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Immunity Patterns of Covid-19 Recovered Patients in Gilgit Baltistan, Pakistan

Huda Khan, Maisoor Ahmed Nafees, Saif Ud Din^{*}, Mehran Kausar, and Raja Imran

Department of Animal Sciences, Karakorum International University, Gilgit Baltistan, Pakistan

Abstract: The main purpose of this study was to assess the immunity of recovered patients in COVID-19 patients. The idea was to unveil the nexus between the onset of COVID-19 coupled with the virulence of the disease and the immune responses of the target population in tandem with the intake of local/traditional foods. In the current study antibody presence and complete blood profile of 100 individuals were studied for COVID-19, from the Gilgit area. Immunity against COVID-19 recovered patients was observed during this investigation after more than eight months of their recovery. In this study, 78.2% of people were positive for Immunoglobin G antibody and 23.7% for Immunoglobin M antibody. The most infected age group recorded was 31-40 years. The mean blood glucose level in random was 143.38 mg/dL and 128.18 mg/dL in males and females, respectively. The mean cholesterol level in males was 148.82 mg/dL while the female cholesterol mean was 126.49 mg/dL. White Blood Cells were observed within their prescribed ranges. The mean hemoglobin level was 14.580 g/dL and 12.022 g/dL while mean RBC was 4.7838×10^6 m/µL and 4.2993×10^6 m/µL and the mean platelets were 238.45×10^3 /µL and 261.91×10^3 /µL in male and female, respectively. It is observed that the A⁺ blood group was more susceptible to infection. About 75% of individuals consume highly rich and nutritious food. About 55% of people engaged themselves with exercise. 25% of participants used their normal regular diet during the infection phase on the other hand 75% of individuals treated themselves with a specific and highly nutritious diet for their rapid and healthy recovery. More than 65% of people were re-infected after COVID-19 vaccination but the symptoms were not severe. Due to the strong innate immunity of people from this region, they recovered more rapidly. The dietary habits, high hemoglobin levels coupled with regular exercise might have a positive impact on early recovery of COVID-19-infected patients.

Keywords: IgG, IgM, Vaccination, Immunity, Susceptibility.

1. INTRODUCTION

Outbreak of a novel coronavirus was reported at the end of 2019, causing respiratory diseases in Wuhan, Hubei, China that disease was officially named as Corona Virus Disease 2019 (COVID-19). The coronavirus that is the contributory agent of these respiratory diseases was identified and the genome of this virus was sequenced [1]. Coronaviruses belong to family Coronaviridae and order Nidovirales. These are RNA based viruses, non-segmented and enveloped in their morphology. They are largely scattered in humans and other mammals [2]. The four types of HCoVs (HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1) worldwide spread in the human race and caused around one-third of common cold infections of human population [3]. These four HCoVs can cause life-threatening

pneumonia and bronchitis especially in elders, children and immune compromised patients in the severe case of viral infection [4-6]. COVID-19 is considered as the third terrifically pathogenic infection of 21st century among mankind after MARS-CoV and SARS-CoV [7]. The first case of coronavirus was reported on 29th December 2019; Chinese authorities recognized a group of similar cases of pneumonia of mysterious etiology in Wuhan City of China, Hubei Province at Huanan seafood market. At that market several animals such like birds and rabbits were on sale before the outbreak of virus [8-9]. A new strain of coronavirus (2019-nCoV) was isolated on 7th of January 2020 from a patient [8]. Most of the initial cases had the epidemiological associations towards a live animal market (Huanan South China Seafood Market), signifying a probable zoonotic base [10].

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^{*}Corresponding Author: Saif Ud Din <saifuddin@kiu.edu.pk>

Though, the ultimate source of the coronavirus is mysterious. Occurrence of infection in at least one family chain in healthcare staff verify the humanto-human mode of transmission, nevertheless the extent of this means of transmission was unclear until 21 January 2020, the WHO recommended that there was potential continual human-to-human transmission mode [10].

Since the epidemic of the COVID-19 in January 2020, the virus has attacked most of countries around the world. This outbreak has devastated several thousands of lives worldwide [11]. The World Health Organization (WHO) announced it as a pandemic in March 2020, and proclaimed as emerging threat. Many parts of countries have been locked down and strict social distancing measures have been announced by the states to stop the virus transmission [11, 12]. Li et al. [13] and Ong et al. [14] stated Novel coronavirus, known as COVID-19 or SARS-Cov-2 has become a global threat and healthcare alarm. Human to human transmission happens through respiratory droplets (coughing or sneezing) and by direct contact with any infected person or indirect contact with fomites in the surroundings Guan et al. [15, 16]. According to WHO [10], the pandemic, COVID-19 has affected above 2.5 million people throughout the globe. The reports of the WHO revealed that the epidemic of COVID-19, has affected over 2164111 people and more than 146,198 people were died in more than 200 countries around the world. Sohrabi et al. [17] emphasized the coverage of the epidemic with the World Health Organization (WHO) affirming the COVID-19 outburst as a worldwide emergency on January 30, 2020. Huang et al. [18] and Guan et al. [16] explained that the Covid-19 is clinically identified on the basis of illness expression and the patient's distinctiveness such as epidemiological conditions, interview of sickness, travel history, among associates, and laboratory conclusion. Huang et al. [18] showed that Covid-19 disease may produce single or numerous symptoms include dyspnea (shortness of breath), cough, chills, fever, headache, myalgia (muscle pain), sore throat, and loss of taste and/or smell. Hu et al. [19] said that the symptoms in the early phase sometimes bear a resemblance to those of the common cold and influenza; thus, health care providers frequently face complexities in distinctive coronavirus infection from the other infections. Furthermore, asymptomatic and subclinical SARS-CoV-2

contagion is comparatively widespread. These factors make satisfactory identification extremely complicated. Intermittently, COVID patients boost pneumonia, which can lead to brutal death and respiratory failure. Arabi *et al.* [20] determined that viral tropism is towards lungs and Communityacquired pneumonia (CAP). Some patients having pneumonia abruptly worsen into severe respiratory failure (SRF) so they require intubation and Mechanical ventilation (MV). This type of patient goes through towering risk of death up to 60% Long *et al.* [21]. Corona Virus 2019 infection explicit IgG and IgM came to top intensities at 17-19 days and 20-22 days after manifestation beginning correspondingly.

In Pakistan, the seroprevalence of SARS-CoV-2 antibodies was 7.1%, according to a research. IgG positivity was found in 6.3% of people, whereas IgM positivity was found in 1.9%. Seroprevalence varied from 0% in Ghotki to 17% in Gilgit. Seroprevalence varied by age category, ranging from 3.9% (0-9 years) to 10.1% (40-59 years) [22]. The purpose of this study was to assess the immunity pattern and reasons behind the Covid-19 recovered patients of mountainous region, to test the blood cell morphology, dietary pattern, age and recovery relationship of recovered patients.

2. MATERIALS AND METHODS

2.1. Study Area and Sampling

This study was conducted at Gilgit Baltistan the very north of Pakistan. A controlled study was conducted on convenient samples of more than 100 recovered patients of COVID-19 by visiting them at declared COVID-isolated centers by Gilgit Government and Isolated patients in homes. All recovered patients of any age group and background were tested. Blood samples were collected from the rehabilitated patients. Sampling was done through proper Biosafety guidelines. The clinical data along with personal data of patients were added in this research. Age, gender, epidemiological history, history of smoking, and co-morbidities, e.g., cancer, blood pressure, high blood cholesterol, hypertension and diabetes were recorded in the personal data of patients. The clinical data included early symptoms, clinical presentation, fundamental signs, remedial drug-use, respiratory assistance, and disease outcome. A pre-planned questionnaire

was also developed to assess the dietary patterns of patients.

2.2. Antibody Test

The Rapid COVID-19 IgM/IgG combine Test Kit is a lateral flow immunoassay proposed for qualitative recognition and discrimination of Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies to SARS-CoV-2 in blood. We used whole blood for antibody tests. "HEALGEN One Step Rapid Testing Kit" was used in this research in order to detect the antibodies presence in recovered patient's blood. About 5 μ l whole Blood specimens were taken and transferred the specimen into the sample well. Added 2 drops of sample buffer (80 μ l) into buffer well immediately. Observed the red colored lines appeared on test window.

2.3. Complete Blood Count (CBC Test)

Blood was collected in EDTA coated CP tubes. EDTA tubes were used to prevent clotting in blood. About 2 μ l blood was drawn from patients using hypodermic disposable syringe. Tubes were placed on shaker machine after 4-5 minutes; the CBC tube was placed in "Sysmex XP-100 Automated Hematology Analyzer" to analyze the sample, as mentioned by Farah *et al.* [23]. The analysis was performed at the Laboratory of hematology Department, District Head Quarter (DHQ) Hospital Gilgit.

2.4. Sugar Test

Portable Glucometer (On Call EZ II) was used to test the blood sugar level of patients at random on the spot. Sugar fasting test was not taken due to different timing of sample collection. Randomly sample was taken during this investigation.

2.5. Cholesterol Tests and Blood Grouping

Cholesterol and blood group testing was done in the Laboratory of DHQ Hospital Gilgit according to their prescribed methods. Cholesterol reagent (Human 100 ml) was added into a simple glass testing tube about 1000 μ l and placed the tube in temperature tank for 10 minutes in 37 °C. Then added 10 μ l blood serum and tested blood cholesterol level in MERCK analyzer (Micro-lab 300 by ELI Tech Group).

2.6. Statistical Analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (Version 22.0, SPSS Inc). Categorical variables were presented as mean and Standard error in each group. ANOVA and LSD was run through the parameters. A p-value less than 0.05 is considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1. Blood Profile of Recovered Patients of COVID-19

The findings were normal after recovery from infection as this was a post disease study; the survivors regain their normal amount of all blood components during recovery phase. Lu and Wang [24] analyzed the changing in blood parameters and their results showed that all the parameters gradually increased to their normal percentages as leading towards the recovery from the disease. The mean Hemoglobin level shown in current research is 14.580 g/dL and 12.022 g/dL in male and female respectively. The average Hemoglobin level was observed with in standard range the individuals, included in the present research, belong to the high altitude where Hb% is generally high than individuals living in lower altitudes. A study by Liu and Li [25] reported that the Hemoglobin molecules become physiologically inactive and no longer play their role as oxygen carriers during SARS-CoV-2 infection. Lanini et al. [26] investigated different blood parameters of individuals suffered from covid-19; They observed average Hb levels were in the normal range in survivors while a mild anaemia was observed at the end in non-survivors.

The average readings of Total leukocyte count (TLC) observed within male individuals was 6913.04/ μ L and the female reading was 9490.89/ μ L. The means recorded in this study lies within the prescribed limits of WBCs. After recovery from infection the blood components regain their optimal range. The highest and lowest observed values were recorded in male individuals were 21100/ μ L and 2420/ μ L, respectively. Platelets mean for male is 238.45×10³/ μ L and 261.91×10³/ μ L for female is observed that SARS-CoV-2 can decrease platelet formation, increase platelet degradation, or more likely, increase platelet consumption due to platelet

activation and thrombosis. Lippi et al. [28] found that lower platelet counts are linked to an elevated risk of in-hospital death in COVID-19 patients. The normal platelet counts ranges from 150,000 to 450,000 per micro-liter of blood. The means for both groups (Male and Female), fall within the normal limits of platelets in this research has been observed. The both upper and lower values were observed in female individuals $5.24 \times 10^3/\mu L$ and 8.0×10³/µL, respectively. Lanini et al. [26] observed in their study that the platelets' amount was inferior in survivor group than that of the non-survivors group of COVID-19 at early infection, meanwhile the count increased along the way of recovery. This study is on the post infection analysis, therefore investigation showed normality in platelets after infection.

3.2. Blood Glucose and Cholesterol

Guo et al. [29] and Zhou et al. [30] worked on diabetes and reported that it is one of basic comorbidities for infection development and mortality by COVID-19. Zaki et al. [31] also suggested a strong association between diabetes and severity of disease in COVID-19. Deng and Peng [32] investigation on patients of Corona, reported that 19% patients had diabetes and diabetic patients contributed to 42% of death rate. In this research sugar was also considered as a part of study. Random sugar level was analyzed in male and female recovered patients and the observed mean was 143.38 mg/dL and 128.18 mg/dL, respectively. The highest observed value was 250 mg/dL in male diabetic patient while lowest value was 85 mg/dL in a female at random. Majority of the population were males who had diabetes in the current investigation. A study by Wu et al. [1], also revealed that diabetes was the second most common co-morbidity among the patients of COVID-19. The observed Cholesterol mean of male is 148.82 mg/dL while female mean for cholesterol is 126.49 mg/dL in current investigation. The standard cholesterol level must be less than 200 milligrams per deciliter (mg/ dL) for adults. The highest recorded cholesterol level in this investigation was 300 mg/dL while the lowest recorded value was 84 mg/dL and both of them are recorded in male patients. Kocar et al. [33] elucidated that the cholesterol levels and the amount of lipoproteins in blood are good indicators for examining the status of viral infection in any individual. Another study by Daniels et al. [34] revealed that the cholesterol levels are key feature in infections of COVID-19.

3.3. Age of Infected Patients by SARS-CoV-2

During this study the most infected age group was 31-40 years; however, Corona virus attacked older people as well. In this study people nearly 90 years old were also observed to be infected by COVID-19. Ho *et al.* [35] investigated that people with older age are more susceptible to the infection. The condition was observed more severe in people more than 60 years. A similar study by Hu *et al.* [19] also reported that the older age was more affected by this pandemic. Another related study by Mahase [36] reported that aged people are more vulnerable to COVID-19 having multiple co-morbidities and weak immune system.

3.4. Antibodies

This research describes the initial and cross-

Blood Group	×/0	No	Sugar mg/ dL	Cholesterol	Hb g/dL	WBCs/µL	Neutro	Lymph	Eosino	Mono	RBCs m/µI	Platelets /µL
A+	37.60%	38	$\begin{array}{c} 140.89 \pm \\ 7.4263^{\rm B} \end{array}$	$\begin{array}{c} 138.65 \pm \\ 5.1689^{\scriptscriptstyle A} \end{array}$	$\begin{array}{c}13.457\pm\\0.3047\end{array}$	$\begin{array}{c} 6654.1 \pm \\ 241.87^{\text{A}} \end{array}$	${\begin{array}{c} 58.811 \pm \\ 1.7782^{\rm A} \end{array}}$	$\begin{array}{c} 33.919 \pm \\ 1.6147^{\rm B} \end{array}$	$\begin{array}{c} 4.4595 \pm \\ 0.3906^{\rm A} \end{array}$	$\begin{array}{c} 2.5946 \pm \\ 0.3582^{\rm A} \end{array}$	$\begin{array}{c} 5435000 \pm \\ 1003000^{\rm A} \end{array}$	${}^{227412\pm}_{12163^{AB}}$
B+	19.80%	20	${}^{140.1\pm}_{9.859^B}$	$142.7 \pm 7.408^{\text{A}}$	13.43 ± 0.442	$\begin{array}{c} 6047.5 \pm \\ 359.1^{\rm A} \end{array}$	$\begin{array}{c} 56.1 \pm \\ 2.442^{\mathrm{A}} \end{array}$	$\begin{array}{c} 36.4 \pm \\ 1.978^{\rm AB} \end{array}$	$\begin{array}{c} 5.6 \pm \\ 0.613^{\rm A} \end{array}$	$\begin{array}{c} 2.5 \pm \\ 0.500^{\rm A} \end{array}$	$\begin{array}{l} 4648000 \pm \\ 126582^{\rm A} \end{array}$	${}^{266400\pm}_{22686^A}$
B-	2%	2	$\begin{array}{c} 106.5 \pm \\ 2.5^{\scriptscriptstyle B} \end{array}$	$114.5\pm18.5^{\rm B}$	13.3 ± 0.1	$\begin{array}{c} 2755 \pm \\ 2745^{\rm B} \end{array}$	$54\pm2.0^{\rm A}$	$\begin{array}{c} 39.5 \pm \\ 1.5^{\rm AB} \end{array}$	$3\pm2.0^{\rm A}$	$3\pm1.0^{\rm A}$	$\begin{array}{l} 4905000 \pm \\ 285000^{\rm A} \end{array}$	$\begin{array}{c} 107650 \pm \\ 107350^{B} \end{array}$
AB+	15.80%	16	${}^{131.38\pm}_{8.371^B}$	$\begin{array}{c} 146.56 \pm \\ 12.15^{\rm A} \end{array}$	$\begin{array}{c} 13.513 \pm \\ 0.53^{\rm A} \end{array}$	${\begin{array}{c} 7181.3 \pm \\ 366.8^{\rm A} \end{array}}$	${\begin{array}{c} 55.063 \pm \\ 1.84^{\rm A} \end{array}}$	${ 39.813 \pm \atop 2.02^{\rm A} }$	$\begin{array}{c} 3.4375 \pm \\ 0.55^{\rm A} \end{array}$	${}^{1.625\pm}_{0.28^{\rm A}}$	$\begin{array}{c} 4514000 \pm \\ 188120^{\rm A} \end{array}$	${}^{231200\pm}_{20803^{AB}}$
O+	22.80%	23	${\begin{array}{c} 124.57 \pm \\ 9.815^{\rm B} \end{array}}$	${}^{128.83\pm}_{5.33^{AB}}$	$\begin{array}{c} 13.191 \pm \\ 0.34^{\rm A} \end{array}$	$\begin{array}{c} 7550.9 \pm \\ 786.8^{\rm A} \end{array}$	$\begin{array}{c} 56.565 \pm \\ 2.41^{\rm A} \end{array}$	$\begin{array}{c} 36.087 \pm \\ 2.06^{\rm AB} \end{array}$	$\begin{array}{c} 4.6957 \pm \\ 0.57^{\rm A} \end{array}$	$\begin{array}{c} 2.7391 \pm \\ 0.436^{\rm A} \end{array}$	$\begin{array}{l} 4342000 \pm \\ 209863^{\rm A} \end{array}$	$\begin{array}{c} 256304 \pm \\ 20051^{\rm A} \end{array}$
О-	2%	2	$245\pm5.0^{\rm A}$	$179.5\pm3.50^{\scriptscriptstyle A}$	${}^{14.85\pm}_{1.05^{\rm A}}$	$\begin{array}{c} 6950 \pm \\ 350^{\rm A} \end{array}$	60.5 ± 2.5	$^{\rm A}_{ m A}34.5\pm2.5^{\rm AB}$	$4.5\pm0.5^{\rm A}$	$0.5\pm0.5^{\rm A}$	$\begin{array}{c} 4945000 \pm \\ 295000^{\rm A} \end{array}$	$\begin{array}{c} 236000 \pm \\ 27000^{\rm AB} \end{array}$

Table 1. Blood group and complete blood profile of patients.

Note: A, B, C, and D showed ANOVA and LSD statistically significant within means at alpha value of $p \le 0.05$.

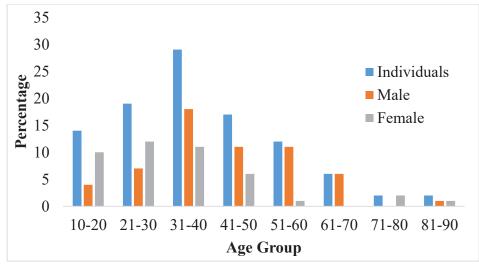


Fig. 1. Total number of individuals and gender wise ratio in different age groups.

sectional results of quantitative and functional SARS-CoV-2 antibodies. The expression of IgM and IgG antibodies varied because of some factors like severity of SARS-CoV-2 infection, and time delay from recovery period started. During this investigation the immunity against COVID-19 was observed up to more than eight to nine months after infection. Huynh *et al.* [37] investigated antibodies against COVID-19 and observed their presence up to 180 days. Bitzogli *et al.* [38] tested for anti-SARS-CoV-2 antibodies in patients survived from ICU found considerably lesser at disease inception, but they gradually lose auto antibodies by onset of pathogens. Antibody IgM and IgG were tested to

check the long lasting and chance of re-infection. It was observed that the patients recovered from severe corona infection have higher immunity and presence of antibodies for long time period in their body. Elslande *et al.* [39] have emphasized that after eight months of infection, antibodies stay more commonly positive in those patients who suffered from severe infection contrasted to the patients gone through mild symptoms during their infectious phase. Ibarrondo *et al.* [40] underlined that severity in infection emphasize the importance of response triggered by immune system, with drastically advanced antibody titers within people with severe Corona infection.

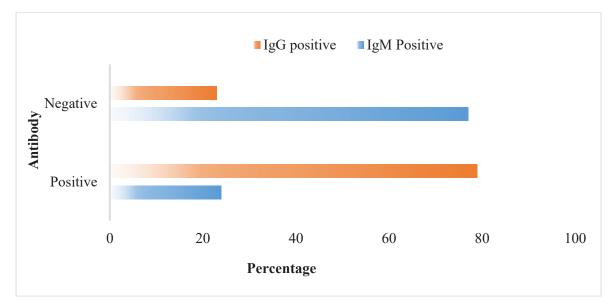


Fig. 2. Antibody status in post recovery stage of individuals.

The observed Immunoglobin G (IgG) positive individuals were 78.23% and 23.6% positive for Immunoglobin M (IgM). Long et al. [21], Adams et al. [41] Gao et al. [42] found during their studies that IgM positive rate was lower than that of IgG rate in patients after recovery Covid infection. Alzaabi et al. [43] suggested that the regular up thrust in antibody and tremendous tiers of IgG titers inside the first five months suggest that immunization is feasible, and the probabilities of re-infection is minimum. They also found that the aged individuals are likely to have higher positive IgG antibody reaction and even higher in the amount of IgG antibody levels comparatively the younger individuals. It might be the fact that the aged patients were frequently gone through more severe conditions.

3.5. Gender Differences in Infection

Male participants were observed to be more affected by COVID-19 than female individuals during this research. Total 55.5% male individuals while 44.5% females were included in this investigation. Huang et al. [18] investigated that men make up more than two third of the total confirmed cases about 73% vs. 27%. A similar study by Jin et al. [44] also reported gender differences in previous studies to affect where male individuals were at a higher infection risk than that of females. Another similar investigation supported the findings of gender difference, and reported that the number of male subjects were double of female patients of COVID-19 [45]. Furthermore, the diabetic patients were observed to be older aged, therefore having another risk that could boost their vulnerability to corona infection.

3.6. Blood Group Susceptibility

The observed prevalent blood group was A⁺ with 37.6% followed by O^+ with 22.8%, B^+ , AB^+ , O^- and B⁻. This investigation showed that the blood group A⁺ is more susceptible towards the COVID-19 infection. Zhou et al. [30] results indicated that the antigen A carrier has drastically high possibility of developing severe or critical infectious cases than that of other blood types. In current investigation A⁺ blood type was most susceptible and O⁺ was second most susceptible group while on contrary most of researches revealed that O blood type is at very low risk of infection. Zhou et al. [30] evaluated ABO blood types distributions in 2173 patients of Corona, and found that blood type O was associated with a lower risk while A was related with higher risk of infection. A similar investigation done by Solmaz and Araç [46] investigated that having blood type O might be defensive, and blood type A might have greater vulnerability towards infection, but this does not affect the way of the disease and is not linked with mortality. Wu et al. [1] found similar results in their research on 187 patients of COVID-19 and Goker et al. [47] in their study on 186 COVID-19 patients.

3.7. Symptoms during Infection Phase

The symptoms of COVID-19 may different from person to person. The most common symptoms are fatigue, fever, cough, headache, and nasal congestion, shortness of breath, nausea, and diarrhea [48]. The current study reveals that 50.5% people showed moderate symptoms, 31.7% and 13.9% individuals were observed with mild and severe symptoms respectively during

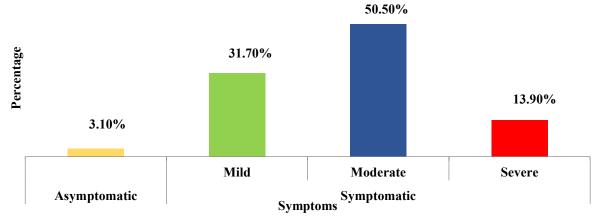


Fig. 3. Percentage of symptoms in people suffered from COVID-19.

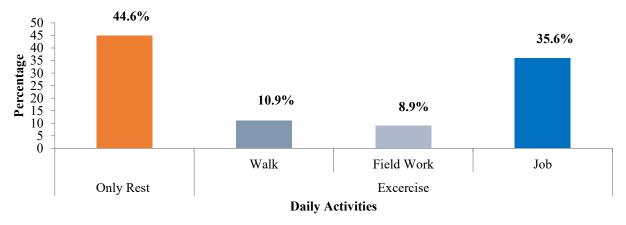


Fig. 4. Daily Life activities of patients during infection period.

infection. About 3.10% people were examined to be asymptomatic. The current study showed that infected people were gone through moderate symptoms more than the other symptoms. Very few were observed to be asymptomatic. During current research the most common observed symptoms were: fever, cough, sneezing, difficulty in breathing, chest pain, headache, nausea, body pain, flu, sour throat, muscle pain, fatigue, loss of taste and Smell. Some of symptoms were observed unusual within this targeted group of people. Those unusual symptoms were: elevated heart beats, digestion issues, abdominal pain, vomiting, shivering, anxiety and depression. Guan et al. [15] and Qin et al. [49] analyzed the symptoms and reported that all those mild or moderate symptoms could steps forward into harsh lung infection and ARDS (Acute Respiratory Distress Syndrome) that will go together with by neutrophilia, lymphopenia, and thrombocytopenia.

3.8. Daily Life Activities and Exercise

Regular exercise boosts up the immunity system and person stay healthy. In broader level the routine and moderate exercise provides protection against any disease or illness and is favourable for healthy immune system. Individuals who are habitual to exercise usually stay fit and active in daily life. An investigation by Walsh *et al.* [50] explained that routine exercise influences the immunity along with the antiviral defense mechanism of immune system. Another similar study by Vina *et al.* [51] considered exercise as one of the most important and repeatedly given treatments in diseased condition as well as in healthy conditions.

The results of the current study revealed that about 44.6 % people get proper rest during their infection phase. Whereas, remaining 55.4% people did different form of exercises of their daily life. From exercise group 35.6% individuals used to continue their jobs, 10.9% used to do their routine walk for exertion while 8.9% people did their field work properly. A study by Sallis et al. [52] analyzed the patients of Corona virus and observed minor danger and having severe outcomes in patients engaged in fewer physical activities comparatively those who were constantly inactive. Davies et al. [53] highlighted that exercises and all other types of physical activities are very important in conserving mass of muscles by making active the process of muscle protein synthesis.

3.9. Dietary Patterns during Infection

Taking balance amount of nutrients and proper diet boost up the immune system, and is essentially required in order to maintain health and appropriate functioning of cells. A healthy immune system can easily defend body against pathogens. Appropriate quantity of highly nutritious food is taken during any infection. Aman and Masood [54] reported that the optimum intake of healthy diet and dietary nutrients influence the immune system of the body. An appropriate diet can make sure that body is in good condition to overcome the viral attack. Afshin *et al.* [55] highlighted that a deprived or bad diet causes more demises worldwide than that of smoking or hypertension.

In the present study, we have investigated the types of food consumed during COVID-19 infection. Individuals are divided into two groups: Regular diet group and Special diet group. About 25% participants used their normal regular diet during their infection phase, on the other hand 75% individuals treated themselves with specific diet for their rapid and healthy recovery. Individuals among these 75% consumed protein rich food in form of meat, meat stock, chicken and fish. Secondly, most intakes were of functional foods (nuts and dry fruits) then vegetables and fresh fruits along with milk and milk products. Deutz et al. [56] investigated in their study and suggested that higher intake of protein possibly will be advantageous to aged people particularly those having any chronic disease. According to WHO [10] a healthy nutrition and water consumption are very essential. A diet in balanced proportion keeps person well and in good physical shape, boost the immune system, and decreases infections and chronic diseases risk. Diet consisting of fresh and organic foods is suggested for daily intake, in order to supply the needed nutrients for the body.

3.10. Reinfection after Vaccination and Types of Vaccines

Results of the current study revealed that more than 65% people get infected even after COVID-19 vaccination. Reinfection was also observed in recovered patients of COVID who are not vaccinated. The vaccinated individuals did not have any severe symptoms. Prado-Vivar *et al.* [57] reported reinfection in an immuno compromised patient of 46 years and a similar study by Tillet *et al.*

[58] reported reinfection in 25-year-old immunodeficient patient.

3.11. Pregnancy Effected by COVID-19

Baud *et al.* [59] reported an instance of unsuccessful labor during the second trimester of pregnancy in a lady with COVID-19, which gave off an impression of being identified with placental disease with SARS-CoV-2. During the current study, among the target group, there were some pregnant females who recovered from infection, one of them gone through miscarriage at first trimester. We also tested child for COVID-19 infection transfer from mother to baby by testing antibody's presence in child after birth as mother suffered from COVID-19 during pregnancy. The new born was not found to be symptomatic for infection. Antibody test by rapid kit was negative.

In a research, Wang *et al.* [60] reported that coronavirus was not spotted in the blood of umbilical cord. Another related study by Schwartz [61] studied pregnant women suffering from COVID infection and reported, that there is no reference of transplacental or intrauterine transmission of virus. Zeng *et al.* [62] investigated immunoglobulin M (IgM) in babies and revealed a high level of antibody IgM in their blood. A contradictory study by Farhat *et al.* [63] reported that about four neonates were positive for PCR test, as their mothers were symptomatic and doubtful for COVID-19 infection during pregnancy.

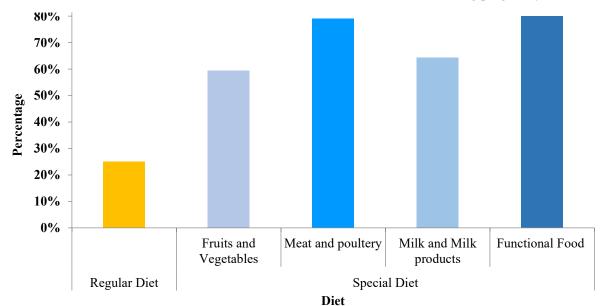


Fig. 5. Diet taken by individuals during their Infection period.

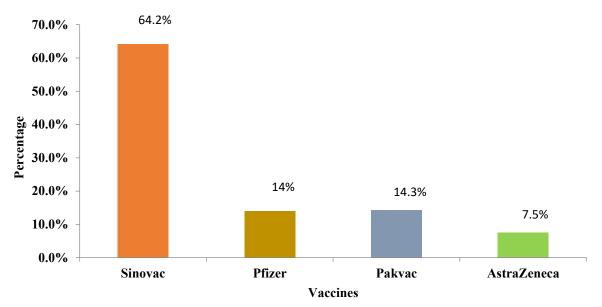


Fig. 6. Types of vaccines given to the followed-up patients.

4. CONCLUSIONS

In conclusion, the results of the present study indicate a sustained and extended positive immune response among recovered patients of COVID-19. Immunity against COVID-19 observed during this investigation was more than eight months after infection. Aged people got severe conditions but the most infected age group recorded was 31-40 years. Males were more infected than female individuals. A+ blood group was observed to be more susceptible towards infection. The high Hb levels being the inhabitants of areas having higher altitudes, may be a factor in retaining higher immunity levels. This is coupled with pure intake of traditional and organic diet and strenuous pattern of life (in terms of life style in vogue). Vaccination prevented people from severity in disease. Due to strong innate immunity of people from this region, they recovered more quickly. The co-morbidities and immuno-compromised condition along with dietary habits and regular exercise was revealed where results showed high hemoglobin levels coupled with healthy and nutrition rich diet, regular exercise. It has a positive impact on early recovery of Covid infected patients. All these factors collectively might have a positive impact on early and rapid recovery of Covid infected patients of this area.

5. RECOMMENDATIONS

According to our knowledge, there are no other studies regarding corona conducted in Gilgit Baltistan, therefore, more research is needed. Specifically, on the recurrent infection on those patients who already recovered from Covid during 1st, 2nd and 3rd wave. Multi-dimensional analysis should be performed related to Covid from this area in broader level in context of the genetic/molecular characteristics. The transfer of Corona virus to neonates needs to be further investigated. No studies suggesting an ethnic preponderance to COVID-19 infection in this area although the disease behavior has been diverse. The increased prevalence in people having blood group A is needed to be explored further in terms of the detailed body immune pre-cursors and their subsequent role portraying higher susceptibility rates.

6. CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest concerning research, authorship, of this article.

7. REFERENCES

- Y. Wu, Z. Feng, P. Li, and Q. Yu. Relationship between ABO blood group distribution and clinical characteristics in patients with COVID-19. *Clinica Chimica Acta* 509: 220-223 (2020).
- D.D. Richman, R.J. Whitley, and F.G. Hayden (Eds.). Clinical virology Fourth Edition. ASM Books, Wiley (2016).
- 3. L.V.D. Hoek. Human coronaviruses: What do they

cause? Antiviral Therapy 12: 651-658 (2007).

- F. Pene, A. Merlat, A. Vabret, F. Rozenberg, A. Buzyn, F. Dreyfus, A. Cariou, F. Freymuth, and P. Lebon. Coronavirus 229E-Related Pneumonia in Immunocompromised Patients. *Clinical Infectious Diseases* 37: 929–932 (2003).
- E.E. Walsh, J.H. Shin, and A.R. Falsey. Clinical impact of human coronaviruses 229E and OC43 infection in diverse adult populations. *The Journal* of *Infectious Diseases* 208(10): 1634-1642 (2013).
- G.J. Gorse, T.Z. O'Connor, S.L. Hall, J.N. Vitale, and K.L. Nichol. Human Coronavirus and Acute Respiratory Illness in Older Adults with Chronic Obstructive Pulmonary Disease. *The Journal of Infectious Diseases* 199: 847–857 (2009).
- 7. L.E. Gralinski, and V.D. Menachery. Return of the Coronavirus: 2019-nCoV. *Viruses* 12(2): 135 (2020).
- W. Tan, X. Zhao, X, Ma, W. Wang, P. Niu, W. Xu, and G. Wu, A novel coronavirus genome identified in a cluster of pneumonia cases Wuhan, China 2019–2020. *China CDC weekly* 2(4):61-62 (2020).
- N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F. Zhan, X. Ma, D. Wang, W. Xu, G. Wu, G. F. Gao, and W. Tan. A novel coronavirus from patients with pneumonia in China, 2019. *The New England Journal of Medicine* 382: 727-33 (2020).
- 10. WHO. Nutrition. Nutrition advice for adults during the COVID-19 outbreak (2020). Available from http://www.emro.who.int/nutrition/ nutritioninfocus/nutrition-advice-for-adults-duringthe-covid-19-outbreak.html
- 11. A. Azar (Ed.). Control Applications for Biomedical Engineering Systems. *Academic Press* (2020).
- 12. F. Brauer, and C. Castillo-Chavez (Eds.). Mathematical models in population biology and epidemiology. *Springer, New York* (2010).
- Q. Li, X. Guan, P. Wu, X. Wang, L. Zhou, Y. Tong, and Z. Feng. Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. *The New England Journal of Medicine* 382(13): 1199–1207 (2020).
- S.W.X. Ong, Y.K. Tan, P.Y. Chia, T.H. Lee, O.T. Ng, M.S.Y. Wong, and K. Marimuthu. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. JAMA 323(16): 1610-1612 (2020).
- W.J. Guan, W.H. Liang, Y. Zhao, H.R. Liang, Z.S. Chen, Y.M. Li, and J.X. He. Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. *European Respiratory Journal* 55(5): 2000547 (2020).
- W.J. Guan, Z.Y. Ni, Y. Hu, W.H. Liang, C.Q. Ou, J.X. He, and N.S. Zhong. Clinical characteristics of coronavirus disease 2019 in China. *The New England Journal of Medicine* 382(18): 1708-1720 (2020).
- C. Sohrabi, Z. Alsafi, N. O'neill, M. Khan, A. Kerwan, A. Al-Jabir, and R. Agha. World Health Organization declares global emergency: A review of the 2019

novel coronavirus (COVID-19). International Journal of Surgery 76: 71-76 (2020).

- C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xian, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wangi, and B. Coat. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395: 497–506 (2020).
- Q. Hu, H. Guan, Z Sun, L. Huang, C. Chen, T. Ai, and L. Xia. Early CT features and temporal lung changes in COVID-19 pneumonia in Wuhan, China. *European Journal of Radiology* 128: 109017 (2020).
- 20. Y.M. Arabi, S Murthy, and S. Webb. COVID-19: a novel coronavirus and a novel challenge for critical care. *Intensive Care Medicine* 46: 833-36 (2020).
- Q.X. Long, B.Z. Liu, H.J. Deng, G.C. Wu, K. Deng, Y.K. Chen, and A.L. Huang. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nature Medicine* 26(6): 845-848 (2020).
- A.Z. Ahmad, K. Shahzad, M. Masood, M. Umar, F. Abbasi and A. Hafeez. COVID-19 seroprevalence in Pakistan: a cross-sectional study. *BMJ Open* 12(4): e055381 (2022).
- F. Farah, A. Mehwish, and H.A. Nafisa. Comparative Study in the Diagnosis of Anemia by SYSMEX KX-21N hematology analyzer with Peripheral Blood Smear. *International Journal of Endorsing Health Science* 1(2): 89-92 (2013).
- 24. G. Lu, and J. Wang. Dynamic changes in routine blood parameters of a severe COVID-19 case. *Clinica Chimica Acta* 508: 98–102 (2020).
- 25. W. Liu, and H. Li. COVID-19: Attacks the 1-beta Chain of Hemoglobin and Captures the Porhyrin to Inhibit Heme Metabolism. *ChemRxiv* (2020). doi:10.26434/chemrxiv-2021-dtpv3-v12
- 26. S. Lanini, C. Montaldo, E. Nicastri, F. Vairo, C. Agrati, N. Petrosillo, and G. Ippolito COVID-19 disease—Temporal analyses of complete blood count parameters over course of illness, and relationship to patient demographics and management outcomes in survivors and non-survivors: A longitudinal descriptive cohort study. *PloS One* 15(12): e0244129 (2020).
- P. Xu, Q. Zhou, and J. Xu. Mechanism of thrombocytopenia in COVID-19 patients. *Annals of Hematology* 99(6): 1205-1208 (2020).
- G. Lippi, M. Plebani, and B.M. Henry. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis. *Clinica Chimica Acta* 506: 145-148 (2020).
- W. Guo, M. Li, Y. Dong, H. Zhou, Z. Zhang, C. Tian, and D. Hu. Diabetes is a risk factor for the progression and prognosis of COVID-19. *Diabetes/Metabolism Research and Reviews* 36(7): e3319 (2020).
- 30. F. Zhou, T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, and B. Cao. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The Lancet*

395(10229):1054-1062 (2020).

- 31. N. Zaki, H. Alashwal, and S. Ibrahim. Association of hypertension, diabetes, stroke, cancer, kidney disease, and high-cholesterol with COVID-19 disease severity and fatality: A systematic review. *Diabetes* & *Metabolic Syndrome* 14(5):1133-1142 (2020).
- S.Q. Deng, and H.J. Peng. Characteristics of and public health responses to the coronavirus disease 2019 outbreak in China. *Journal of Clinical Medicine* 9(2):575 (2020).
- E. Kocar, T. Rezen, and D. Rozman. Cholesterol, lipoproteins, and COVID-19: Basic concepts and clinical applications. *Biochimica et Biophysica Acta-Molecular and Cell Biology of Lipids* 1866(2): 158849 (2021).
- 34. L.B. Daniels, A.M. Sitapati, J. Zhang, J. Zou, Q.M. Bui, J. Ren, and K. Messer. Relation of statin use prior to admission to severity and recovery among COVID-19 inpatients. *The American Journal of Cardiology* 136: 149-155 (2020).
- 35. F.K. Ho, F. Petermann-Rocha, S.R. Gray, B.D. Jani, S.V. Katikireddi, C.J. Niedzwiedz, and J.P. Pell. Is older age associated with COVID-19 mortality in the absence of other risk factors? General population cohort study of 470,034 participants. *PLoS One* 15(11): e0241824 (2020).
- 36. E. Mahase. Covid-19: Why are age and obesity risk factors for serious disease. *BMJ* 371: m4130 (2020).
- 37. A. Huynh, D.M. Arnold, J.W. Smith, J.C. Moore, A. Zhang, Z. Chagla, B.J. Harvey, H.D. Stacey, J.C. Ang, R. Clare, N. Ivetic, V.T. Chetty, D.M.E. Bowdish, M.S. Miller, J.G. Kelton, and I. Nazy. Characteristics of anti-SARS-CoV-2 antibodies in recovered COVID-19 subjects. *Viruse* 13(4): 697 (2021).
- K. Bitzogli, E. Magira, L. Chatzis, E. Jahaj, H. Alexopoulos, M. Dalakas, and P. Vlachoyiannopoulos. AB0697 ANTI-SARS-COV-2 antibodies and autoantibodies in covid-19 patients survived after icu admission, 6 months later. *Annals of the Rheumatic Diseases* 80: 1381 (2021).
- 39. J. Elslande, M. Oyaert, S. Ailliet, M. Van Ranst, N. Lorent, Y.V. Weygaerde, and P. Vermeersch. Longitudinal follow-up of IgG anti-nucleocapsid antibodies in SARS-CoV-2 infected patients up to eight months after infection. *Journal of Clinical Virology* 136: 104765 (2021).
- F.J. Ibarrondo, J.A. Fulcher, D. Goodman-Meza, J. Elliott, C. Hofmann, M.A. Hausner and O.O. Yang. Rapid decay of anti–SARS-CoV-2 antibodies in persons with mild Covid-19. *The New England Journal of Medicine* 383(11): 1085-1087 (2021).
- 41. E.R. Adams, M. Ainsworth, R. Anand, M.I. Andersson, K. Auckland, J.K. Baillie, E. Barnes, S. Beer, J.I. Bell, T. Berry, S. Bibi, M. Carroll, S.K. Chinnakannan, R.J. Ploeg, and A. Pollard. Antibody testing for COVID-19: A report from the National COVID Scientific Advisory Panel. *Wellcome Open Research* 5(5):139 (2020).
- 42. H.X. Gao, Y.N. Li, Z.G. Xu, Y.L. Wang, H.B. Wang, J.F. Cao, and E.H. Dai. Detection of serum immunoglobulin M and immunoglobulin

G antibodies in 2019 novel coronavirus infected patients from different stages. *China Medical Journal* 133: 1479–1480 (2020).

- 43. A.H. Alzaabi, L.A. Ahmed, A.E. Rabooy A.A. Zaabi, M, Alkaabi, F. AlMahmoud, and K.A. Mazrouei. Longitudinal changes in IgG levels among COVID19 recovered patients: A prospective cohort study. *PLoS One* 16(6): e0251159 (2021).
- 44. J.M. Jin, P. Bai, W. He, F. Wu, X.F. Liu, D.M. Han, and J.K. Yang. Gender differences in patients with COVID-19: focus on severity and mortality. *Frontiers in Public Health* 8:152 (2020).
- 45. F. Mauvais-Jarvis. Aging, male sex, obesity, and metabolic inflammation create the perfect storm for COVID-19. *Diabetes* 69(9): 1857-1863 (2020).
- I. Solmaz, and S. Araç. ABO blood groups in COVID-19 patients; cross-sectional study. *International Journal of Clinical Practice* 75(4): e13927 (2021).
- 47. H. Goker, E.A. Karakulak, H. Demiroğlu, C.M.A. Ceylan, Y. Büyükaşik, A.C. Inkaya, and S. Ünal. The effects of blood group types on the risk of COVID-19 infection and its clinical outcome. *Turkish Journal* of Medical Sciences 50(4): 679 (2020).
- T.P. Velavan, and C.G Meyer. The COVID-19 epidemic. *Tropical Medicine & International Health* 25(3):278 (2020).
- C. Qin, L. Zhou, Z. Hu, S. Zhang, S. Yang, Y. Tao, C. Xie, K. Ma, K. Shang, W. Wang, and D.S. Tian. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clinical Infectious Diseases* 71(15): 762-768 (2020).
- N.P. Walsh, M. Gleeson, R.J. Shephard, M. Gleeson, J.A. Woods, N. Bishop, and P. Simon. Position statement. part one: Immune function and exercise. *Exercise Immunology Review* 17: 6-63 (2011).
- 51. J. Vina, F. Sanchis-Gomar, V. Martinez-Bello, and M.C. Gomez-Cabrera. Exercise acts as a drug; the pharmacological benefits of exercise. *British Journal of Pharmacology* 167(1): 1-12 (2012).
- 52. R. Sallis, D.R. Young, S.Y. Tartof, J.F. Sallis, J. Sall, Q. Li, and D.A. Cohen. Physical inactivity is associated with a higher risk for severe COVID-19 outcomes: a study in 48440 adult patients. *British Journal of Sports Medicine* 55(19): 1099-1105 (2021).
- K.A.B. Davies, S. Pickles, V.S. Sprung, G.J. Kemp, U.Alam, D.R. Moore, and D.J. Cuthbertson. Reduced physical activity in young and older adults: metabolic and musculoskeletal implications. *Therapeutic Advances in Endocrinology and Metabolism* 10: 1-15 (2019).
- F. Aman, and S. Masood. How Nutrition can help to fight against COVID-19 Pandemic. *Pakistan Journal of Medical Sciences* 36(COVID19-S4): S121-23 (2020).
- 55. A. Afshin, P.J. Sur, K.A. Fay, L. Cornaby, G. Ferrara, J.S. Salama, and C.J. Murray. Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* 393(10184): 1958-1972 (2019).

- 56. N.E. Deutz, J.M. Bauer, R. Barazzoni, G. Biolo, Y. Boirie, A. Bosy-Westphal, and P.C. Calder. Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN Expert Group. *Clinical Nutrition* 33(6): 929-936 (2014).
- 57. B. Prado-Vivar, M. Becerra-Wong, J.J. Guadalupe, S. Marquez, B. Gutierrez, P. Rojas-Silva, and P. Cardenas. COVID-19 Re-Infection by a Phylogenetically Distinct SARS-CoV-2 Variant, First Confirmed Event in South America. SSRN (2020). https://doi.org/10.2139/ssrn.3686174.
- R.L. Tillett, J.R. Sevinsky, P.D. Hartley, H. Kerwin, N. Crawford, A. Gorzalski, and M. Pandori. Genomic evidence for reinfection with SARS-CoV-2: a case study. *The Lancet Infectious Diseases* 21(1): 52-58 (2021).
- 59. D. Baud, G. Greub, G. Favre, C. Gengler, K. Jaton, E. Dubruc, and L. Pomar. Second-trimester miscarriage in a pregnant woman with SARS-

CoV-2 infection. JAMA 323(21): 2198-2200 (2020).

- X. Wang, Z. Zhou, J. Zhang, F. Zhu, Y. Tang, and X. Shen. A case of 2019 Novel Coronavirus in a pregnant woman with preterm delivery. *Clinical Infectious Diseases* 71(15): 844–6 (2020).
- 61. D.A. Schwartz. An analysis of 38 pregnant women with COVID-19, their newborn infants, and maternal-fetal transmission of SARS-CoV-2: maternal coronavirus infections and pregnancy outcomes. *Archives of Pathology & Laboratory Medicine* 144(7): 799-805 (2020).
- H. Zeng, C. Xu, J. Fan, Y. Tang, Q. Deng, W. Zhang, and X. Long. Antibodies in infants born to mothers with COVID-19 pneumonia. *JAMA* 323(18): 1848-49 (2020).
- A.S. Farhat, S.J. Sayedi, F. Akhlaghi, A. Hamedi, and A. Ghodsi. Coronavirus (COVID-19) infection in newborns. *International Journal of Pediatrics* 8(6): 11513-11517 (2020).