
Indoor ventilation	
Yes	4 (6.2)
No	60 (93.8)
Use face mask	
Yes	11 (17.2)
No	53 (82.8)
Hand washing	
Yes	13 (20.3)
No	51 (79.7)
Handshaking	
Yes	56 (87.5)
No	8 (12.5)
Visit to crowded places	
Yes	58 (90.6)
No	6 (9.4)

Most (84.4%) of the positive patients were noticed to live in close contact settings and be deficient in indoor ventilation (93.8%). The majority of the COVID-19 patients (82.8%) were not using face masks, frequently (87.5%) involved in handshaking, and not washing 79.7% or desensitizing their hands after handshaking (Table 3).

3.4. Comorbidities

Results showed that (1.6%) of cardiac patients, (3.1%) diabetic, and (1.6%) chronic lung disease had COVID-19 infection. Noticeably, (10.9%)

Table 4. Comorbidities in relation to COVID-19 infection.

Characteristics	Frequency (%)
Cardiac disease	
Yes	1 (1.6)
No	63 (98.4)
Diabetes	
Yes	2 (3.1)
No	62 (96.9)
Obesity	
Yes	7 (10.9)
No	57 (89.1)
Chronic Lung disease	
Yes	1 (1.6)
No	63 (98.4)
Asthma	
Yes	5 (7.8)
No	59 (92.2)

Table 5. Psychological stress.

Characteristics	Frequency (%)
Psychological trauma	
Yes	25 (39.1)
No	39 (60.9)
Stress	
Yes	28 (45.4)
No	35 (54.6)
Anxiety	
Yes	17 (26.6)
No	47 (73.4)
Depression	
Yes	8 (12.5)
No	56 (87.5)

were obese, and (7.8%) of asthma patients had COVID-19 disease (Table 4).

3.5. Psychological Trauma

Analysis showed 12.5% depression, 26.6% anxiety, and 45.4% stress in COVID diseased patients. Overall, psychological trauma was realized in 39.1% of cases (Table 5).

3.6. Haematological Profile

To analyze the effect of COVID-19 infection on haematological parameters, patients were grouped into 15–30 years, 31–50 years, and >50 years. An increase, but within the normal range of leucocyte count was realized in the age group 31–50 years; however, a significant increase was observed in the older age group (>50 years) (Fig 1A). The age group 15–30 years showed 64.4% neutrophils, followed by 68.31% in the age group 31–50 years, and the highest neutrophil count (77.69%) was realized in the age group >50 years. The lymphopenia (18.13%) was realized in the age group above 50 years. The midcells, which consist of monocytes, eosinophils, and basophils, showed no significant effect on COVID-19 infection (Fig 1B).

The results further showed a significant effect of the SARS-CoV-2 infection on haemoglobin concentration. The effect was realized in all three age groups, showing Hb% of 9.4 g/dl, 9.2 g/dl, and 8.02 g/dl in age groups 15–30 years, 31–50 years, and above 50 years, respectively. However, this effect was more prominent in the elderly (>50 years) (Fig. 2A). A significant impact was seen on the red blood cell count ($3.481x10^{12}$/l) specifically in the elder age group (>50 years) (Fig. 2B). However, this effect was non-significant on patients in the age groups of 15–30 years and 31–50 years.

The result showed an impact on the indices of RBCs such as hematocrit or packed cell volume (Fig 2C). The PCV was significantly reduced in all three age groups. However, the effect was more prominent in the age group >50 years. Similarly, reduced MCV was observed in all three age groups in COVID-19-diseased patients. This ultimately led to a reduced value for MCH. while a mild effect on the MCHC levels was realized. Results showed platelet counts of $230.8x10^9$ /l, $170.3x10^9$ /l, and $128.5x10^9$ /l in the age groups of 15–30 years, 31–50 years, and >50 years, respectively (Fig. 2D).

4. DISCUSSION

During the pandemic, several COVID-19 disease waves affected the Makran region. The current research revealed the molecular epidemiology of the COVID-19 infection and identified the risk factors that contributed to facilitating the development of the disease in humans. A higher positivity rate (11.16%) correlates with the associated risk factors, which are linked with COVID-19-infected patients before, during, and after infection, which ultimately leads to the spread of the disease in the community. It is interesting to mention that in this study, female participants were more infected than males,

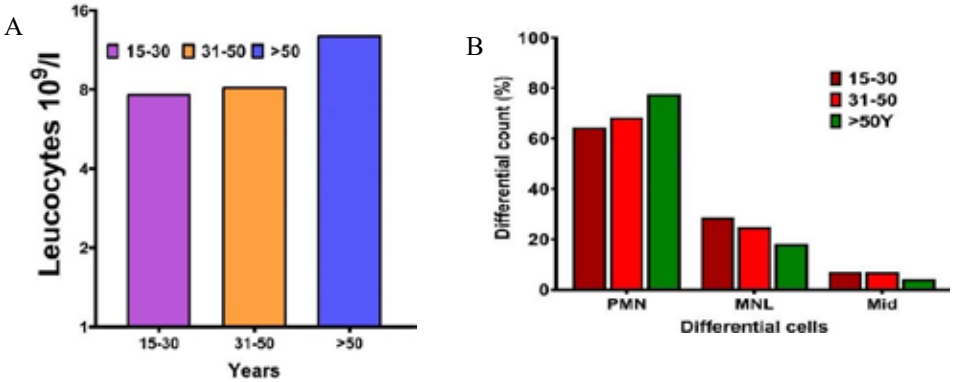

Fig. 1 (A & B). Effect of COVID-19 infection on leucocytes and differential count The blood samples of the COVID-19 positive patients were investigated by a hematology analyzer and the results were analyzed in the Graph Pad, for statistical analysis. (A) shows the effect of COVID-19 infection on leucocytes and (B) shows the effect on the differential count of blood.

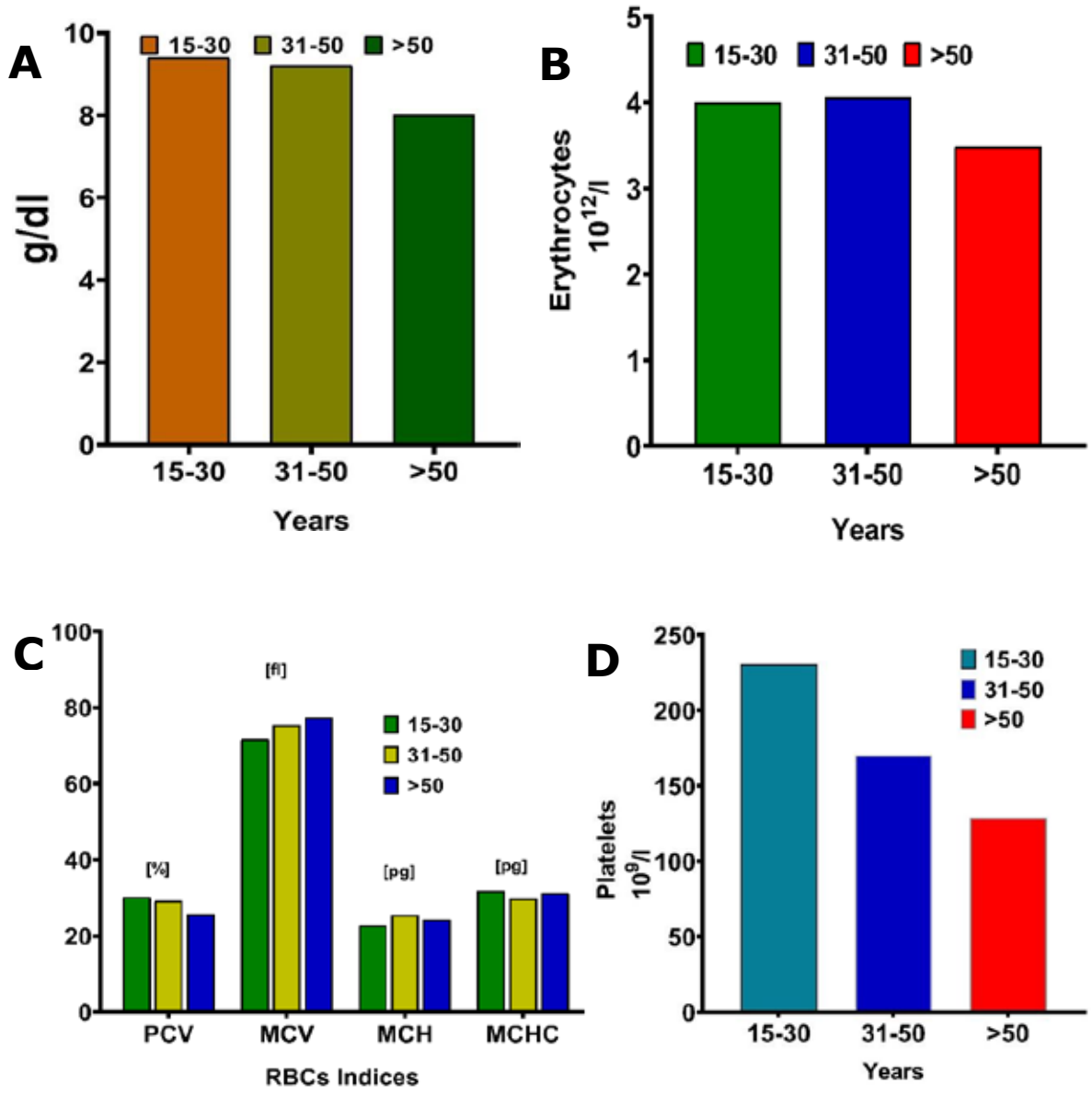

Fig. 2 (A-D). Effect of SARS-CoV-2 infection on hematological profile.
The effect of coronavirus disease was statistically analyzed in the Graph Pad Prism software. The effect of SARS-CoV-2 on Haemoglobin (A), Erythrocytes (B), RBC Indices (C), and Platelets (D) are shown here.

suggesting that females are more vulnerable to infection. This is most probably due to the changes in daily life activities. Because in the Mekran region, the females are also involved in small-scale businesses, they are in contact with the public and get infected by COVID-19 from other individuals. However, the results of this study are in contrast to other studies [20-25], which determined that males are more susceptible to the virus and are more affected by COVID-19 infection than females. It is assumed that due to poor hygienic conditions, nutritional deficiency, and a weak immune system, the females are more vulnerable to the diseases. However, it is determined that gender-specific hormones also play a role in immunity against viral disease [26].

In contrast to previous studies, our study showed a higher prevalence rate of COVID-19 infection in younger individuals as compared to older people. The possible reason for this could be that individuals of this age are in close contact with their educational institutes and classrooms, which puts them at greater risk of getting infections. Additionally, individuals in this age group are more responsible and struggle to earn for their

families, due to which they are more in contact with the community and are at greater risk of getting infection. Another possible assumption is that the prevalent variants of SARS-CoV-2 in the region could be more transmissible to children and infectious.

Ginnah which is located close to Turbat city, is more populated and crowded. The people daily visit Turbat city for work and daily requirements which ultimately communicate and in contact with each other. This close contact with undiagnosed COVID-19 patients may cause or spread the infection, due to which a higher positivity rate was seen in this study. This suggests that the prevalent serotype may be more contagious, infectious, and harmful. This research demands that a study be designed to investigate the available variants of SARS-CoV-2 that are involved in causing COVID-19 disease. This is in agreement with the study of Zhang and Atkinson (2008), who determined that urbanization promotes the transmission of infectious diseases due to increased contact rates [27]. In contrast to Ginnah, the areas of Dasht, Tijaban, and Sharak are far from Turbat City and less populated and scattered, due to which COVID-19 infection was less prevalent. Our study findings are in concordance with previous studies, where an increase in COVID-19 infection was determined in urban areas as described above. Factors such as access to healthcare in urban areas and inadequate surveillance and monitoring in rural areas also affect the data on COVID-19 infection [28].

Overall, data showed that the majority of the positive patients were not following the standard operating procedure (SOPs). An increase in the positivity rate of the COVID-19 infection is related to the non-vaccination against SARS-CoV-2. In addition, an incomplete vaccination is also a possible reason not to protect an individual against the infection. Individuals are more likely to get infections when they smoke or consume alcohol. However, our data determined that about 10% of the positive patients were involved in smoking. Therefore, at some point, smoking could not be considered the only significant factor in inducing COVID-19 infection. Data further showed that the majority of the patients had a history of visiting crowded places before and after infection. Visiting crowded places such as bazaars, marriage ceremonies, and social gatherings was identified as a major risk factor for causing infection.

This study showed that social distancing was not followed due to the cultural trend of social interactions at the workplace and other social occasions that make individuals more susceptible to infection. This correlates with another study [29] carried out previously. While not following quarantine and living in close living conditions and improper indoor ventilation are also considered risk factors involved in causing infection in this study. Another risk factor involved is the trend of joint family systems in the study area. Large families live together within one boundary in a close-contact setting, which places the individuals at risk and causes the spreading of infection among the community. Hand washing or the use of hand sanitizer was not in practice specifically after handshaking**.**

Patients with coinfections such as asthma and chronic obstructive pulmonary disease (COPD) showed critical health conditions and increased risk of hospital admission [30], which suggests that SARS-CoV-2 most likely infects comorbid patients [13]. This correlates with our study, which revealed that patients with chronic lung disease and asthma were prone to COVID-19 infection. Chronic blood sugars result in a weakened immune system, with a possible link to angiotensin-converting enzyme 2 (ACE2) expressions in cardiac tissues [31]. Patients with renal disease and those on dialysis were reported to have increased mortality when infected with the SARS-CoV-2 virus [32]. ACE2 expression in kidney cells is a unique target for SARS-CoV-2, causing tubular and glomerular damage [33].

Fever and cough, which are considered to be the most common symptoms of an infection, were realized in this study. The appearance of these symptoms shows the organ's response to infections [34] and is considered the most common clinical manifestation [35, 1]. The results of this study showed clinical symptoms of vomiting, nausea, and diarrhea. These symptoms have been reported previously in other studies in COVID-19-positive patients [36, 37]. This indicates that cell types in the GIT are potentially susceptible to SARS-CoV-2 infection, and the study indicates that diarrhea is

associated with COVID-19 infection [38].

Moreover, the association between chest distress and shortness of breath with COVID-19 infection is in agreement with previous studies [39, 40]. Additionally, the clinical manifestations of body aches, fatigue, and loss of smell and taste were also realized in the COVID-19-infected patients. Including damage to the human body, stress, and fear seriously disturbing the mental health of the people during the pandemic [14, 15, 41]. The COVID-19 pandemic has shown a negative effect on the economy, social life, and human health globally [42], and among health issues, the mental health of people has been seriously disturbed. Psychological traumas like stress, anxiety, and depression have been realized in this study, which correlates to previous studies carried out in different regions globally [43, 44].

The haematological abnormalities are associated with the severity and type of disease and are used to facilitate the early diagnosis or prognosis and disease severity [45]. Haematological abnormalities in COVID-19-positive patients are a major cause of disease progression, severity, and mortality. Thrombocytopenia, an abnormal coagulation profile, and lymphopenia are associated with disease progression, severity, and risk of mortality [18]. The leucocytosis was realized at an older age compared to a younger age. The increase in leucocytes is according to the previously published study [13], however, leucopenia is also reported in a study [46]. A decrease in lymphocyte count (16.9%) was previously reported [47]. Lymphopenia occurs in viral disease, which is associated with the cytopathic effect [48]. Another explanation of lymphopenia is an increased inflammatory response of the granulocytes and apoptosis of the lymphocytes [49].

Patients infected with COVID-19 infection had reduced haemoglobin concentrations in all three age groups, and this effect was more prominent in the older (>50 years) age. The low haemoglobin concentration is associated with the severity of the disease [50]; however, this was also linked with older age, at which the immune system becomes weakened and the individuals become more prone to infections. Moreover, the reduced count of RBCs due to the effect of coronavirus disease is also studied elsewhere [51]. Analysis showed that the effect of COVID-19 infection on platelets becomes more severe with age, due to weakened immune systems. This ultimately disrupts the blood cells, such as leucocytes, red blood cells, and platelets. It was determined that SARS-CoV-2 has an impact on megakaryocyte maturation. The COVID-19 infection increases platelet aggregation, which leads to platelet consumption in the microcirculation and damages lung tissue. Additionally, the novel coronavirus inhibits erythropoiesis in the bone marrow, which consequently causes thrombocytopenia by reducing platelet production [52]. Previous studies also reported thrombocytopenia in COVID-19-positive patients and considered as biomarkers that facilitate the diagnosis and severity of the disease [15].

5. CONCLUSIONS

The molecular analysis revealed the occurrence of coronavirus disease in the study area. The high-risk factors involved in spreading the SARS-CoV-2 virus include lack of social distancing, visits to crowded places, living in close contact settings, and not using face masks. The common clinical features, such as fever, cough, nausea, and diarrhea, and the less common symptoms, like chest distress, shortness of breath, and psychological stress, were observed. Noticeably, coronavirus disease was mainly associated with obesity and asthma, as well as diabetes and cardiovascular disease, and showed a significant effect on hematological profile. The study suggests that the large population of the study area is infected by SARS-CoV-2, appealing to the need for surveillance on a large scale and the implementation of preventive measures described in this study to control further dissemination of the disease.

6. ACKNOWLEDGMENTS

The authors would like to thank the Higher Education Commission (HEC) Islamabad and the University of Turbat, Kech for funding under the UOTCRF project and we also appreciate the District Head Quarter Hospital Turbat for providing a laboratory facility to carry out the research investigations.

7. CONFLICT OF INTEREST

The authors declared no conflict of interest.

8. FUNDING

The Study is funded by the Higher Education Commission, Pakistan and University of Turbat, Kech.

9. REFERENCES

1. C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu and X. Gu. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet* 395 (10223): 497–506 (2020).
2. W.B. Yu, G.D. Tang, L. Zhang and R.T. Corlett. Decoding the evolution and transmissions of the novel pneumonia coronavirus (SARS-CoV-2/ HCoV-19) using whole genomic data. *Zoological Research* 41(3): 247 (2020).
3. Q. Li, X. Guan, P. Wu, X. Wang, L. Zhou, Y. Tong, R. Ren, K.S.M. Leung, E.H.Y. Lau, and J.Y. Wong. Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. *New England Journal of Medicine* 382 (13): 1199-1207 (2020).
4. G. Huynh, T.N. Nguyen, V.K. Tran, K.N. Vo, V.T. Vo, L.A. Pham. Knowledge and attitude toward COVID-19 among healthcare workers at District 2 Hospital, Ho Chi Minh City. *Asian Pacific Journal of Tropical Medicine*. 13(6):260-65 (2020).
5. WHO. WHO coronavirus disease (COVID-19) dashboard. 2020. [Accessed 9 December 2020] https://covid19.who.int/.
6. Word Health Organization (WHO), report 2023, https://covid19.who.int/region/emro/country/pk
7. H.N. Chalise. South Asia is more vulnerable to the COVID-19 pandemic. *Arch Psychiatr Ment Health* 4: 46-47 (2020).
8. N. Ilyas, R. E. Azuine, and A. Tamiz. COVID-19 pandemic in Pakistan. *International Journal of Translational Medical Research and Public Health* 4 (1): 37-49 (2020).
9. S. ul Haq, P. Shahbaz, and I. Boz. Knowledge, behavior, and precautionary measures related to COVID-19 pandemic among the general public of Punjab province, Pakistan. *The Journal of Infection in Developing Countries* 14 (08): 823-835 (2020).
10. A. Ali, M. Sohaib, S. Iqbal, K. Hayat, A.U. Khan, and M.F. Rasool. Evaluation of COVID-19 disease awareness and its relation to mental health, dietary habits, and physical activity: a cross-sectional study from Pakistan. *The American journal of tropical medicine and hygiene* 104 (5): 1687 (2021).
11. A. Sarwer, B. Javed, E.B. Soto and Z.U.R Mashwani. Impact of the COVID-19 pandemic on maternal health services in Pakistan. *The International Journal of Health Planning and Management* 35 (6): 1306-1310 (2020).
12. C. Menni, A.M. Valdes, M.B. Freidin, C.H. Sudre, L.H. Nguyen, D.A. Drew, ... and T.D. Spector. Real-time tracking of self-reported symptoms to predict potential COVID-19. *Nature medicine* 26 (7): 1037-1040 (2020).
13. N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, Y. Qiu, J. Wang, Y. Liu, and Y. Wei. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet*, 395 (10223): 507–513 (2020).
14. N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, and P. Niu. A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine* 382(8): 727–733 (2020).
15. Y. Yang, W. Shang, and X. Rao. Facing the COVID-19 outbreak: What should we know and what could we do? *Journal of Medical Virology* 92 (6): 536–537 (2020).
16. J.P. Teixeira, S. Barone, K. Zahedi, and M. Soleimani. Kidney injury in COVID-19: epidemiology, molecular mechanisms and potential therapeutic targets. *International Journal of Molecular Sciences* 23(4): 2242 (2022).
17. D.S. Harbuwono, D.O. Handayani, E.S. Wahyuningsih, N. Supraptowati, F. Kurniawan, S. Wafa, and D.L. Tahapary. Impact of diabetes mellitus on COVID-19 clinical symptoms and mortality: Jakarta's COVID-19 epidemiological registry. *Primary Care Diabetes* 16(1): 65-68 (2022).
18. D. Lillicrap. Disseminated intravascular coagulation in patients with 2019-nCoV pneumonia. *Journal of Thrombosis and Haemostasis* 18(4)*:* 786 (2020).
19. V.J. Clemente-Suárez, M.B. Martínez-González, J.C. Benitez-Agudelo, E. Navarro-Jiménez, A.I. Beltran-Velasco, P. Ruisoto, and J.F. Tornero-Aguilera. The impact of the COVID-19 pandemic on mental disorders. A critical review. *International Journal of Environmental Research and Public Health* 18(19): 10041 (2021).
20. E.E. Overton, D. Goffman and A.M. Friedman. The epidemiolog y of COVID-19 in pregnancy. *Clinical Obstetrics and Gynecology* 65(1): 110 (2022).
21. E. Shim, A. Tariq, W. Choi, Y. Lee, and G. Chowell. Transmission potential and severity of COVID-19 in South Korea. *International Journal of Infectious Diseases* 93: 339-344 (2020).
22. R. De La Vega, R. Ruíz-Barquín, S. Boros and A. Szabo. Could attitudes toward COVID-19 in Spain render men more vulnerable than women?. *Global Public Health* 15(9)*:* 1278-1291 (2020).
23. F. Raimondi, L. Novelli, A. Ghirardi, F.M. Russo, D. Pellegrini, R. Biza, and F. Di Marco. Covid-19 and gender: lower rate but same mortality of

severe disease in women—an observational study. *BIOMED CENTRAL Pulmonary Medicine* 21: 1-11 (2021).
24. G.M. Bwire. Coronavirus: why men are more vulnerable to Covid-19 than women?. *SN Comprehensive Clinical Medicine* 2(7)*:* 874-876 (2020).
25. P. Mishra, R.K. Singh, A. Nath, S. Pande, A. Agarwal, O.P. Sanjeev, and R.K. Dhiman. A novel epidemiological scoring system for the prediction of mortality in COVID-19 patients. *Transactions of The Royal Society of Tropical Medicine and Hygiene* 116(5): 409-416 (2022).
26. J. Fischer, N. Jung, N. Robinson, and C. Lehmann. Sex differences in immune responses to infectious diseases. *Infection* 43: 399-403 (2015).
27. P. Zhang, and P.M. Atkinson. Modelling the effect of urbanization on the transmission of an infectious disease. *Mathematical Biosciences* 211(1): 166-185 (2008).
28. F. Shahbazi, M. Solgi, and S. Khazaei. Predisposing risk factors for COVID-19 infection: A case-control study. *Caspian Journal of Internal Medicine* 11(1): 495 (2020).
29. G.L. Hooper, and S.A. Quallich. Health seeking in men: A concept analysis. *Urologic Nursing* 36 (4): 163-173 (2016).
30. P. Aveyard, M. Gao, N. Lindson, J. Hartmann-Boyce, P. Watkinson, D. Young, C.A.C. Coupland, P.S. Tan, A.K. Clift, and D. Harrison. Association between pre-existing respiratory disease and its treatment, and severe COVID-19: a population cohort study. *The Lancet Respiratory Medicine* 9(8): 909–923 (2021).
31. Y.Y. Zheng, Y.T. Ma, J.Y. Zhang, and X. Xie. COVID-19 and the cardiovascular system. *Nature Reviews Cardiology* 17 17(5): 259–260 (2020).
32. F. Alberici, E. Delbarba, C. Manenti, L. Econimo, F. Valerio, A. Pola, C. Maffei, S. Possenti, S. Piva, and N. Latronico. Management of patients on dialysis and with kidney transplantation during the SARS-CoV-2 (COVID-19) pandemic in Brescia, Italy. *Kidney International Reports* 5(5): 580–585 (2020).
33. B. Diao, C. Wang, R. Wang, Z. Feng, J. Zhang, H. Yang, Y. Tan, H. Wang, C. Wang, and L. Liu. Human kidney is a target for novel severe acute respiratory syndrome coronavirus 2 infection. *Nature Communications* 12(1): 1–9 (2021).
34. J.J.G. Plaza, N. Hulak, Z. Zhumadilov and A. Akilzhanova. Fever as an important resource for infectious diseases research. *Intractable & Rare Diseases Research* 5(2): 97–102 (2016).
35. J. Xie, Q. Wang, Y. Xu, T. Zhang, L. Chen, X. Zuo, J. Liu, L. Huang, P. Zhan, and T. Lv. Clinical characteristics, laboratory abnormalities and CT findings of COVID-19 patients and risk factors of severe disease: a systematic review and meta-analysis. *Annals of Palliative Medicine* 10 (2): 1928–1949 (2021).
36. S. Murat, B.D. Karatekin, A. Icagasioglu, C. Ulasoglu, S. İçten, and O. Incealtin. Clinical presentations of pain in patients with COVID-19 infection. *Irish Journal of Medical Science* 190: 913–917 (2021).
37. M.S. Asghar, S.J.H. Kazmi, N.A. Khan, M. Akram, S.A. Khan, U. Rasheed, M. Hassan, and G.M. Memon. Correction: clinical profiles, characteristics, and outcomes of the first 100 admitted COVID-19 patients in Pakistan: a single-center retrospective study in a tertiary care hospital of Karachi. *Cureus* 12(6): (2020).
38. F. Hikmet, L. Méar, Å. Edvinsson, P. Micke, M. Uhlén, and C. Lindskog. The protein expression profile of ACE2 in human tissues. *Molecular Systems Biology* 16(7): e9610 (2020).
39. R. Alizadehsani, Z.A. Sani, M. Behjati, Z. Roshanzamir, S. Hussain, N. Abedini, F. Hasanzadeh, A. Khosravi, A. Shoeibi, and M. Roshanzamir. Risk factors prediction, clinical outcomes, and mortality in COVID-19 patients. *Journal of Medical Virology* 93(4): 2307–2320 (2021).
40. C. Zhao, Y. Bai, C. Wang, Y. Zhong, N. Lu, L. Tian, F. Cai, and R. Jin. Risk factors related to the severity of COVID-19 in Wuhan. *International Journal of Medical Sciences* 18(1): 120 (2021).
41. M.I. Hossain, N. Mehedi, I. Ahmad, I. Ali, and A. Azman. Psychosocial stress and trauma during the COVID-19 pandemic: Evidence from Bangladesh. *Asian Social Work and Policy Review* 15(2): 145-159 (2021).
42. D. Roy, R. Ghosh, S. Dubey, M.J. Dubey, J Benito-León, and B.K. Ray. Neurological and neuropsychiatric impacts of COVID-19 pandemic. *Canadian Journal of Neurological Sciences* 48(1):9–24 (2021).
43. A.M. Ali, A.A. Alkhamees, E.S. Abd Elhay, S.M. Taha, and A.O. Hendawy. COVID-19-Related Psychological Trauma and Psychological Distress Among Community-Dwelling Psychiatric Patients: People Struck by Depression and Sleep Disorders Endure the Greatest Burden. *Frontiers in Public Health* 9:799812 (2022).
44. S.K Brooks, R.K Webster, L.E. Smith, L. Woodland, S. Wessely, N. Greenberg, and G.J. Rubin. The psychological impact of quarantine and how to reduce it: Rapid review of the evidence. *The Lancet* 395(10227): 912–920 (2020).
45. S. Araya, M. Wordofa, M.A. Mamo, Y.G. Tsegay, A. Hordofa, A.E. Negesso, T. Fasil, B. Berhanu, H. Begashaw, A. Atlaw, and T. Niguse. The magnitude of hematological abnormalities among COVID-19 patients in Addis Ababa, Ethiopia. *Journal of*

Multidisciplinary Healthcare 545-554 (2021).
46. R.K. Gujar, A. Meena, S.S. Chouhan, and K. Likhar. Hematological profiles of COVID-19 patients at the Ratlam district, Madhya Pradesh State. India. *Bioinformation* 17(7): 686 (2021).
47. J Mao, R. Dai, R.C. Du, Y. Zhu, L.P. Shui, and X.H. Luo. Hematologic changes predict clinical outcome in recovered patients with COVID-19. *Annals of Hematology* 100(3):675–689 (2021).
48. R. Xu, B. Cui, X. Duan, P. Zhang, X. Zhou, Q. and Yuan. Saliva: potential diagnostic value and transmission of 2019-nCoV. *International Journal of Oral Science* 12(1): 11 (2020).
49. L. Tan, Q. Wang, D. Zhang, J. Ding, Q. Huang, Y.Q. Tang, Q. Wang, and H. Miao. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduction and Targeted Therapy* 5(1): 33 (2020).
50. M. Karimi Shahri, H.R. Niazkar and F. Rad. COVID-19 and hematology findings based on the current evidences: A puzzle with many missing pieces. *International Journal of Laboratory Hematology* 43(2): 160-168 (2021).
51. G. Wang, C. Wu, Q. Zhang, F. Wu, B. Yu, J. Lv, Y. Li, T. Li, S. Zhang, C. Wu, and G. Wu, and Y. Zhong. C-reactive protein level may predict the risk of COVID-19 aggravation. *Open forum infectious diseases Oxford University Press* 7(5) 153 (2020).
52. M. Al-Nbaheen, R. Vishnubalaji, D. Ali, A. Bouslimi, F. Al-Jassir, M. Megges, A. Prigione, J. Adjaye, M. Kassem, and A. Aldahmash. Human stromal (mesenchymal) stem cells from bone marrow, adipose tissue and skin exhibit differences in molecular phenotype and differentiation potential. *Stem Cell Reviews and Reports* 9: 32-43 (2013).