
بسم الله الرحمن الرحيم

IMMUNE RESPONSE TO SARS-COV-2 IN HEALTHCARE WORKERS

By

ASAD ABBAS

Armed Forces Post Graduate Medical Institute, Rawalpindi

National University of Medical Sciences, Rawalpindi

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IMMUNE RESPONSE TO SARS-COV-2 IN HEALTHCARE WORKERS

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ASAD ABBAS

Armed Forces Post Graduate Medical Institute, Rawalpindi

National University of Medical Sciences, Rawalpindi

TO: THE BOARD OF UNDERGRADUATE STUDENTS

*The members of the committee appointed to examine the research thesis of **Mr. ASAD ABBAS** finds it satisfactory and recommend that it be accepted for presentation*

Clinical Supervisor

LT. COL SAIF ULLAH KHAN NIAZI

Consultant Virologist

Armed Forces Institute of Pathology, Rawalpindi

Co-Supervisor

MAJ. MISBAH NOOR

Consultant Virologist

Armed Forces Institute of Pathology, Rawalpindi

Statistical supervisor

MISS MARYAM ABBAS

Assistant Research officer

Armed Forces Post Graduate Medical Institute, Rawalpindi

COMPLETION OF CERTIFICATE

This is certified that **Mr. ASAD ABBAS,** student of B.Sc. (Hons) final year, Medical Laboratory Technology has completed his research work contained in this thesis titled **''IMMUNE RESPONSE TO SARS-COV-2 IN HEALTHCARE WORKERS''** at Armed Forces Institute of Pathology (AFIP), Rawalpindi under my supervision.

Clinical supervisor_____

LT. COL SAIF ULLAH KHAN NIAZI

Consultant Virologist

Armed Forces Institute of Pathology, Rawalpindi

Co-Supervisor_____

MAJ. MISBAH NOOR

Consultant Virologist

Armed Forces Institute of Pathology, Rawalpindi

Statistical supervisor_____

MISS MARYAM ABBAS

Research officer

Armed Forces Post Graduate Medical Institute, Rawalpindi

DEDICATION

I would like to dedicate this thesis first of all to my **ALLAH ALMIGHTY**, with endless gratitude, for the guidance, strength, power of mind, skills & healthy life has given me.

I also dedicate this thesis to my **Parents, Brothers, Respected Teachers & Close Friends Circle** who have been my source of inspiration & give me strength when I thought of giving up, who continuously provided their moral, spiritual & support, love, prayers and encouragement has made my voyage contented and smooth.

DECLARATION

This is to certify that Thesis entitled, **''IMMUNE RESPONSE TO SARS-COV-2 IN HEALTHCARE WORKERS''** is my work produced under supervision towards the awards of B.Sc. (Hons) Degree in Allied Health Sciences (Medical Lab Technology) and that, to the best of my knowledge, it has not been published in part or whole by anyone for degree elsewhere. All references herein have been duly acknowledged.

ASAD ABBAS

ABSTRACT

Background

A respiratory illness emerged in Wuhan City, Hubei Province, China in early December 2019 and spread worldwide. The China Health Authority (CHA) alerted the World Health Organization (WHO) to several cases of pneumonia of unknown etiology on 31st December 2019. The WHO declared the outbreak as a Public Health Emergency of International Concern (PHEIC) on 30th January 2020 and finally declared a pandemic on 10th March 2020. A novel coronavirus was identified as the clinical cause of the sickness generally named Coronavirus Disease 2019 (COVID-19) and may manifest either as an asymptomatic infection, a mild upper respiratory tract infection, or severe viral pneumonia with respiratory failure and death.

Objective

To determine the overall SARS-CoV-2 antibody prevalence during the outbreak of COVID-19 in HCWs at AFIP Rawalpindi.

Study Design & duration

Descriptive cross-sectional study between Jun to Dec 2020.

Study Site & Setting

The study was conducted in the Department of Virology, Armed Forces Institute of Pathology, Rawalpindi, and a training institute affiliated with Armed Forces Post Graduate Medical Institute (AFPGMI), Rawalpindi.

Material and Methods

A descriptive cross-sectional study was conducted at the Department of Virology AFIP, Rawalpindi. The sample was taken from 191 HCWs, 160 males and 31 females between Jun to Dec 2020. Detection of SARS-CoV-2 IgG antibodies was carried out by CLIAs based auto analyzers.

Results

There were 78 (40.84%) seropositive samples out of 191 HCWs. Among 78 seropositive samples, 67 were males and 11 females. The overall detection of SARS-CoV-2 IgG antibodies in males and females HCWs was 85.90% and 14.10% respectively.

Conclusion

This study showed that the SARS-CoV-2 IgG antibodies were predominance among the males that is 85.90%. This shows more male HCWs were exposed or infected due to COVID-19 disease. Prevention from COVID-19 depends on strictly following Standard Operating Procedure (SOP) related to precautionary measures of COVID-19 and by using proper Personal Protective Equipment (PPE).

Key Words

- COVID-19
- Outbreak
- Corona Virus
- SARS-CoV-2
- Healthcare Workers
- IgG Antibody
- SOPs
- ChemiLuminescence ImmunoAssays

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

SARS-CoV-2	Severe Acute Respiratory Syndrome Corona Virus 2
COVID-19	Corona Virus Disease 2019
HCWs	Healthcare Workers
IgG	Immunoglobulin G
RNA	Ribonucleic Acid
SsRNA	Single Stranded Ribonucleic Acid
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization
CHA	China Health Authority
PHEIC	Public Health Emergency of International Concern
SOB	Shortness of Breath
AFIP	Armed Forces Institute of Pathology
MERS	Middle East Respiratory Syndrome
SARS	Severe Acute Respiratory Syndrome
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
SOP	Standard Operating Procedure
RLU	Relative Light Units

CHAPTER - 1

INTRODUCTION

A respiratory illness emerged in Wuhan City, Hubei Province, China in early December 2019 and spread worldwide. The China Health Authority (CHA) alerted the World Health Organization (WHO) to several cases of pneumonia of unknown etiology on 31st December 2019 [1]. The WHO declared the outbreak as a Public Health Emergency of International Concern (PHEIC) on 30th January 2020 and finally declared a pandemic on 10th March 2020. A novel coronavirus was identified as the clinical cause of the sickness generally named Coronavirus Disease 2019 (COVID-19) and may manifest either as an asymptomatic infection, a mild upper respiratory tract infection, or severe viral pneumonia with respiratory failure and death [1].

Table 1.1: Clinical features of patients with a varying degree of disease [2]

Mild Disease	Severe Disease	Critical Disease
Dry Cough	Fever	Respiratory failure
Fever	Tachypnea	Fever
Sour Throat	Dyspnea	Decreases blood oxygen saturation
With or without nasal congestion	-	Septic shock
Generalized body aches	-	Multiple organ failure
Headache	-	-
Malaise and fatigue	-	-

COVID-19 outbreaks cause significant mortality and morbidity. The signs and symptoms at illness onset include fever, cough, fatigue, anorexia, shortness of breath (SOB), and myalgia. Age and several co-morbidities (diabetes, cardiovascular or respiratory chronic diseases) are strong risk factors for severe illness, complications, and death. Transmission occurs mostly from person-to-person via respiratory droplets among close contacts [3].

Coronaviruses are a large family of viruses that are known to cause illnesses ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the strain of coronavirus that causes the COVID-19. This is a new coronavirus that has not been previously identified in humans [4].

The COVID-19 has triggered an unexpected and exceptional global crisis, which has rapidly overwhelmed the responsive capacity of the entire system of health care, thus also including laboratory diagnostics [5, 6]. Since this virus is new and any approved drug for the treatment of disease is not available but the vaccine is available, there is an urgent need for highly specific and sensitive diagnostic measures to identify infected people and isolate them to avoid the further spread of the virus [7].

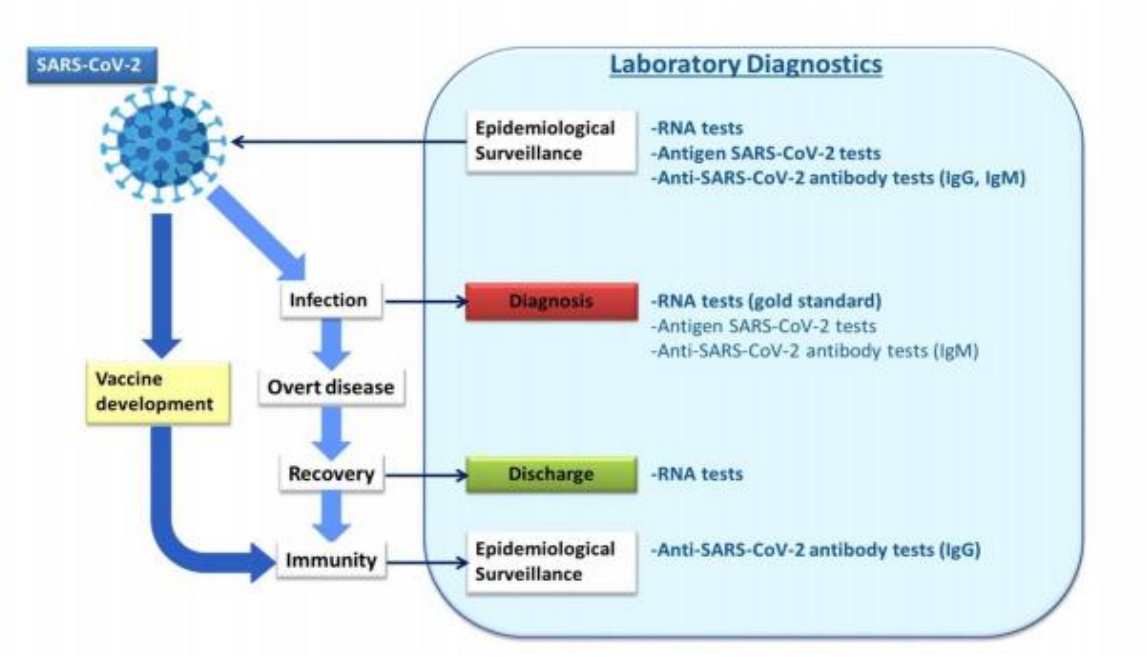

Figure 1: Testing in the context of COVID-19 disease [8]

SARS-CoV-2 infection can be detected in the laboratory by three methods:

i. Molecular test
ii. Antigen test
iii. Antibody test

Detection of the viral nucleic acid-based on a reverse transcriptase-polymerase chain reaction (RT-PCR), in samples from the upper and lower respiratory tract, is the most reliable laboratory diagnosis. Viral RNA shedding is greatest at the time of symptom onset and declines throughout infection. The detection of RNA during the period of recovery does not necessarily indicate the presence of the viable infectious virus. The sample type and collection procedure as well as the method of extraction may impact the recovery of viral RNA and lead to false-negative results.

In antigen tests, different proteins on the surface of the virus are detected and generally available in a rapid test form that could be used at the point of care.

Antibody tests detect antibodies against the viral proteins. Several relevant uses of serological tests have been pointed out: as an aid in the diagnosis of patients with several days of evolution, or in suspected cases with repeatedly negative RNA results,

in epidemiological serosurveys to determine the precise rate of infection, in the identification of individuals who could serve as donors for plasma immunotherapy strategies, to determine the immune status of individuals, especially in Healthcare Workers (HCWs) to limit their risk of exposure or inadvertent spread of the virus. The spike protein and the nucleoprotein have been suggested as the main targets for the measurement of antibody responses. The tests that detect two antibody types at the same time (IgG and IgM) are superior to the ones testing for only one antibody. Automatic analyzers like ARCHITECT i2000 SR, and Cobas e 411, etc. are being used for antibody tests in laboratories.

Table 1: Predicted indications on a person's COVID-19 status from testing different targets [2, 8]

COVID-19 phase (Duration)	RNA test	Antigen test	IgM test	IgG test
Before infection	Negative	Negative	Negative	Negative
The first phase of infection (0-4 days)	Positive	Positive later than RNA test	Negative	Negative
The second phase of infection (5-8 days)	Positive	Positive	Positive	Negative
Last phase of infection (9-13 days)	Positive	Positive	Positive	Positive
After infection (14 days to onwards)	Negative	Negative	Positive and later negative	Positive

Amid the COVID-19 crisis, HCWs are at an exceptionally high risk of infection as they work on the frontline of the pandemic [9]. To ensure the health and safety of HCWs certain timely preventive measures along with Standard Operating Procedures (SOPs)

were taken during the outbreak at the Armed Forces Institute of Pathology (AFIP), Rawalpindi. We planned to investigate the progression of the immune response to SARS-COV-2 after the outbreak of COVID-19 in HCWs at AFIP, Rawalpindi and we tested serum samples to examine the presence of antibodies (IgG) against COVID-19.

1.1 Objectives

The main aim was to determine the overall SARS-CoV-2 antibody prevalence during the outbreak of COVID-19 in HCWs at AFIP, Rawalpindi. We determined the serologic response of SARS-CoV-2 in HCWs using fully automated analyzers based on ChemiLuminescence ImmunoAssays (CLIAs).

1.2 Hypothesis

The standard operating procedures (SOPs) and protective measures prevent the transmission of SARS-CoV-2 resulting in a fewer number of infections in HCWs during the pandemic.

CHAPTER - 2

REVIEW OF LITERATURE

2.1 Virology

2.1.1 Origin, Classification, and Genome

SARS-CoV-2 is a member of the family Coronaviridae and order Nidovirales. The family consists of two subfamilies, Coronavirinae and Torovirinae, and members of the subfamily Coronavirinae are subdivided into four genera:

(i) Alpha coronavirus contains the human coronavirus (HCoV)-229E and HCoV-NL63
(ii) Beta coronavirus includes HCoV-OC43, Severe Acute Respiratory Syndrome human Coronavirus (SARS-HCoV), HCoV-HKU1, and Middle Eastern Respiratory syndrome coronavirus (MERS-CoV)
(iii) Gamma coronavirus includes viruses of whales and birds
(iv) Delta coronavirus includes viruses isolated from pigs and birds [10].

SARS-CoV-2 belongs to Beta coronavirus together with two highly pathogenic viruses, SARS-CoV and MERS-CoV. SARS-CoV-2 is an enveloped and positive-sense single-stranded RNA (+ssRNA) virus [11]. Phylogenetic analysis of the SARS-CoV-2 genome indicates that the virus is closely related (with 88% identity) to two bat-derived SARS-like coronaviruses collected in 2018 in eastern China (bat-SL-CoVZC45 and bat-SL-CoVZXC21) and genetically distinct from SARS-CoV (with about 79% similarity) and MERS-CoV [12]. The studies suggest that bats might be the original host of this virus [13, 14]. However, a study is needed to elucidate whether any intermediate hosts have facilitated the transmission of the virus to humans. Bats are unlikely to be the animal that is directly responsible for the transmission of the virus to humans for several reasons [14]:

(i) There were various non-aquatic animals (including mammals) available for purchase in Huanan Seafood Wholesale Market but no bats were sold or found.
(ii) SARS-CoV-2 and its close relatives, bat SL-CoVZC45 and bat-SL-CoVZXC21, have a relatively long branch (sequence identity of less than 90%), suggesting those viruses are not direct ancestors of SARS-CoV-2.

(iii) In other coronaviruses where the bat is the natural reservoir such as SARS-CoV and MERS-CoV, other animals have acted as the intermediate host (civets and possibly camels, respectively).

Nevertheless, bats do not always need an intermediary host to transmit viruses to humans. For example, the Nipah virus in Bangladesh is transmitted through bats shedding into raw date palm sap [15].

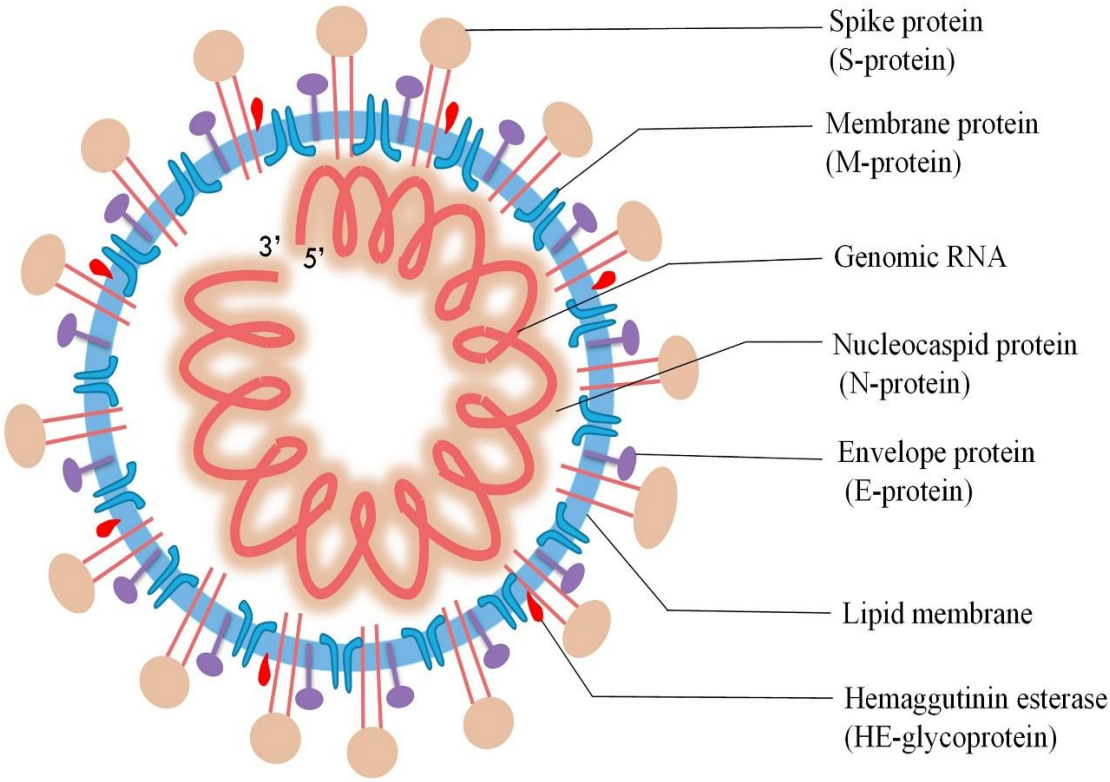

Figure 2.1: Schematic of the structure of SARS-CoV-2 [34].

Diagram showing the single-stranded RNA genome and proteins present in coronavirus.

Coronaviruses share structural similarities and are composed of 16 nonstructural proteins and 4 structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N) [16].

2.1.2 Physicochemical Properties

The virus particle has a diameter of 60~100 nm and appears round or oval [17]. Most of the knowledge about the physicochemical properties of CoVs comes from SARS-CoV and MERS-CoV. SARS-CoV-2 can be inactivated by UV or heated at 56 °C 30

min, and also sensitive to most disinfectants such as diethyl ether, 75% ethanol, chlorine, peracetic acid, and chloroform [17]. It has been reported that SARS-CoV-2 was more stable on plastic and stainless steel than on copper and cardboard, and a viable virus was detected up to 72 hr after application to these surfaces. The half-life of SARS-CoV-2 was longer than that of SARS-CoV and the longest viability of both viruses was on stainless steel and plastic [21].

2.1.3 Receptor Interactions and Cell Entry

Human angiotensin-converting enzyme 2 (ACE2) is a functional receptor used by SARS-CoV-2 for cell entry, similar to SARS-CoV [19]. ACE2 is a type I membrane protein expressed in the lung, heart, kidney, and intestine mainly associated with cardiovascular diseases. The full-length ACE2 consists of an N-terminal peptidase domain (PD) and a C terminal Collectrin-like domain (CLD) that ends with a single transmembrane helix and an ~40-residue intracellular segment. In addition to cleavage of angiotensin (Ang) I to produce Ang-(1-9), ACE2 also provides a direct binding site for the S proteins of CoVs [20]. The S protein of CoVs exists in a metastable pre-fusion conformation that undergoes a dramatic structural rearrangement to fuse the viral membrane with the host cell membrane [21]. This process is triggered by the S1 subunit and a host–cell receptor binding, which destabilizes the pre-fusion trimmer, resulting in the S1 subunit shedding and the S2 subunit transition to a highly stable post-fusion conformation [21]. To engage a host–cell receptor, the receptor-binding domain (RBD) of S1 undergoes hinge-like conformational movements that transiently hide or expose the determinants of receptor binding [22]. To figure out the potential of SARS-CoV-2 to infect humans, the receptor-binding domain (RBD) of its S protein, which is in contact with ACE2, was analyzed. The biophysical and structural evidence suggests that SARS-CoV-2 S protein likely binds to human ACE2 with a 10–20 fold higher affinity than SARS-CoV [22]. Another structural evidence suggests that the ACE2-B0AT1 complex can bind two S proteins simultaneously [23].

2.2 Pathogenesis

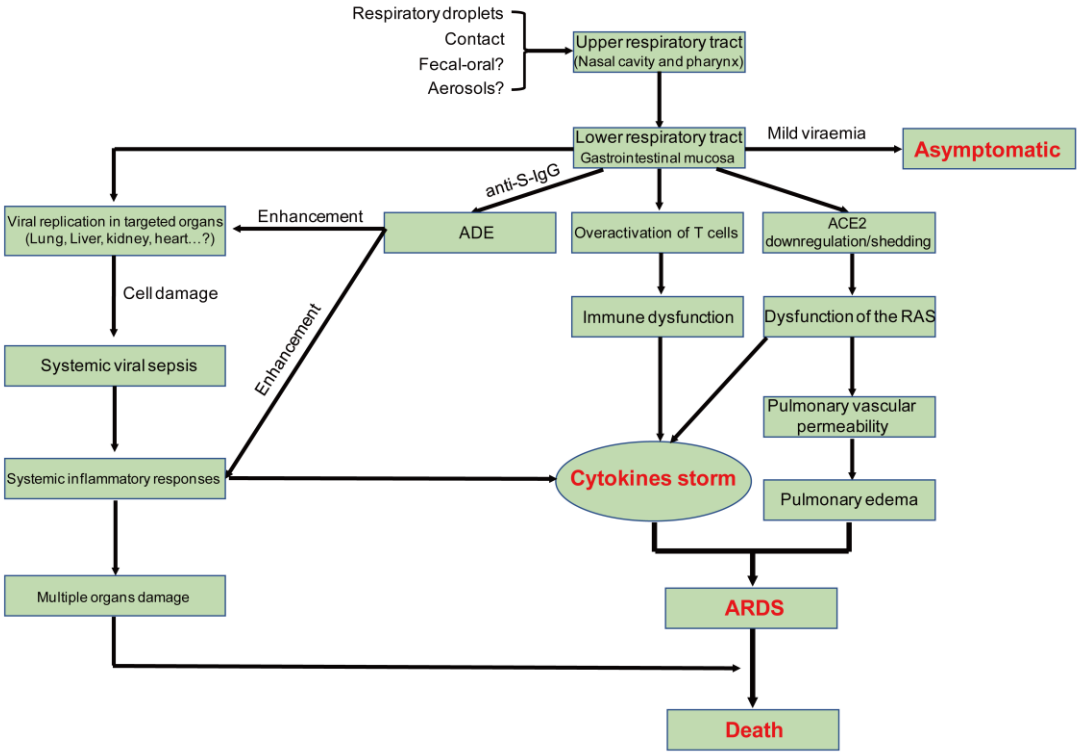

Figure 2.2: Postulated pathogenesis of SARS-CoV-2 infection. Antibody-dependent enhancement (ADE); ACE2: angiotensin-converting enzyme 2; RAS: renin-angiotensin system; ARDS: acute respiratory distress syndrome [24].

2.2.1 Virus Entry and Spread

SARS-CoV-2 is transmitted predominantly via respiratory droplets, contact and indirect contact from surfaces, etc. Primary viral replication is presumed to occur in the mucosal epithelium of the upper respiratory tract (nasal cavity and pharynx), with further multiplication in the lower respiratory tract, giving rise to a mild viremia. Few infections are controlled at this point and remain asymptomatic. Some patients have also exhibited non-respiratory symptoms such as acute liver and heart injury, kidney failure, diarrhea, implying multiple organ involvement. ACE2 is broadly expressed in the nasal mucosa, bronchus, lung, heart, esophagus, kidney, stomach, bladder, and ileum, and these human organs are all vulnerable to SARS-CoV-2. Recently, potential

pathogenicity of the SARS-CoV-2 to testicular tissues has also been proposed by clinicians, implying fertility concerns in young patients [24]. The postulated pathogenesis of SARS-CoV-2 infection is graphed in Figure 2.2 [24].

2.2.2 Pathological Findings

The first report of pathological findings from a severe COVID-19 showed pulmonary bilateral diffuse alveolar damage with cellular fibromyxoid exudates. The right lung showed evident desquamation of pneumocytes and hyaline membrane formation, indicating acute respiratory distress syndrome. The left lung tissue displayed pulmonary edema with hyaline membrane formation, suggestive of early-phase acute respiratory distress syndrome (ARDS). Interstitial mononuclear inflammatory infiltrates, dominated by lymphocytes, could be observed in both lungs. Multinucleated syncytial cells with atypical enlarged pneumocytes characterized by large nuclei, amphophilic granular cytoplasm, and prominent nucleoli were identified in the intra-alveolar spaces, indicating viral cytopathic-like changes. These pulmonary pathological findings extremely resemble those seen in SARS and MERS. Moderate microvascular steatosis and mild lobular and portal activity were observed in liver biopsy specimens, which might be caused by either SARS-CoV-2 infection or drug use. Besides, only a few interstitial mononuclear inflammatories infiltrate were found in the heart tissue, which means that SARS-CoV-2 might not directly impair the heart. Massive mucus secretion in both lungs was found in death cases with COVID-19, which was different from SARS and MERS [24].

2.2.3. Acute Respiratory Distress Syndrome (ARDS)

ARDS is a life-threatening lung condition that prevents enough oxygen from getting to the lungs and into the circulation, accounting for mortality of most respiratory disorders and acute lung injury. In fatal cases of human SARS-CoV, MERS-CoV, and SARS-CoV-2 infections, individuals exhibit severe respiratory distress requiring mechanical ventilation, and the histopathology findings also support ARDS. Previous studies have found that genetic susceptibility and inflammatory cytokines were closely related to the occurrence of ARDS. More than 40 candidate genes including ACE2, interleukin 10 (IL-10), tumor necrosis factor (TNF), and vascular endothelial growth factor (VEGF) among others have been considered to be associated with the development or outcome

of ARDS. Increased levels of plasma IL-6 and IL-8 were also demonstrated to be related to adverse outcomes of ARDS. The above biomarkers suggest both a molecular explanation for the severe ARDS and a possible treatment for ARDS followingSARS-CoV-2 infection [25, 26].

2.2.4 Cytokine Storm

Clinical findings showed exuberant inflammatory responses during SARS-CoV-2 infection, further resulting in uncontrolled pulmonary inflammation, likely a leading cause of case fatality. Rapid viral replication and cellular damage, virus-induced ACE2 down-regulation and shedding, and antibody-dependent enhancement (ADE) are responsible for aggressive inflammation caused by SARS-CoV-2, as concluded in a recently published review article [26]. SARS-CoV-2 hijacks the same entry receptor, ACE2, as SARS-CoV for infection, suggesting the likelihood of the same population of cells being targeted and infected [27]. The initial onset of rapid viral replication may cause massive epithelial and endothelial cell death and vascular leakage, triggering the production of exuberant pro-inflammatory cytokines and chemokines. Loss of pulmonary ACE2 function has been proposed to be related to acute lung injury because ACE2 down-regulation and shedding can lead to dysfunction of the renin-angiotensin system (RAS), and further enhance inflammation and cause vascular permeability. For SARS-CoV, one confusing issue is that only a few patients, particularly those who produce neutralizing antibodies early, experience persistent inflammation, ARDS, and even sudden death, while most patients survive the inflammatory responses and clear the virus [26]. The above phenomenon also exists in the SARS-CoV-2 infection. A possible underlying mechanism of antibody-dependent enhancement (ADE) has been proposed recently [26]. ADE, a well-known virology phenomenon, has been confirmed in multiple viral infections. ADE can promote viral cellular uptake of the infectious virus–antibody complexes following their interaction with Fc receptors (FcR), FcγR, or other receptors, resulting in enhanced infection of target cells. The interaction of FcγR with the virus-anti-*S* protein-neutralizing antibodies (anti-*S*-IgG) complex may facilitate both inflammatory responses and persistent viral replication in the lungs of patients [27, 28].

2.2.5 Immune Dysfunction

Peripheral CD4 and CD8 T cells showed reduction and hyper-activation in a severe patient. High concentrations of pro-inflammatory CD4 T cells and cytotoxic granules CD8 T cells were also determined, suggesting antiviral immune responses and over-activation of T cells. Additionally, several studies have reported that lymphopenia is a common feature of COVID-19, suggestive of a critical factor accounting for severity and mortality [29].

2.3 Prevalence

Serological surveys have been conducted to establish the prevalence of COVID-19 antibodies in various cohorts and communities, reporting a wide range of outcomes. The prevalence of such antibodies among HCWs, presumed at higher risk for infection, has been increasingly investigated, more studies are needed to better understand the risks and infection transmission in different healthcare settings.

The study on seroprevalence was conducted on HCWs at a regional hospital system in Orange County, California, during May and June 2020. Study subjects were recruited from the entire hospital employee workforce and the independent medical staff. Data were collected for job duties and locations, COVID-19 symptoms, a PCR test history, travel record since January 2020, and the existence of household contacts with COVID-19. A blood sample was collected from each subject for serum analysis for IgG antibodies to SARS-CoV-2. Of 2,992 tested individuals, a total of 2,924 with complete data were included in the analysis. The observed prevalence of 1.06% (31 antibody-positive cases), the adjusted prevalence of 1.13% for test sensitivity and specificity were identified. Significant group differences between positive vs. negative were observed for age ($z = 2.65$, $p = .008$), race ($p = .037$), presence of fever ($p < .001$), and loss of smell ($p < .001$), but not for occupations ($p = .710$) [30].

A study was conducted for seroprevalence against SARS-CoV-2 in a random sample of HCW from a large hospital in Spain. Of the 578 participants recruited from 28 March to 9 April 2020, 54 (9.3%, 95% CI: 7.1–12.0) were seropositive for IgG against SARS-CoV-2 [31].

Between 15 to 23 April 2020, a study was conducted in Denmark, they screened 29,295 HCWs for SARS-CoV-2 immune response, of whom 28,792 (98·28%) provided their test results. They identified 1163 (4·04% [95% CI 3·82–4·27]) seropositive HCWs. Frontline HCWs working in hospitals had a significantly higher seroprevalence (779 [4·55%] of 16 356) than HCWs in other settings (384 [3·29%] of 11 657; RR 1·38 [1·22–1·56]; p<0·001). [32]

Another study followed 166 HCWs of the University Perinatal Care Center, Regensburg, Germany approximately 12 weeks after a COVID-19 outbreak. 27 of the subjects had previously tested positive for the presence of SARS-CoV-2 by PCR testing and developed COVID-19. Serologic responses were tested with two independent commercially available test kits. 77.8 % of COVID-19 study subjects developed a specific IgG response [36].

2.4 Immune response to the virus and its role in protection

Covid-19 leads to an antibody response to a range of viral proteins, but the spike (S) protein and nucleocapsid are those most often used in serological diagnosis. Few antibodies are detectable in the first four days of illness, but patients progressively develop them, with most achieving a detectable response after four weeks. A wide range of virus-neutralizing antibodies have been reported, and emerging evidence suggests that these may correlate with severity but wane over time. The duration and protectivity of antibody and T cell responses remain to be defined through studies with longer follow-up. CD-4 T cell responses to endemic human coronaviruses appear to manifest cross-reactivity with SARS-CoV-2, but their role in protection remains unclear [26].

CHAPTER 3

MATERIAL AND METHODS

3.1 Study Design & Settings

Descriptive cross-sectional study design.

Sampling Technique

The non-probability consecutive sampling technique.

Study Duration

The duration was between Jun to Dec 2020.

Study Site & Setting

This study was carried out at the Virology Department of Armed Force Institute of Pathology, Rawalpindi. AFIP is the training institute of Armed Forces Post Graduate Medical Institute (AFPGMI) Rawalpindi.

Selection Criteria of Study

I. Inclusion Criteria

All the HCWs at AFIP were included in this study.

II. Exclusion Criteria

All the staff members other than HCWs of AFIP, Rawalpindi were excluded from this study.

Study Population

HCWs of AFIP serving during the pandemic crisis of COVID-19.

Sample Size

A total of 191 serum samples were tested during the study period.

3.2 SARS-CoV-2 testing

The SARS-CoV-2 IgG assay is designed to detect immunoglobulin class G (IgG) antibodies to the nucleocapsid protein of SARS-CoV-2 in serum and plasma from individuals who are suspected of COVID-19 and others which may have been infected by SARS-CoV-2. Antibody response to SARS-CoV-2 was now evaluated by using fully automated SARS-CoV-2 Antibody immunoassay analyzers based on ChemiLuminescence ImmunoAssays (CLIAs). Tests were performed according to the manufacturer's protocol.

ARCHITECT i2000 SR

The ARCHITECT i2000 SR analyzer is fully automated and its performed assay is a two-step immunoassay for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma using Chemiluminescent ImmunoAssay (CLIA) technology. The presence or absence of IgG antibodies to SARS-CoV-2 in the sample is determined by comparing the chemiluminescent RLU in the reaction to the calibrator RLU, which is calculated by the system as an Index (S/C). According to the manufacturer's recommendations, all the results with < 1.40 were considered as negative and all the results with ≥ 1.40 were considered as positive.

Cobas e 411

The Cobas e 411 analyzer is a fully automated analyzer that uses a patented ElectroChemiLuminescence (ECL) technology for immunoassay analysis. It is designed for both quantitative and qualitative in vitro assay determinations for a broad range of applications. According to the manufacturer's recommendations, all the results with cutoff Index (COI) < 1.0 were considered as non-reactive or negative and all the results with COI ≥ 1.0 were considered as reactive or positive.

Ethical Consideration

Institutional consent was taken from the institutional ethical committee, AFIP, Rawalpindi. Results were included to keep confidential & strictly for academic purposes. Clinical specimens were given specific identification numbers to maintain anonymity.

HCWs Consent

Data was taken under the legal consent of the HCWs.

3.3 Statistical Analysis

Data were analyzed using Statistical Packager for Social Sciences (SPSS) version 26.0. The data were analyzed using descriptive statistics, frequencies, and percentages.

CHAPTER 4

RESULTS

After the outbreak of the COVID-19 pandemic, at AFIP, serologic testing of HCWs was performed to detect the immune response to SARS-CoV-2 using fully automated analyzers based on CLIAs. A total of 191 HCWs were the participants of the study. Antibody testing identified 78 (40.84%) samples positive while the other 113 (59.16%) were negative.

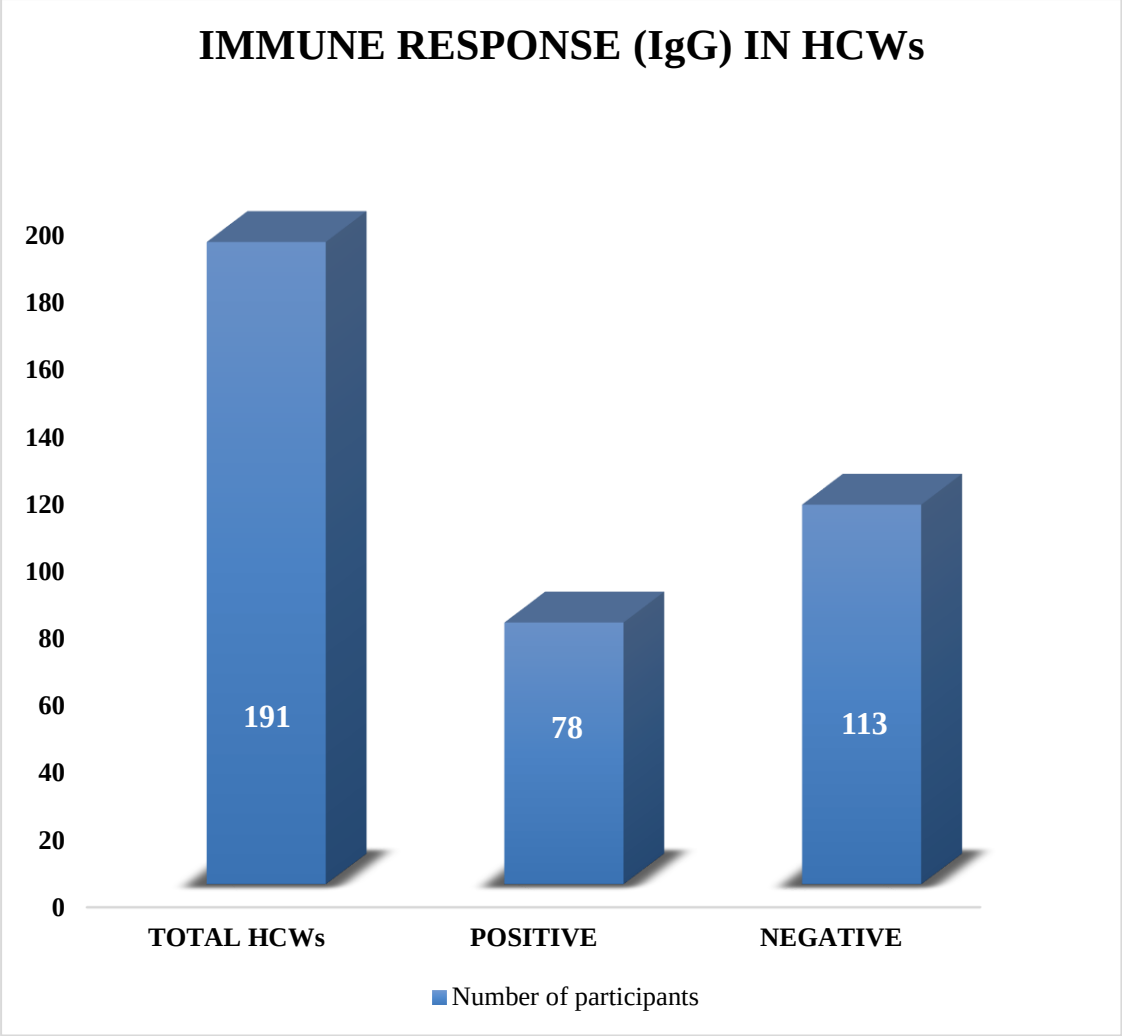

Figure 4.1: The graphical distribution of immune response (IgG) to SARS-CoV-2 in HCWs.

Interpretation

The above graphical representation of results is indicating that a total of 191 HCWs were part of the study and 78 (40.84%) HCWs have developed the immune response to the virus and were found IgG positive against the virus. And 113 (59.16%) HCWs were found as IgG negative against the virus.

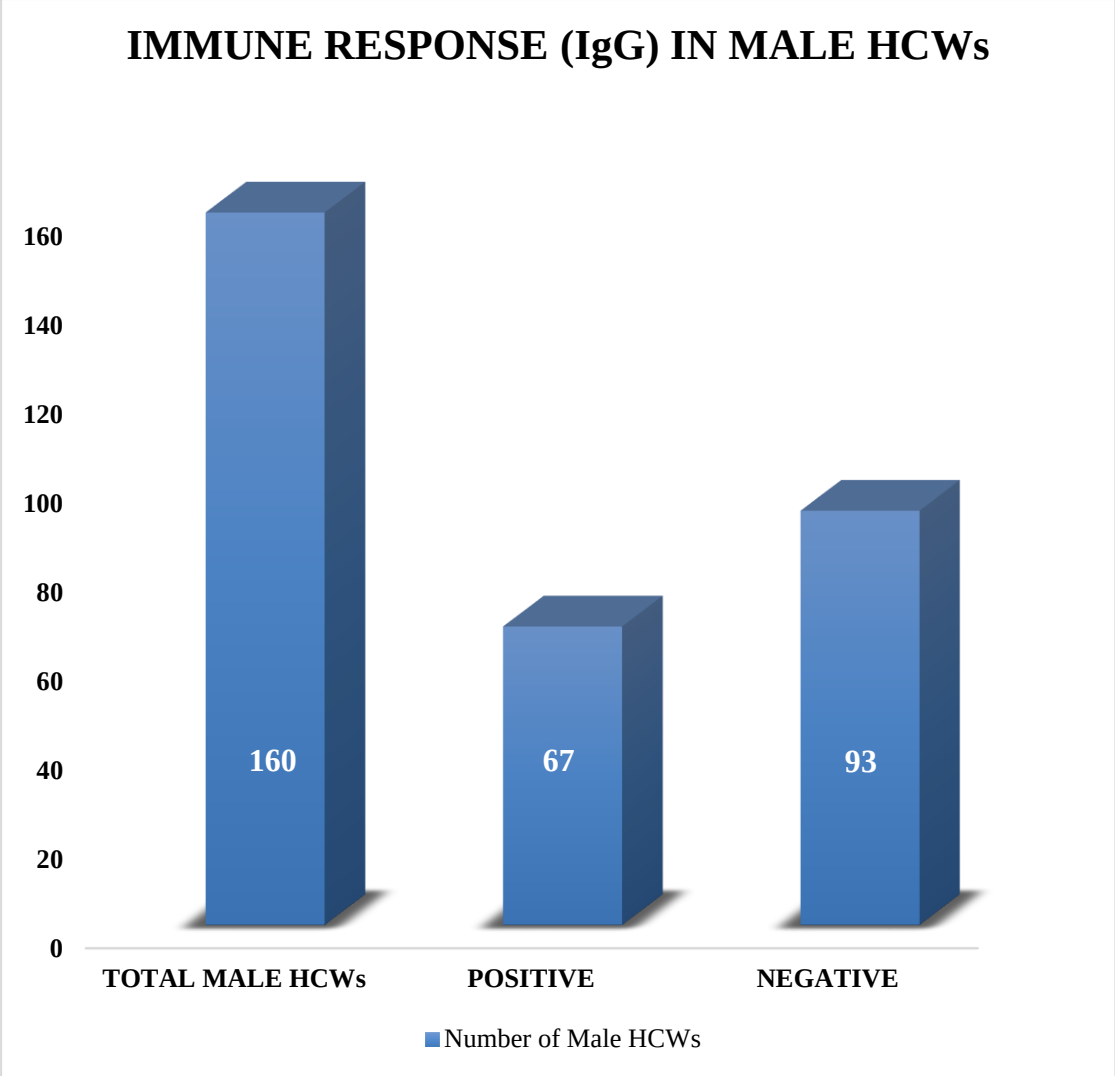

Figure 4.2: The graphical distribution of immune response (IgG) to SARS-CoV-2 in Male HCWs.

Interpretation

The above graphical representation of results is indicating that a total of 160 male HCWs were part of the study and 67 (41.88%) male HCWs have developed the immune response to the virus and were found IgG positive against the virus that means they were exposed to the SARS-CoV-2. The other 93 (58.12%) male HCWs were not exposed to the virus and they were found as IgG negative against the virus.

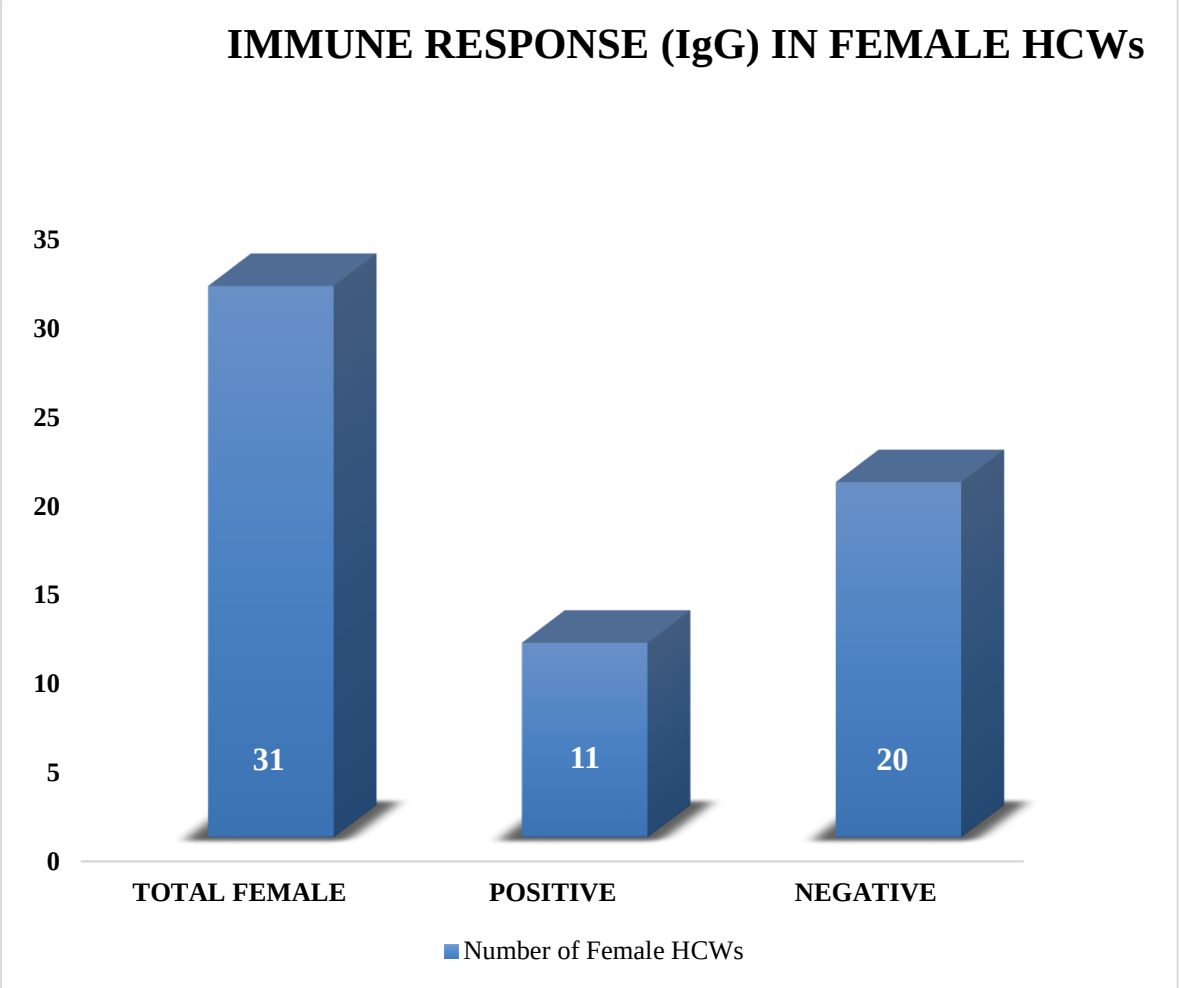

Figure 4.3: The graphical distribution of immune response (IgG) to SARS-CoV-2 in Female HCWs.

Interpretation

The above graphical representation of results is indicating that a total of 31 female HCWs were part of the study and 11 (35.48%) female HCWs have developed the immune response to the virus and were found IgG positive against the virus that means they were exposed to the SARS-CoV-2. The other 20 (64.52%) female HCWs were not exposed to the virus and they were found as IgG negative against the virus.

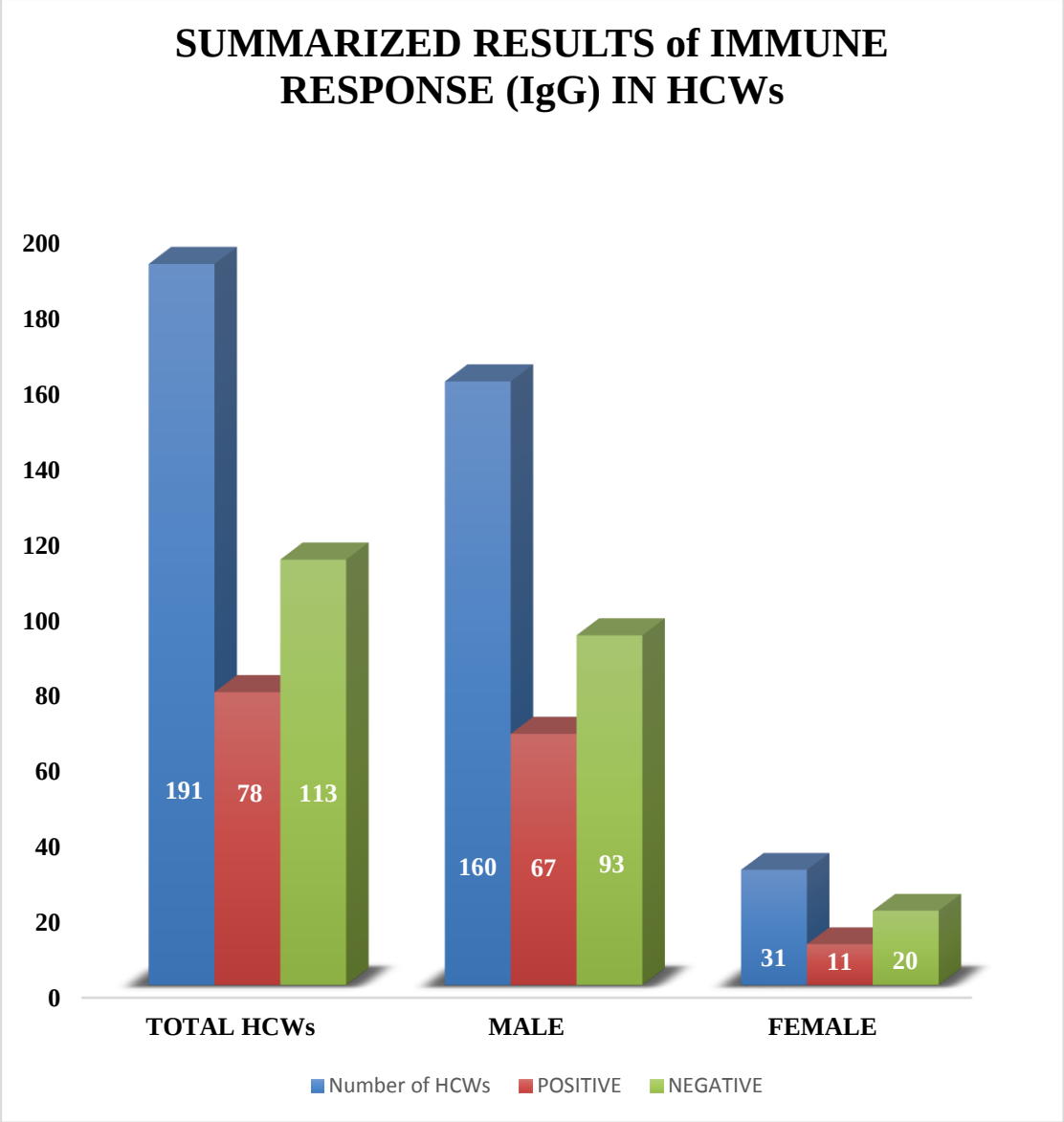

Figure 4.4: The graphical distribution of HCWs according to gender and immune response (IgG) results.

Table 2: The data distribution of HCWs according to gender and immune response (IgG) results

VARIABLE	MALE HCWs		FEMALE HCWs		TOTAL HCWs	
	n1	**%**	**n2**	**%**	**N**	**%**
Number of HCWs	160	83.77	31	16.23	191	100
POSITIVE	67	85.90	11	14.10	78	40.84
NEGATIVE	93	82.30	20	17.70	113	59.16

n1= Number of Male HCWs in data

n2= Number of Female HCWs in data

N= Total Number of HCWs in data

Interpretation

The above graphical representation and table results are indicating that overall 191 total number of HCWs were part of the study of the immune response to SARS-CoV-2 in HCWs. There were 160 (83.77%) male HCWs and 31 (16.23%) female HCWs.

There were a total of 78 (40.84%) HCWs who have developed the immune response to the virus and were found as IgG positive against the virus and the other 113 (59.16%) HCWs haven't developed any immune response to SARS-CoV-2 and they were found as IgG negative against the virus.

The number of IgG positive male and female HCWs was 67 (85.90%) and 11 (14.10%) out of total positive HCWs respectively and the number of IgG negative male and female HCWs was 93 (82.30%) and 20 (17.70%) out of total negative HCWs respectively.

There were 160 (83.77%) male HCWs out of which 67 (41.88%) HCWs were SARS-CoV-2 IgG positive and 93 (58.12%) HCWs were negative and there were 31 (16.23%) female HCWs out of which 11 (35.48%) HCWs were SARS-CoV-2 IgG positive and 20 (64.52%) HCWs were negative.

Inference

Seroprevalence was seen higher in male HCWs (85.90%) than in female HCWs (14.10%).

CHAPTER 5

DISCUSSION

In this research study between Jun to Dec 2020, overall 191 total number of HCWs were part of the study of the immune response to SARS-CoV-2 out of which a total of 78 (40.84%) HCWs were found as IgG positive by CLIAs based auto analyzers, their immune response has developed against the virus. And 113 (59.16%) HCWs have not developed antibodies to SARS-CoV-2 and they were found as IgG negative against the virus. While in the study on seroprevalence conducted on HCWs at a regional hospital system in Orange County, California, during May and June 2020 2,992 tested individuals, a total of 2,924 with complete data were included in the analysis. The observed prevalence of 1.06% (31 antibody-positive cases), the adjusted prevalence of 1.13% for test sensitivity and specificity were identified [30]. This shows that a higher prevalence is present in our study as compared with the mentioned study regional hospital system of Orange County, California.

This study was held between Jun to Dec 2020 at AFIP, Rawalpindi, in which 78 (40.84%) out of 191 HCWs were found seropositive against SARS-CoV-2 while in the study conducted for seroprevalence against SARS-CoV-2 in a random sample of HCW from a large hospital in Spain, 54 (9.3%) out of 578 participants recruited from 28 March to 9 April 2020, were seropositive for IgG against SARS-CoV-2 [31]. This comparison shows that a higher prevalence is seen in our study as compared with the study of a large hospital in Spain.

In our study, 191 total HCWs were part of the study of the immune response to SARS-CoV-2 out of which a total of 78 (40.84%) were seropositive and 113 (59.16%) were found as IgG negative against the virus. There were 160 (83.77%) male HCWs out of which 67 (41.88%) HCWs were SARS-CoV-2 IgG positive and there were 31 (16.23%) female HCWs out of which 11 (35.48%) HCWs were seropositive while between 15 to 23 April 2020, a study was conducted in Denmark, they screened 29,295 HCWs for SARS-CoV-2 immune response, of whom 28,792 (98.28%) provided their test results. They identified 1163 (4.04%) seropositive HCWs. Frontline HCWs working in hospitals had a significantly higher seroprevalence (779 [4.55%] of 16 356) than HCWs

in other settings (384 [3.29%] of 11 657 [32]. Here it is also seen that more seroprevalence is found in our stud as compared with the study conducted in Denmark.

In this study 191 total number of HCWs were recruited to determine immune response to SARS-CoV-2 out of which a total of 78 (40.84%) HCWs were found as IgG positive by CLIAs based auto analyzers which is the indication of the development of immune response against the virus and 113 (59.16%) HCWs have not developed an immune response to SARS-CoV-2 and they were found as IgG negative against the virus while in a study of the University Perinatal Care Center, Regensburg, Germany followed 166 HCWs approximately 12 weeks after a COVID-19 outbreak. 27 of the subjects had previously tested positive for the presence of SARS-CoV-2 by PCR testing and developed COVID-19. Serologic responses were tested with two independent commercially available test kits. 77.8 % of COVID-19 study subjects developed a specific IgG response [33]. This study contains more seroprevalence in HCWS as compared to our study.

Control and prevention strategies

COVID-19 is a serious disease of global concern. By some estimates, it has a higher reproductive number than SARS [35] and more people reported to become infected or died from SARS-CoV-2 than SARS [36]. Similar to SARS-CoV and MERS-CoV, disrupting the chain of transmission is considered key to stopping the spread of disease [37]. Different strategies should be implemented in health care settings and at the local and global levels. Health care settings can, unfortunately, be an important source of viral transmission. Applying triage, following correct infection control measures, isolating the cases and contact tracing are key to limit the further spreading of the virus in clinics and hospitals [37].

Suspected cases presenting at healthcare facilities with symptoms of respiratory infections (e.g. runny nose, fever, and cough) must wear a face mask to contain the virus and strictly adhere to triage procedure. They should not be permitted to wait with other patients seeking medical care at the facilities. They should be placed in a separated, fully ventilated room and approximately 2 m away from other patients with convenient access to respiratory hygiene supplies. Besides, if a confirmed COVID-19 case requires hospitalization, they must be placed in a single patient room with negative

air pressure – a minimum of six air changes per hour. Exhausted air has to be filtered through high-efficiency particulate air (HEPA) and medical personnel entering the room should wear personal protective equipment (PPE) such as gloves, gown, disposable N95, and eye protection. Once the cases are recovered and discharged, the room should be decontaminated or disinfected and personnel entering the room need to wear PPE particularly facemask, gown, eye protection [38].

In a community setting, isolating infected people is the primary measure to interrupt transmission. For example, immediate actions taken by Chinese health authorities included isolating the infected people and quarantining suspected people and their close contacts [39]. Also, educating the public to recognize unusual symptoms such as chronic cough or shortness of breath is essential therefore that they could seek medical care for early detection of the virus. If large-scale community transmission occurs, mitigating social gatherings, temporary school closure, home isolation, close monitoring of symptomatic individual, provision of life supports (e.g. oxygen supply, mechanical ventilator), personal hand hygiene, and wearing personal protective equipment such as facemask should also be enforced [40].

In a global setting, locking down Wuhan city was one of the immediate measures taken by Chinese authorities and hence had slowed the global spread of COVID-19. Air travel should be limited for the cases unless severe medical attention is required. Setting up temperature checks or scanning is mandatory at the airport and border to identify the suspected cases. Continued research into the virus is critical to trace the source of the outbreak and provide evidence for future outbreaks [40].

CONCLUSION

In this study, the seroprevalence was seen higher as compared to other studies and in the present study more seroprevalence is seen in male HCWs (85.90%) than in female HCWs (14.10%). The possible explanation for this prevalence in HCWs, high SARS-CoV-2 infection rate includes a relative lack of awareness of timely procurement of personal protective equipment, rigorous employee education, patient triage, and treatment protocol development and implementation. It was identified that seropositive HCWs were not following recommended SOPs of COVID-19 prevention and were not wearing proper personal protective equipment (PPE). In addition to prevention from infection purposes, the spread of the COVID-19 disease can be controlled by strict following of recommended SOPs of COVID-19 preventive measures and use of proper PPE.

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