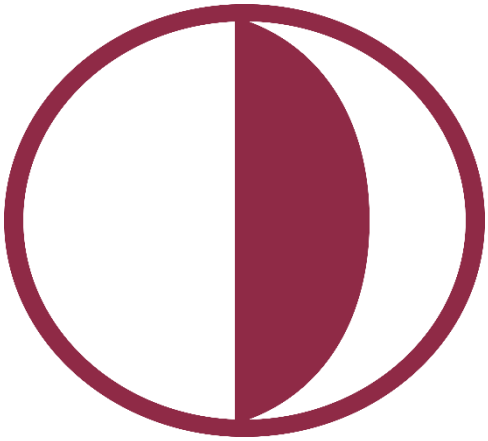

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NEAR EAST UNIVERSITY
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SERO-PREVALENCE OF ANTI-SARS-CoV-2 ANTIBODIES AMONG DIFFERENT AGE GROUPS IN POPULATION OF PROVINCE PUNJAB, PAKISTAN

ADIL ABBAS

MASTERS THESIS

DEPARTMENT OF MEDICAL AND CLINICAL MICROBIOLOGY

MENTOR

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NICOSIA

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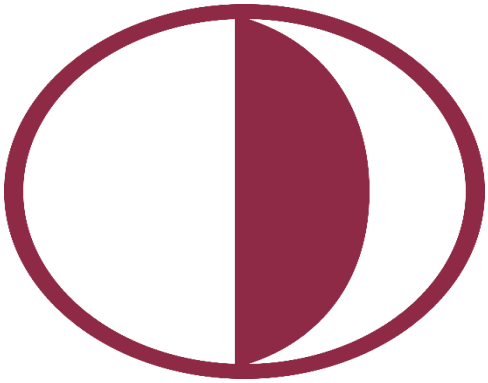

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APPROVAL

The Directorate of Health Sciences Institute, / INSTITUTE OF GRADUATE STUDIES

This study has been accepted by the Thesis Committe in Medical and Clinical Microbiology Program as a Master of Science Thesis.

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According to the relevant articles of Near East University Postgraduate Study-Education and Examination Regulations, this thesis has been approved by the above mentioned members of the thesis committee and the decision of the Board of Directors of the Institute.

Professor K. Hüsnü Can BAŞER, PhD

Director of Graduate School of Health Sciences

STATEMENT (DECLARATION)

Hereby I declare that this thesis study is my own study. I had no unethical behaviour in all stages from planning of the thesis until writing thereof, I obtained all the information in this thesis in academic and ethical rules, I provided reference to all of information and comments which could not be obtained by this thesis study and took these references into the reference list and had no behaviour of breeching patent rights and copyright infringement during the study and writing of this thesis.

Adil Abbas

Signature

ACKNOWLEDGEMENTS

All praises be to Almighty Allah , the most beneficent, the only to be praised, whose blessings and exaltations flourished my thoughts and enabled me to improve my knowledge in such a stage.Who enabled me to produce such a document. I offer my humblest and sincerest words of thanks to His Holy Prophet (S.AW), who is forever a torch of guidance and knowledge of humanity.

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ADIL ABBAS

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LIST OF ABBREVIATIONS

ATPase	Adenosine Triphosphatase
ACE2	Angiotensin Converting Enzyme2
ARDS	Acute Respiratory Distress Syndrome
ALT	Alanine Transaminase
AST	Aspartate Transaminase
CSG	Coronavirus Study Group
CDC	Chinese Center for Disease Control and Prevention
CVD	Cardiovascular Disease
ELISA	Enzyme Linked Immunosorbent Assay
HCoVs	Human Coronaviruses
HE	Hemagglutinin esterase
ICTV	International Committee on Taxonomy of Virus
ORF	Open Reading Frame
PaO2/FiO2	Ratio of Partial Pressure of Arterial Oxygen to Fractional Inspired Oxygen
RBD	Receptor Binding Domain
RCA	Rolling Circle Amplification
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
TMPRSS2	Transmembrane Serine Protease 2

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Department: Medical and Clinical Microbiology

SUMMARY

Aim

- Aim of study is to find out seropositivity (no. of cases) in different cities of Punjab province in Pakistan.
- To find out the prevalence according to gender wise.
- To find out antibody titers in different age groups.
- To find out which age group in Punjab province has the greatest number of antibodies against Coronavirus.

Materials and Methods

This study was conducted at Jinnah hospital Lahore and government quarantine center Lahore in province of Punjab, Pakistan from November 10, 2020, to February 10, 2021. Blood samples were taken by venipuncture technique from the symptomatic patients. The ELISA test was performed by using the OmniPATHTM COVID-19 Total Antibody ELISA Test kit manufactured by Thermo Fisher Scientific company, United States.

Findings and Results

A high seroprevalence of 57% was recorded in the city of Lahore in Punjab province, Pakistan. There was not a big difference of SARS-CoV-2 cases gender wise. Patients of age between 31 to 60 years had recorded a high number of cases. However, children were found to have a smaller number of cases or less susceptibility to SARS-CoV-2 infection. Highest number of antibody titers were found in patients with age between 11 to 30.

Keywords: SARS-CoV-2, Seropositivity, Antibody titers, Age groups, Susceptibility

ABSTRACT

At the end of 2019, there was an outbreak of coronavirus in Wuhan, China which killed more than 1800 people and more than 70,000 people were infected during the first 5 days. Chinese researchers named it as a novel coronavirus disease 2019. Up to 17.30 million cases have been reported in Asia and the number of deaths were more than 2 million. Number of COVID cases in Pakistan is increasing rapidly with 413,191 total cases and a death toll of 8000 so far. This virus is unique because of a single-stranded positive sense RNA genomic structure and containing a size of about 30 kb. This study was conducted at Jinnah hospital Lahore and government quarantine center Lahore in the province of Punjab, Pakistan from November 10, 2020, to February 10, 2021. Blood samples were taken by venipuncture technique from the symptomatic patients. ELISA test was performed by using the kit of OmniPATHTM COVID-19 Total Antibody ELISA Test kit manufactured by Thermo Fisher Scientific company, United States. We have found that the highest number of reactive patients were found in the city of Lahore which were 57% (n=442). From Sialkot 25% (n=194) positive cases were recorded and 18% (n=135) were recorded from Gujrat. We made gender wise distribution according to which out of 84% (n=771) positive reacted participants 40% (n=313) of adult males and 43% (n=337) adult females were reactive against SARS CoV2. Among children 15% (n=121) were reactive against SARS CoV2. We also made age wise distribution among participants to find out how many cases were present in different age groups. We found 11% (n=80) reactive patients in age group (1-10), 10% (n=76) were reactive in age group (11-20), 13% (n=103) were reactive in age group (21-30), 16% (n=124) were found reactive in age group (31-40), highest no of patients 17% (n=132) were found reactive in age group (41-50), 16% (n=126) were reactive in age group (51-60), 11% (n=88) of patients were reactive in age group (61-70). In age group (>71), 6% (n=42) of patients were recorded positive. We had taken the mean of antibody titer according to different age groups and we found the highest mean of antibody titer which is 171 in age group (21-30) followed by age group (11-20) and age group (31-40). In conclusion SARS-CoV-2 had affected Lahore city the most in Punjab, Pakistan and in population of Punjab people of age group (41-50) had affected the most with SARS-CoV-2 and highest number of antibody development was recorded in age group (21-30) which was 171 and was far above than cut off value which was 1.

Keywords: Outbreak, Coronavirus, Punjab province, Seropositivity, Age groups

CHAPTER I

Introduction

Tyrell and Bynoe started history in 1965 (Tyrrell & Bynoe, 1966) when they found that they can pass through a virus named as B814. This virus was obtained from the respiratory tract of an adult which was suffering from a cold and then it was cultured on human embryonic tracheal organs. Inoculation of media from these cultures found the presence of infectious agents which was revealed but Tyrell and Bynoe were not able to grow that infectious particle in tissue culture at that time. Hamre and Procknow (Hamre & Procknow, 1966) at the same time have taken the samples from medical students suffering with cold and grow the virus with very uncommon properties in tissue cultures. Through National Institute of Health in the lab of Robert Chanock (McIntosh, Dees, et al., 1967) multiple strains of sensitive agents were recovered which was obtained from the respiratory tract of humans by the technique which was similarly used by Tyrrell and Bynoe .The term "OC" was selected for these viruses to nominate that they were cultured in organ medium.

At the end of 1960 a group of virologists which was led by Tyrell were working on number of animal viruses and human strains which include bronchitis infectious virus, mouse hepatitis virus and swine virus of transmissible gastroenteritis and all of these were revealed to be morphologically same by taking a look from electron microscope (McIntosh, Becker, et al., 1967; Witte et al., 1968). A new group of virus was given a name as coronavirus and after on it was accepted as a new genus of viruses officially (Tyrrell et al., 1975).

1.1. Genome of SARS-CoV-2

The coronaviruses belong to a class of enveloped viruses, and they create identical structures in the cytoplasm of host cells. This virus is unique because of a single-stranded positive sense RNA genomic structure and containing a size of about 30 kb. Its structure consists of a cap at 5′ and a polyadenylation tract at 3′. At the time of infection in host cell translation of 5′ which is ORF (open reading frame) of viral genome has done and forms and then encoded proteases of virus cleaved that polyprotein to produce various non-structural proteins which includes an adenosine

triphosphatase (ATPase) and RNA-dependent RNA polymerase. These proteins facilitate replication of the viral genome, it also generates nested transcripts. These transcripts are involved in viral protein (Lai & Cavanagh, 1997; Sawicki & Sawicki, 1998). Membrane proteins of virus which includes the main spike (S) and membrane (M) proteins are inserted into Golgi compartment and endoplasmic reticulum while completely replicated RNA plus strand make an assembly with the nucleocapsid (N) protein. RNA-protein complex is formed which is associated with the membrane protein (M) fixed in the membranes of the endoplasmic reticulum. Virus particles transform to make nucleocapsid buds in the lumen of the endoplasmic reticulum (ER). Viruses eventually exit from the cell by migrating through the Golgi complex by exocytosis.

1.2. Epidemiology

At the end of year 2019, there was an outbreak of coronavirus in Wuhan, China which killed more than 1800 people and more than 70,000 people were infected during the first 5 days. Chinese researchers named it as a novel coronavirus disease 2019. The international committee on Taxonomy of viruses (ICTV) has given the name of this virus as SARS-COV-2 (Lai et al., 2020).

Novel SARS-CoV-2 creates pandemic as of February 10, 2021 and it has reached 219 countries and taken the lives of more than 2 million lives and the number of confirmed cases around the globe was above 100 million (https://www.worldometers.info/coronavirus/, accessed 10 February 2021). According to the last update about coronavirus in Pakistan on February 10, 2021, suspected corona cases reported in Pakistan were 8,288,091 in which 559,093 were tested positive for SARS-CoV-2. Of the 8,288,091 total cases, 516,683 patients were recovered from the virus while 30,225 remain critical and 12,185 died (http://covid.gov.pk/, accessed 10 February 2021).

1.3. Mode of Transmission

People who have symptoms from a virus like coughing and sneezing can transmit the infection to other people by contact. Generally, corona virus was thought to spread through zoonotic droplets. Virus causes infection when it replicates in ciliated epithelium and causes severe damage to cells. A research article was published in 2019

according to which coronavirus uses an Angiotensin converting enzyme 2 (ACE.2) which is a membrane exopeptidase present in the receptor for entry into host cells (de Souza Luna et al., 2007). When an infected person is exposed for a long time and short exposure to a person who is symptomatic is at higher risk of transmitting the virus while a short exposure to people who do not show symptoms have less chances of infection (Chu et al., 2020). Another possible reason for transmission is through a surface with a virus on it. As tiny droplets stay in air so inhaling that contaminated air may result in transmission but it is ambiguous that this is the important source of infection or not in humans (Bourouiba, 2020; Lewis, 2020). Nucleic acids can be detected in air or aerosols exist in physiological states like coughing. It does not make sense that particles in air are infectious (Chia et al., 2020). It is believed that there is very low risk of transmission of SARS-CoV-2 from mother to children and in most of the reported case series, mother get infection with SARS-CoV2 during pregnancy in third trimester with none of reported deaths of mother as well as neonates (Dashraath et al., 2020).

1.4. Phylogenetic Analysis

WHO named this disease as coronavirus disease 2019 on the date of 11 February,2020 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). Coronavirus Study Group suggested in their manuscript bioRixiv which suggests that novel coronavirus as severe acute respiratory syndrome coronavirus 2 after analysis of phylogeny of closely associated coronaviruses (Gorbalenya et al., 2020).

CSG announced that there were no intentions to get any recommendation to SARS once declaring yet another virus which has a name obtained from the word SARS whereas SARS is just the name of disease and by naming a new virus which is SARS-CoV-2 literally indicates that it is associated with severe acute respiratory syndrome particularly to scientists who don't have enough knowledge relevant to virology and also to the public. The new name COVID-19 is also not stable with the name of disease and SARS-CoV-2 is a natural existing virus which distinguishes it from other SARS associated coronaviruses and it is mainly characterized by its genome sequence (Jiang et al., 2020).

If this is the case, then this virus will have very bad effects on the community, and it will destroy the economic progress in those countries where this virus is spreading extensively and may be the whole of the world.

1.5. Pathogenesis

COVID19 infected patients have leukocytes high in numbers, showed high levels of pro-inflammatory cytokines in plasma and unusual respiratory results. According to one case report a patient infected with SARS-CoV-2 showed a fever for five days along with symptoms of cough, lungs producing unusual breathing sounds and body temperature reached up to 39.0 °C. The laboratory findings showed abnormal findings with leukopenia and leukocyte number of 2.91 × 10^9 cells/L and 70% were of neutrophils. Additionally, a count of blood C-reactive protein was measured which is 16.16 mg/L and crossed over the normal range (0–10 mg/L). Side by side ESR (erythrocyte sedimentation rate) and small fragment protein called D-dimer were also very increased (Lei et al., 2020). SARS-CoV-2 has a major pathogenesis of targeting the lungs and causing serious pneumonia and RNAaemia connected with acute cardiac arrest. High concentration of cytokines and chemokines including IL1-β, IL8, IL9, IL10, IL1RA, IL7, IFNγ, IP10, MCP1, MIP1α, MIP1β, PDGFB, basic FGF2, GCSF, GMCSF, TNFα, and VEGFA were reported in patients infected with SARS-CoV-2 infection while few of the cases which were referred to ICU (intensive care unit) reported elevated pro-inflammatory cytokines levels in plasma which include IL2, IL10, IL7, IP10, TNFα, GCSF, MIP1α, and MCP1 promoting the severity of disease (C. Huang et al., 2020).

The mechanism on which SARS-CoV-2 causes injury is unknown however a disease model was suggested which consists of 3 phases i.e. replication of virus, immune hyperactivity of immune system and then destruction of lungs (Tsui et al., 2003). Pathogenesis of SARS is associated with severe damage of alveoli, proliferation of epithelial cells and macrophages are increased in number. Spleen shows white pulp atrophy and hemophagocytosis of the lungs in patients infected with SARS. It was reported that hemophagocytosis supports deregulation of cytokines (Fisman, 2000). It is examined that SARS is associated with pneumonia whereas persons affected with SARS also show symptoms gastrointestinal (To et al., 2004) along with atrophy of

spleen and lymphadenopathy (Ding et al., 2003). Frequently found in patients of SARS is diarrhea in about 30-40 percent of patients.in enterocytes replication of SARS-CoV and minimum destruction occurs without changing intestinal structure. Antiapoptotic cellular response epithelial cells of intestine and increased regulation of transforming growth factor β has been associated with absence of intestinal inflammation (Cheng et al., 2004). Recently work done on autopsies taken from SARS patients suggests that this is systemic disease with widely destructing pulmonary system and results in high proportion of virus in urine, stool, secretions from respiratory tract and also in sweat (Farcas et al., 2005).

1.6. Clinical Features

Clinical features of patients who have SARS-CoV-2 are asymptomatic or may develop septic shock leading to multiorgan dysfunction (Cascella et al., 2020). Classification of SARS-CoV-2 is premised on severity of disease. It can be divided into mild, moderate, severe and critical. Widely shown symptoms in patients with SARS-CoV-2 are fever, fatigue, diarrhea and dry cough (Liu et al., 2020).

1.6.1 Mild Disease

Patients when in the mild stage of disease experienced symptoms relevant to upper respiratory tract infection which include fever, nasal blockage, sore throat, dry cough and muscle pain. Serious symptoms such as dyspnea are absent. Most cases with SARS-CoV-2 are in mild stage (Cascella et al., 2020). Radiographic findings are also absent in milder (Liu et al., 2020) however patients can transform into severe or critical in no time.

1.6.2 Moderate Disease

Patients in moderate stages of disease show no signs of severe disease however cough, tachypnea and shortness of breath is reported (Cascella et al., 2020).

1.6.3 Severe Disease

Patients who develop severe forms of disease showed symptoms of acute respiratory distress syndrome (ARDS), pneumonia, sepsis or septic shock (Cascella et al., 2020). It can be diagnosed clinically and with the help of radiological findings complications can be excluded. Clinical findings include tachypnea, severe dyspnea and respiratory disorders. However, fever can be present or absent in severe forms of

disease (Cascella et al., 2020). Additionally, only five percent of patients enter a critical phase with cardiac injury, septic shock and multiple organ dysfunction. According to the data taken from the Chinese Centers for Disease Control and Prevention (CDC) 49% of patients have case fatality (Cascella et al., 2020). High case fatality has been found in patients with pre-existing comorbidities. According to one study 7.3% patients with diabetes, 6.5% patients with respiratory disease, 10.5% patients with cardiovascular disease and 6% patients with hypertension have high case fatality whereas patients without any comorbidity have low case fatality (Liu et al., 2020).

1.6.4 Acute Respiratory Distress Syndrome

ARDS development in a person indicates a very worse condition of respiratory failure and it occurs in one week of clinical results. ARDS is distinguished on the basis of varying degrees of hypoxia and for this purpose values of PaO2/FiO2 (ratio of partial pressure of arterial oxygen to fractional inspired oxygen) are used. If the values of PaO2/FiO2 are less than or equal to 100 mmHg it indicates severe ARDS. If values of PaO2/FiO2 ranged to 100-200 mmHg, it indicates moderate ARDS and if values of PaO2/FiO2 are between 200 mm Hg or 300 mmHg, it is the indication of mild type of ARDS. Whereas aspartate transaminase (AST) and alanine transaminase (ALT) enzyme concentration in serum has a correlation with clinical degradation to ARDS at a time of admission therefore at the time of admission higher levels of these enzymes will lead to clinical degradation to ARDS (Cascella et al., 2020).

1.6.5 Sepsis and Septic Shock

COVID-19 infected patients at the most critical stage acquire sepsis or septic shock. The dysfunction of multiorgan results due to the dysregulation of host response against infection. The major signs which show dysfunctioning of organs are hypotension, skin mottling, altered mentation along with laboratory findings of dysregulation of homeostasis including high lactate, hyperbilirubinemia, acidosis and coagulopathy (Cascella et al., 2020).

1.7. Coronavirus co morbidities

The definition of comorbidity in medical sciences is mainly defined as two or more diseases or medical conditions in a patient. In very simple words comorbidity is defined as an individual might have effects of all other situations and have other

primary conditions of interest and the condition may be physiological or psychological (Das et al., 2020)

1.7.1 Diabetes can be a great threat for SARS-CoV-2 infection

An important risk factor associated with mortality is diabetes which is triggered by SARS-CoV-2. A patient with diabetes is closely characterized by impaired immune system and it is assumed to lead to high susceptibility to SARS-CoV-2 especially those patients which have high concentration of glucose in blood. Cardiovascular disease and diabetes are collectively a significant risk factor to SARS-CoV-2 morbidity (N. Chen et al., 2020).

Chinese Center for Disease Control and Prevention (China CDC) investigated a study 20,982 COVID-19 patients which have approximately 5.3% pf pre-existing diabetes, 4.2% of CVD as well as 12.8% of hypertension (Epidemiology Working Group, 2020). Onder et al. also reported a study in which was conducted in Italy and stated that out of total 355 patients with SARS-CoV-2 infection36% of patients had diabetes and 43% patients had a profile of CVD (Onder et al., 2020). In another Italian study Bhatraju et al. also reported 58% of patients out of 24 patients are associated with diabetes (Bhatraju et al., 2020).

1.7.2 Co infection of SARS CoV 2 with tuberculosis

Coinfection of tuberculosis along with SARS CoV-2 is of main worry because of many reasons. First, diagnosis of tuberculosis and SARS-CoV-2 is mixed due to wide ranging clinical features and also absence of radiological findings which are specific to tuberculosis. Furthermore, immunomodulators in moderate or severe stages of disease reactivate the latent tuberculosis in highly endemic areas of India (Pathak et al., 2020). Moreover, previously existing tuberculosis disease and basic condition of lungs will mainly affect the severity of SARS-CoV-2. Ultimately active co-existing tuberculosis may result in serious illness (Tadolini et al., 2020).

1.8. Diagnostic Techniques

1.8.1 Techniques for diagnosis SARS-CoV-2 with new evolution

For quantification of antigen-antibody interconnection. immunoassays methods are used and they give us precise data about earlier exposure and dynamic of virus infections (Lee et al., 2020) however antibodies are more resistant as compared to viral

RNA and during there is very less chance of deterioration during transportation storage and collection (Younes et al., 2020).

1.8.2 ELISA method

Among developed methods for detection of SARS-CoV-2 ELISA is one of them. The ELISA method is used to locate a particular antigen or antibody in the sample. This method can be done by making use of direct and indirect techniques. Direct method relies upon enzyme linked antibodies to determine antigen. Indirect method uses an antigen linked to the primary antibody and antigen is coated on microplate. At the end secondary enzyme-labeled antibody is put on which distinguishes primary antibody (Sheikhzadeh et al., 2020).

1.8.3 Immunochromatographic assay technique

Immunochromatographic assay method is used to locate analytes qualitatively and it is also called lateral flow immunoassay. With the help of this assay, semi-quantitative data can be produced by coupling it with a reader (Xiang et al., 2020).

1.8.4 Lateral flow immunoassay technique

By the study of Li et al. identification of antibody IgM and antibody IgG in samples of blood was done by using lateral flow immunoassay (Z. Li et al., 2020). The strip of test contains a test line for detection of IgG and IgM antibody and one control line, both are coated with mouse anti human IgG, respectively.

1.9 Amplification Technique

1.9.1 RT-PCR (Reverse transcription polymerase chain reaction)

PCR (Polymerase chain reactions) works on the principle of amplification of RNA transcripts and genes separated from samples. The PCR test kit contains necessary components which include primers, DNA samples, polymerase enzymes and deoxynucleoside triphosphates. PCR methods amplify the genes and their respective RNA transcripts separated from samples. DNA polymerase, extracted DNA, primers and deoxynucleoside are the main ingredients. Another type of PCR is reverse transcription PCR which uses an enzyme called reverse transcriptase enzyme which transforms RNA molecules to cDNA molecules. cDNA then works as a template in PCR reaction. A quantitative type of PCR fluorescent dye or DNA probe such as

TagMan (fluorophore-attached DNA probe) is used to determine DNA molecules (Shahi et al., 2018).

1.9.2 Isothermal nucleic acid amplification

PCR requires complex methods and requirements such as providing multiple temperatures in each cycle. To avoid this complexity isothermal nucleic acid amplification technique is used for amplification of nucleic acids at constant temperature in each (Martzy et al., 2019). There are many techniques previously developed for isothermal amplification of nucleic acids such as rolling circle amplification (RCA), Loop mediated isothermal amplification (LAMP), transcription mediated amplification (LMA) however, for reverse transcription, LAMP method is used to detect SARS-CoV (Hong et al., 2004).

1.10. Treatment

Children infected with human coronaviruses, supportive treatment should be given in which a diet containing good calorie, oxygen supplements and sufficient fluid is included. The focus is to save people from organ failure, nosocomial infections and ARDS and if there is suspected bacterial infection then broad spectrum usually second or third generation antibiotics such as cephalosporins can be given to patients.

1.10.1 SARS-CoV

For the treatment of SARS-CoV antiviral drugs of broad spectrum such as ribavirin or interferon alpha or beta can be given to treat SARS-CoV. However, Ribavirin shows ineffectiveness or it may cause liver dysfunction or hemolytic anemia (Stockman et al., 2006).

1.10.2 SARS-CoV-2

Many clinical trials are going on but there is no evidence or result based on which treatment can be done of patients affecting SARS-CoV-2. Main recommended treatment for children is to treat with nebulized interferon alpha 2b along with the combination of corticosteroids for severe complications like septic shock, ARDS and encephalitis (Wu, Zhao, et al., 2020). However, these therapies have not shown a clear beneficial outcome in treatment of SARS-CoV-2.

1.11. Vaccines

Many vaccines against human coronaviruses are in progressive stages with the objective of protecting people from infection and minimizing the impacts of disease.

Spike glycoprotein (S) and its receptor binding domain (RBD) are main antigens for development of vaccines (He et al., 2006). However, mutation and recombination ability of coronaviruses rapidly is a main problem for invention of vaccines (Su et al., 2016).

1.12. Aims and Objectives

- Aim of study is to find out seropositivity (no. of cases) in different cities of Punjab province in Pakistan.
- To find out the prevalence according to gender wise.
- To find out antibody titers in different age groups.
- To find out which age group in Punjab province has the greatest number of antibodies against coronavirus.

CHAPTER II

Literature Review

2.1. General Information

At the end of 1960, Tyrrell was a group leader of virologists, and they were operating the human strains and a variety of animal viruses. Infections included were mouse hepatitis virus, bronchitis virus and gastroenteritis virus of swine. These viruses were found to be morphologically the same by taking a look through electron microscopy (Witte et al., 1968). This was named as coronavirus and a new group of virus which was accepted as a new genus of viruses officially (Tyrrell et al., 1975).

2.2. Genome

Coronaviruses have a non-segmented single stranded positive sense RNA genome which has a size of approximately 30 kb (Kahn & McIntosh, 2005). RNA strand contains a 5′ end cap structure, replicase genes which code for the non-structural proteins which comprises two-thirds of the genome and the structural protein genes S (spike), E (envelope), M (membrane) and N (nucleocapsid) and variety of accessory genes interspersed within the structural genes at the 3′ end of RNA strand (Fehr & Perlman, 2015). Accessory proteins have no utility in replication still they play a comprehensive in viral pathogenesis (Zhao et al., 2012).

2.3. Transmission

People can get infected with this virus when they closely interact with a person who has developed symptoms from the coronavirus including coughing and sneezing. Normally, corona virus spreads through airborne zoonotic droplets. After the virus replication in the ciliated epithelium, it caused cellular damage and infection at a site of infection. Long exposure to a person who is infected and a short exposure to a symptomatic person are associated with a very high risk for transmission as compared to those persons who are asymptomatic (Chu et al., 2020).

2.4. Pathogenesis

SARS-CoV-2 infected persons have a high number of leukocytes, abnormal respiratory results and high levels of pro-inflammatory cytokines in plasma. According to one case report on SARS-CoV-2 a patient with a fever from 5 days along with cough, temperature and breathing sounds of both lungs. The sputum of the patient showed a

positive test report of polymerase chain reaction that confirmed SARS-CoV-2 infection (Lei et al., 2020). SARS-CoV-2 infection has a main pathogenesis of targeting the respiratory system as pneumonia, RNAaemia combined with acute cardiac injury (C. Huang et al., 2020). SARS-CoV-2 pathology regarding lungs has been associated with diffusion of alveoli, proliferation of epithelial cells and an increased number of macrophages. Putative syncytium-like formation which is the characteristic of many coronavirus infections can be seen due to infiltration of multinucleate giant-cell (Nicholls et al., 2003).

2.5. First outbreak in China

The first outbreak only occurred in the city of Wuhan and its surroundings in the province of Hubei, China on 8 December 2019 (Herroelen et al., 2020). When the first case was reported in Tibet in the month of January 2019, an outbreak of SARS-CoV-2 had spread all over 31 provinces of China. Until 11 February 2020, reported cases of SARS-CoV-2 were 44,672 and among these cases 0.2% (100% in Hubei) on 31 December 2019, 1.7% (88.5% in Hubei) on 10 January 2020, 13.8% (77.6% in Hubei) on 20 January 2020 , and 73.1% (74.7% in Hubei) of cases were recorded before February 2020 (http://www.nhc.gov.cn/xcs/yqtb/202002/26fb16805f024382bff1de80c918368f.shtml , accessed 12 February 2021).

The total death rate among 44,672 of the total confirmed cases was 2.3% while the death rate in the province of Hubei and its neighborhood areas was 2.9% and 0.4%. In all patients aged 80 years or more, the death rate was as high as 14.8% (Epidemiology Working Group, 2020).

2.6. Epidemiology Worldwide

According to an update on February 10, 2021, SARS-CoV-2 cases have crossed over 106,170,395 cases worldwide among which more than 25,914,230 were still active and more than 2,315,014 deaths. The United States is on the top with most confirmed cases of SARS-CoV-2 which is 27,444,459 and half of these cases are in New York state. In the number of deaths, the United States again was on the top and most affected country as of February 10,2021 with 471,566 total deaths. In the European continent, Russia ranks first and is the most affected region having total SARS-CoV-2 cases of 3,951,233 followed by UK, France, Spain, Italy. In the number of deaths, the UK is on the top

with the highest death rate of 112,092 followed by Italy having 91,003 death rates. Spain is behind Italy and following Italy's curve with total cases of 2,971,914 and 61,386 deaths. All of these countries have crossed China's death toll of 4,636 reported cases. All European countries have SARS-CoV-2 cases, and most countries have at least one death. All data regarding SARS-CoV-2 cases and deaths were taken from Worldometer (https://www.worldometers.info/coronavirus/, accessed 10 February 2021).

2.7. Epidemiology in Asia

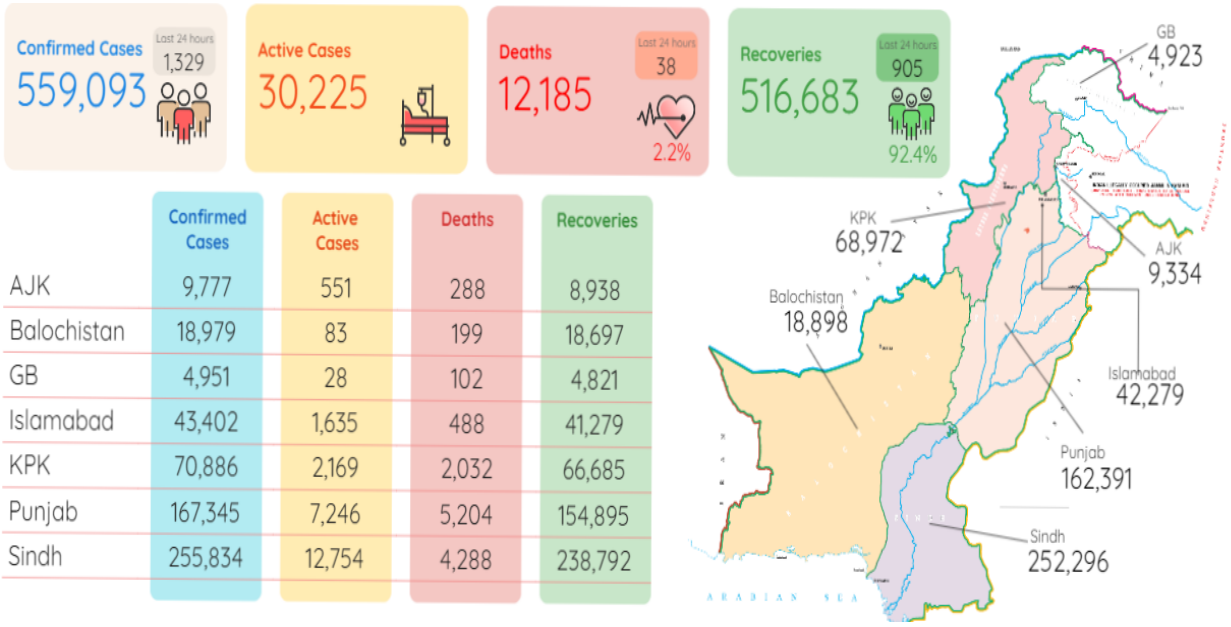
In Asia cases of SARS-CoV-2 are increasing and have reached 23,512,645 with a total death rate of 378,668. India ranked on top with total cases of 10,826,560 and total number of 155,018 deaths as of February 6,2021. Turkey is following India with 2,524,786 total cases and Iran lies after Turkey followed by Iran, Indonesia, Israel, Iraq and Pakistan. Iran has the highest number of deaths after India, having a death toll of 58,412 (https://www.worldometers.info/coronavirus/, accessed 10 February 2021).

2.8. Situation in Pakistan

According to the last update about coronavirus in Pakistan on February 10, 2021, suspected corona cases reported in Pakistan were 8,288,091 in which 559,093 were tested positive for SARS-CoV-2. Of the 8,288,091 total cases, 516,683 patients were recovered from the virus while 30,225 remain critical and 12,185 died. Sindh recorded the highest number of incidences (45.61%) followed by Punjab which has an incidence of 29.73%. No. of cases recorded from Khyber Pakhtunkhwa (KPK) is 12%, 7% from Islamabad, 3.3% from Balochistan,1.70% from Azad Jammu Kashmir (AJK), 0.87% from Gilgit Baltistan (GB). Gilgit Baltistan recorded the lowest number of incidences (0.88%) followed by Azad Jammu Kashmir (1.74%) and Islamabad (7.71%). The highest rate of recovery was reported in Gilgit Baltistan (GB) which was (97.4%), while lower rate of mortality was found in which was (1.04%) compared with other regions of Pakistan (http://covid.gov.pk/, accessed 10 February 2021).

From November 10, 2020 to February 10, 2021, the highest increase in cumulative reported cases of COVID is recorded in Azad Jammu Kashmir (AJK) is increased by 90% (4911 cases to 9334 cases) in Islamabad cases are increased by 88% (22,432 cases to 42,279 cases), in KPK reported cases are increased by 67% from (41,067 cases to 68,972 cases) the reported cases of SARS-CoV-2 in Sindh is increased by 66.69%

(151,352 cases to 252,296 cases), in Punjab cases are increased by 50% (107831 cases to 162,391 cases) and in Gilgit Baltistan cases are increased by 12% (4394 cases to 4923) as shown in Figure 2.1.



	Confirmed Cases	Active Cases	Deaths	Recoveries
AJK	9,777	551	288	8,938
Balochistan	18,979	83	199	18,697
GB	4,951	28	102	4,821
Islamabad	43,402	1,635	488	41,279
KPK	70,886	2,169	2,032	66,685
Punjab	167,345	7,246	5,204	154,895
Sindh	255,834	12,754	4,288	238,792

Figure 2.1 Pakistan situation report (February 10, 2021)

2.9. Mode of Pathogenesis

On the surface of coronavirus there is a spike glycoprotein (S) which is compulsory for entry of virus with the help of binding angiotensin converting enzyme 2 receptor and for combination of virus with the host cell. The glycoprotein (S) makes a homotrimeric-like structure in which each protomer is made up of two subunits named as S1 and S2 (Chen et al., 2005). ACE2 receptor when bound with the receptor-binding domain (RBD)which is present in the S1 subunit sets off a symmetrical change in the glycoprotein (S) that commences fusion of membrane with the host cell. The humoral immunity receptor binding domain (RBD) is a primary target for antibody response and it is also believed that it is a primary antigenic region in SARS-CoV-2 offering protection (Chen et al., 2005). By the use of cryogenic electron microscopy (cryo-EM) the prefusion structure of glycoprotein (s) was recently determined (Wrapp et al., 2020) and it was revealed overall similarity with the SARS-CoV-2. Whereas most monoclonal antibodies were tested which were targeting the receptor binding domain of SARS-CoV showed failure of attachment with the receptor binding domain of SARS-CoV-2 which suggests that these two viruses have different antigenicity to the RBD.

As shown in Figure 1A, SARS-CoV-2 virion has a genome size of 29.9 kb similar to other β-CoVs (http://www.nhc.gov.cn/xcs/yqtb/202002/26fb16805f024382bff1de80c918368f.shtml , accessed 12 February 2021) contains a nucleocapsid which is composed of genomic RNA and phosphorylated nucleocapsid (N) protein . The nucleocapsid is protected by two variants of spike glycoprotein proteins i.e. the spike glycoprotein trimmer (S) which is present in all coronaviruses, and hemagglutinin-esterase (HE) which is only present among some coronaviruses and this nucleocapsid is embedded inside phospholipid bilayers .The envelope protein (E) and membrane (M) protein are located in viral envelope among the S proteins (Wu, Zhao, et al., 2020). The SARS-CoV-2 genome has terminal sequences at 5′ and 3′ end which are of β-CoVs as shown in figure 1B.

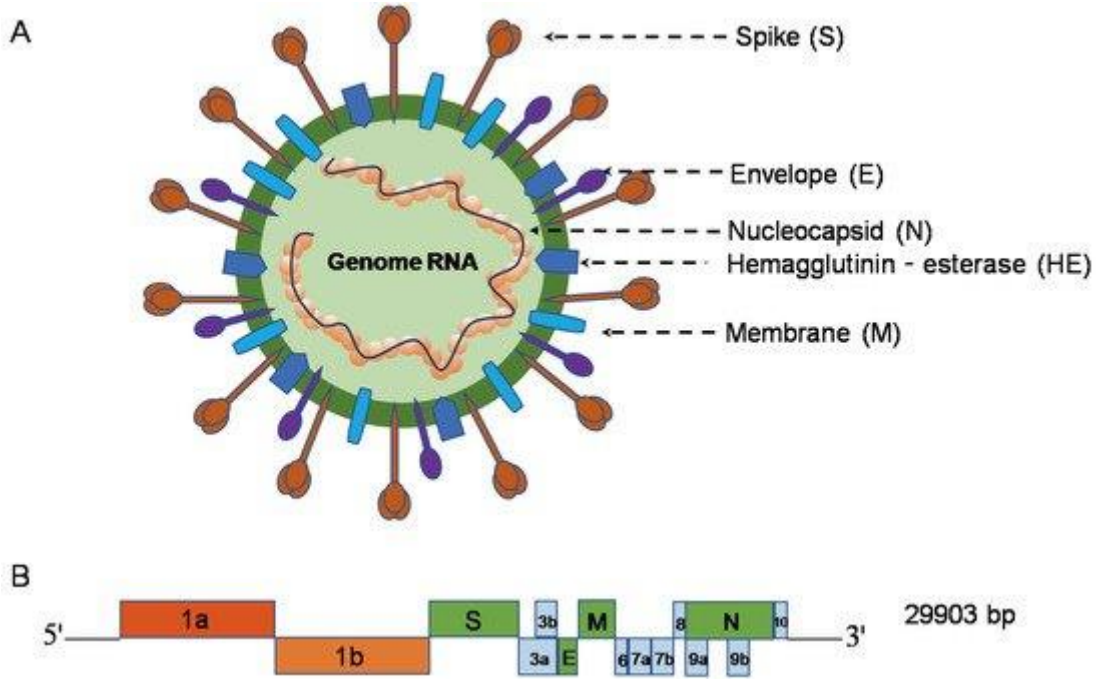


Figure 2.2 β-coronavirus particle and its genome (Jin et al., 2020) (A)
5′ and 3′ terminal sequences of the SARS-CoV-2 genome (B)

2.10. Antibody Response

Experience taken from other species of human coronavirus prescribes that immunity is developed partially after infection. However, this immunity is of short duration usually lasting for a year or two (A. T. Huang et al., 2020). There is very little data found for SARS-CoV-1 which shows that neutralizing antibodies have capacity which can stop viral infection. These antibodies remain effective up to a duration of 17 years

after infection (Anderson et al., 2020). Clinically done, some studies suggest that the onset of response generated by any antibody after acute infection of SARS-CoV-2 seems to be the same as in the case of various kinds of human coronaviruses. Antibody response is generated to counter the nucleocapsid (N) and spike (S) proteins in which the receptor binding domain is present on the spike protein of S1 subunit. Antibodies have different responses against different antigens and have different neutralizing effects. A study done on research of vaccines and its medicinal use of recovered plasma existence of neutralizing antibodies (nAb) has been shown greatly (A. T. Huang et al., 2020). Lessons from previous epidemiological studies about MERS-CoV and SARS-CoV-1 indicate that there is a decline in the protection level of the population from reinfection within a short time duration but it depends on initial disease severity (Kellam & Barclay, 2020). Neutralizing antibodies will serve as an effective key measure to fight with viral infections like SARS-CoV-2. Variability and immunity role of antibodies for the population in the long term needs to be deliberated thoroughly (A. T. Huang et al., 2020). Moreover, perception of mechanistic correlation of the protective immunity in humans is found to be less prevalent which includes antibody titer and specificity required to offer cover (Tay et al., 2020)

2.11. Kinetics Involved in Antibody Response

2.11.1 Seroconversion time of antibodies

Numerous studies regarding seroconversion have given knowledge about seroconversion of IgG, IgA and IgA antibodies. The main results indicated here talk about isotopes of one kind of antibody. For IgG antibodies, after symptoms rise, 12-15 days was the mean and median time for antibodies (Borremans et al., 2020). Great variation was found in the first to last detection of IgG from 4- 73 days after symptoms onset. Methods of reporting varied by study (Gniffke et al., 2020). For IgM antibody, mean or median time to seroconversion ranged from 4-14 days after symptoms arose (Zhao et al., 2020). Variations in methods reported, quality of study and sample size which results in unreliability in findings. Seroconversion time duration for IgG was calculated in very few studies which ranges between 4-24 days after first appear of symptoms while most of them were ranged between 4-11 days (Herroelen et al., 2020) along with some deviations, which also include two studies about seroconversion from 24 days to first detection (Solbach et al., 2020; Sun et al., 2020)

2.11.2 Antibody dynamics over time

Dynamics of IgG antibody shows a sometime high titers, plateau, and prolonged existence with very low levels. When IgG antibody titers go to a maxima between 3rd and 7th week after rise of symptoms (Du et al., 2020). Few studies had reported no change in virus-specific IgG after the third week. The levels were not reported well after the peak value (Xu et al., 2020). In week eight, decreased antibody levels were reported after onset of symptoms according to two studies (Vabret et al., 2020).

IgM antibody dynamics show sometimes spike and sometimes downfall with a peak recorded between 02 to 05 weeks after symptoms being shown then decrease with time to below the limit of detection (Q. Li et al., 2020). After the peak, reported level of IgM was consistently decreasing from start of two to three weeks (Padoan et al., 2020) to the end of eight weeks (C. C. Jin et al., 2020) after onset of symptoms with most of the studies have reported this decline between three to five weeks (Xie et al., 2020).

Very few studies demonstrate IgA dynamics in comparison to IgG and IgM antibodies. Levels of IgA titer are highly described between 16-22 days after symptoms rise. However, no agreement on shift over time has been reached (To et al., 2020).

2.12. Protective Immunity

2.12.1 Neutralizing antibody kinetics

After the disease onset neutralizing antibodies were detected in 07 to 15 days continuously increases between 14–22 days before plateauing (Espejo et al., 2020) and declines in a period of six weeks (Brochot et al., 2020). Several studies suggest specific neutralizing antibodies. Majority of these studies have diversity between assay limits, comparison between findings and of variable but moderate quality. Rogers et al. conducted a study about a significant characteristic of receptor binding domain antibodies in neutralization in pseudovirus assay with the help of a re-challenge model which is a very effective model of an animal which supports the findings (Rogers et al., 2020). Findings also indicated that SARS-CoV-2 infection posits powerful immunity against the glycoprotein (S) protein. Few antibodies were neutralizing, in line with other findings (Cao et al., 2020).

Some of the studies reported B cell responses thoroughly. Nineteen hospitalized patients were studied by Galson et al, the study demonstrated well about induction of

memory response by B cells and also clonal expansion, but primary expansion was seen in the population of naïve B cells (Galson et al., 2020).

2.12.2 Antibodies correlation with viral load

Few studies indicated strong relationship between IgG antibodies of SARS-CoV-2 and viraemia and co-existence of viral RNA and antibodies or the co-existence viral RNA and antibodies (Brochot et al., 2020; Y. Jin et al., 2020). According to one cohort study, the presence of anti-N IgG SARS-CoV-2 was in correlation with reduced viral load (Bryan, Fink, et al., 2020). Another cohort study had the same findings but quantification of viral load was not discussed (Zhao et al., 2020). Findings from both of these studies suggests that RNA detection persists despite clinical recovery and in the convalescent phase viral loads were generally reduced. Also detectable specific IgG antibodies of SARS-CoV-2 and detectable RNA of SARS-CoV-2 coincide with each other and they could be present in fewer patients until 50 days following seroconversion (Wang et al., 2020).

2.12.3 Reappearance of SARS-CoV-2 infection

Few research articles are relevant to the reappearance of SARS-CoV-2 which dealt with seven animal studies. These studies were of varying quality and very limited. Two themes were broadly deliberated in which exposure after getting primary infection with SARS-CoV-2 (Brocato et al., 2020) following re-exposure and passively transferring of neutralizing antibodies . Re-challenged time of primary infection differing between 20-43 days after inoculation. One study about nine macaques (Ryan et al., 2020) demonstrated the protection from infection to some extent and indicated a significant reduction in viral titer and minimum clinical symptoms (Chandrashekar et al., 2020).

2.12.4 Cross-reactivity of SARS-CoV 2 with other coronaviruses

Abundant evidence was not found about cross reactivity of antibodies specific to other coronavirus (Prévost et al., 2020). Several in vitro studies have been done by using a variety of assays which explored cross reactivity of antibody binding and their response and cross neutralization which occurred between SARS-CoV-2 and MERS-CoV, HCoVs and SARS-CoV-1. Highest antibody-binding response due to cross reactivity seems to be in SARS-CoV-1 and SARS-CoV-2 (Wu, Wang, et al., 2020). While in one study it is found that HCoVs are abundant in the human population and

serum exposure to HCoVs is 10% and it demonstrates again cross reactivity with small scale neutralization activity (Chaolin Huang et al., 2020).

CHAPTER III

Methodology

3.1. Study Site

This study was conducted at Jinnah hospital Lahore and government quarantine center Lahore in province of Punjab, Pakistan from November 10, 2020, to February 10, 2021. Samples were collected from patients visiting the hospital and quarantine center of different communities with symptoms of fever, dry cough, tiredness and shortness of breath by taking informed consent from every patient and from the guardian in case of a child.

3.2. Inclusion Criteria

Patients visited Jinnah hospital and government quarantine center from November 10,2020 to February 10, 2021, with the suspected symptoms and those who were willing to take part in this study were enrolled in this study.

3.3. Exclusion Criteria

Patients with no suspected symptoms of COVID and those who do not want to participate in this study were excluded from this study.

3.4. Sample Collection

Blood samples were taken by venipuncture technique from the symptomatic patients. (Sign and symptoms of SARS CoV2 difficulty breathing that lasts for 2 weeks, loss of taste, fever and taste).

3.5. Storage

Samples that were collected from patients inside the quarantine center were transported to the Wah Clinical Laboratory, Wah Cantt by storing the samples at a temperature of 2-8^{o}C for no longer than 48 hours.

3.6. Serum Separation

For serum separation about 3ml of whole blood was taken aseptically by venipuncture technique and put into serum separator tube or gel tube for isolation of serum. After clotting, the serum was separated by centrifugation at 3000 rpm for 5 minutes.

3.7. Test Kit

The test kit used for Elisa was **OmniPATHTM COVID-19 Total Antibody ELISA Test** kit manufactured by **Thermo Fisher Scientific company, United States.** The intended use of this kit is for detection of total antibodies (including IgM, IgA and IgG) qualitatively to SARS-CoV-2 in human serum. The total antibodies test detects all types of antibodies which includes IgA, IgG, and IgA. **OmniPATHTM** measures antibodies that bind to the spike glycoprotein (S protein) which is the major surface protein that SARS-CoV-2 uses to bind to a receptor and invade cells. Specifically, it targets the RBD of the S1 subunit of the S protein, which is the major target of neutralizing antibodies. Targeting the RBD- a unique region with less similarity to the other coronaviruses and it helps to eliminate cross reactivity and improve assay specificity. According to conventional knowledge we came to know that IgM is a marker of an early antibody response while IgG denotes a late response. However, in COVID-19 infections it has been shown that IgM appears at about the same time when IgG appears thus, the rationale for total antibody testing.

3.8. ELISA Machine

The ELISA machine used in testing was **Dynex AGILITY automated ELISA machine** made by Dynex Technologies, United States. This machine has a flexible throughput that allows up to12 plates on board at once and it has a high precision due to the electronic processing that provides premium precision with minimal manual operations.

3.9. Procedure of Test Followed

ELISA test was performed by following procedure which was according to the test protocol. Reagents were brought to the temperature of 15-30^{o}C for a time duration of at least 30 minutes before use. For quality control, two positive, two negative controls were applied per run. For control wells 50ul of control solution was directly applied to individual wells and for sample wells 50ul of sample serum was applied to each well. Mixing was done by shaking softly and then microplate wells were covered with an adhesive sealer. Incubation was done for a time duration of 30 minutes at 37^{o}C. Liquid in the wells were discarded and each well was filled with a wash buffer with the amount of 300ul. After that the wash buffer was left for 5 seconds in each well of microplate and then the wash buffer was removed from the wells. 100ul of Enzyme Conjugate was added to each well of microplate with the same amount and in the same way as

the samples. Microplate was covered with a sealer adhesive in nature and incubation was done for a time duration of 30 minutes at 37ºC. Wash the microwells again as described before Chromogenic Reagent A in the amount of 50ul and 50ul of Chromogenic Reagent B was added in each well with the same amount and in the same way as the addition of patient specimens. Again, mixing was done by gentle shaking microwells that were covered with adhesive sealer. Incubation was done for 30 minutes at 37ºC protected from sunlight. After that 50ul of stop solution was added to each well with the same amount and in the same way as the Chromogenic Reagents. Microwells were taped well to make sure that the reagents were mixed thoroughly. At the end microwell readers were set to a wavelength of 450nm for measurement of optical density with the help of spectrophotometer of each well against the mean of two Reagent Blank wells. Microplate was readed within a time duration of 10 minutes after the stop solution was added. Specimen results were determined using specimen index ratio. If the specimen index ratio was <1.0 the result was considered as negative and if it was >1.0 then it was considered as a positive result.

3.10 Statistical analysis

3.10.1 Calculation of P value

We calculate the P values by making relationship between the number of male and female patients and their antibody titer in cities Lahore, Sialkot and Gujrat to know that how much probability of antibody titers are there in male and females among cities.

We also calculate the P values by making relationship between total male and female patients in the study and their respective antibody titers. P values calculation can be seen in chapter IV.

3.10.2 Probabilities

We calculated the probabilities by making correlation between no. of total patients and reactive male and female patients to find out how much chance of coronavirus was there in reactive patients. Also, we calculated probabilities between total no. of patients and non-reactive male and non-reactive female patients to find out that how much chance was there of no infection in the non-reactive patients.

3.10.3 Regression line and Correlation graph

Regression line and Correlation graph was made between no. of patients in different age groups and no. of reactive patients to calculate the estimated value of correlation which shows about strength of correlation between these two variables and also tells us that how they are dependent on each other.

3.10.4 Box Plot or Whisker Plot

Box plot graph of antibodies was made to find out the quartiles and percentile distribution of antibodies in four quartiles of box plot.

CHAPTER IV

Findings and Results

In the present research study about 919 SARS-CoV-2 suspected patients were enrolled based on their history and signs and symptoms from different areas of Punjab Pakistan. Patients were enrolled not considering their age and gender. Among these patients 42% (n=382) were adult females and 43% (n=399) were adult males. Among children category from 1to15 years old we found 15% (n=138) of children as depicted in (table 1.1) and (Figure 1.1)

Table no 4.1 Total participants enrolled in this study

Total Participants	Male participants	Female participants	Children participants
919	43% (n=399)	42% (n=382)	15% (n=138)

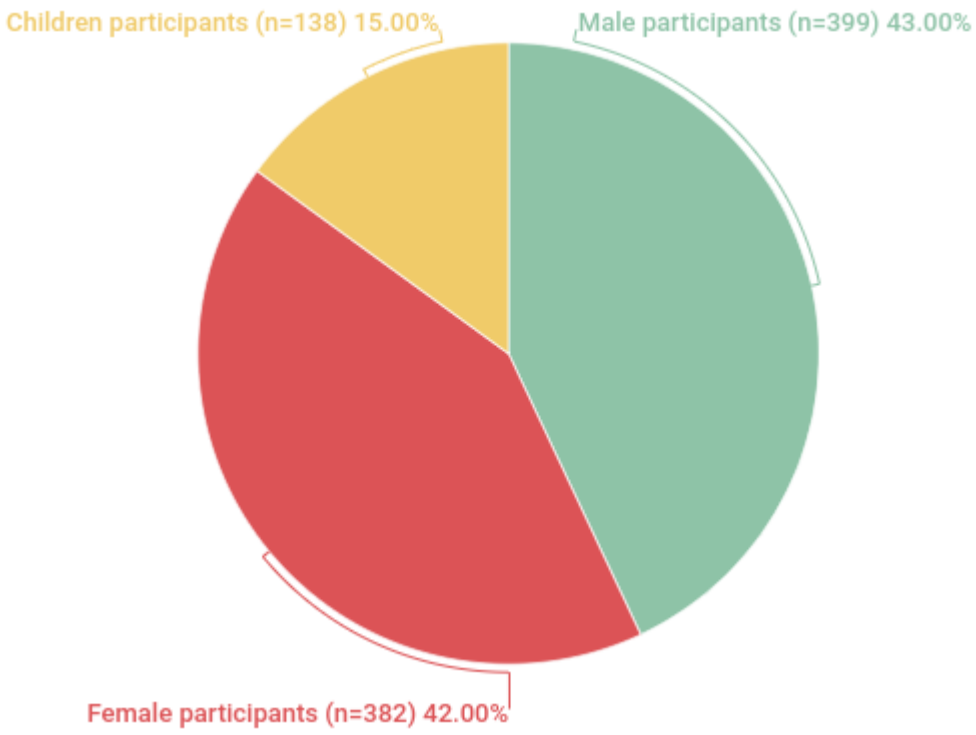


Figure 4.1 Total participants enrolled in this study

4.1. Clinical Profile of Study Subjects

About 50% (n=450) of participants were having dry coughs with or without complaints and 27% (n=250) were having symptoms of fever with or without complaints. About 12% (n=112) of participants were having symptoms of sore throat and headaches, 5% (n=48) of participants were having symptoms of tiredness and body pain and 4% (n=42) of participants were having symptoms of shortness of breath and 2% (n=17) of participants were asymptomatic. The duration of illness ranges from 1 day to 15 days as shown in (Table 4.2) and (Figure 4.2)

Table 4.2 Clinical profile of study subjects

Disease	**Associated with complaint**	**Only complaint**
Dry cough	26%	24%
Fever	21%	6%
Sore throat and headache	4%	1%
Tiredness and body pain	3%	1%
Asymptomatic	2%	

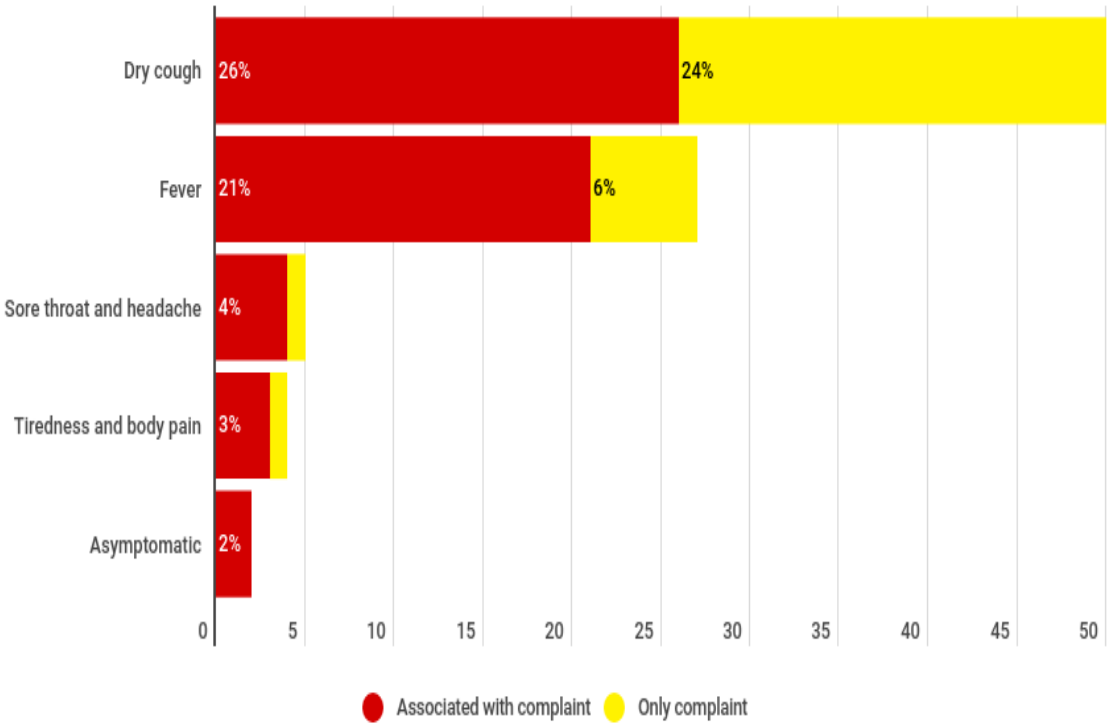


Figure 4.2 Clinical profile of study subjects

4.2. Total Antibody Testing

Participants were tested for SARS CoV2 total antibodies (IgA, IgG, and IgM) by using the ELISA method. Result of the test was the titer of total antibodies as IgM and IgG develop in SARS-CoV-2 patients at the same time, so we went for total antibody testing.

4.3. City Wise Distribution of Cases across Punjab Pakistan

Across Punjab, Pakistan samples were taken from three different cities which were Lahore, the capital of Punjab, Sialkot and Gujrat. Out of 84% (n=771) total reactive patients, the highest number of patients were recorded from Lahore which were 57% (n=442). From Sialkot 25% (n=194) positive cases were recorded and 18% (n=135) were recorded from Gujrat. Tabular form and graphical form are as below in (Table 4.3) and (Figure 4.3)

Table 4.3 City wise distribution of cases across Punjab Pakistan

Total SARS CoV2 positive cases	Lahore	Sialkot	Gujrat
84% (n=771)	57%(n=442)	25%(n=194)	18%(n=135)

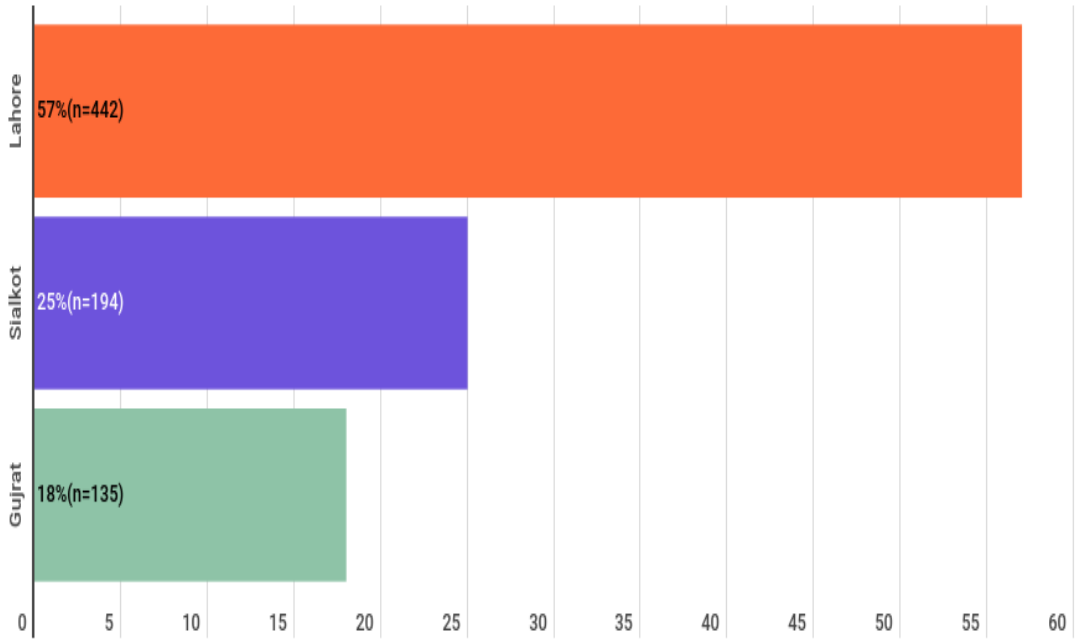


Figure 4.3 City wise distribution of cases across Punjab Pakistan

4.4 Calculation of p value:

4.4.1 Lahore city (males):

Average antibody titer of 442 patients (null hypothesis H_o) = μ = 74.669

No. of male patients n = 204

Average antibody titer of male patients $\bar{x}$ = 9.625

Standard deviation σ = 20.864

Confidence = 0.95

Significance level α = 1-C (1-0.95) = 0.05

Z value (Z_c) = $\bar{x}-\mu_o / S/\sqrt{n}$

Z = - 44.525

P value <0 .0001 which is significant if P< .005 and we reject the null hypothesis that average antibody titer of males in Lahore city is 74.669 but its more than that.

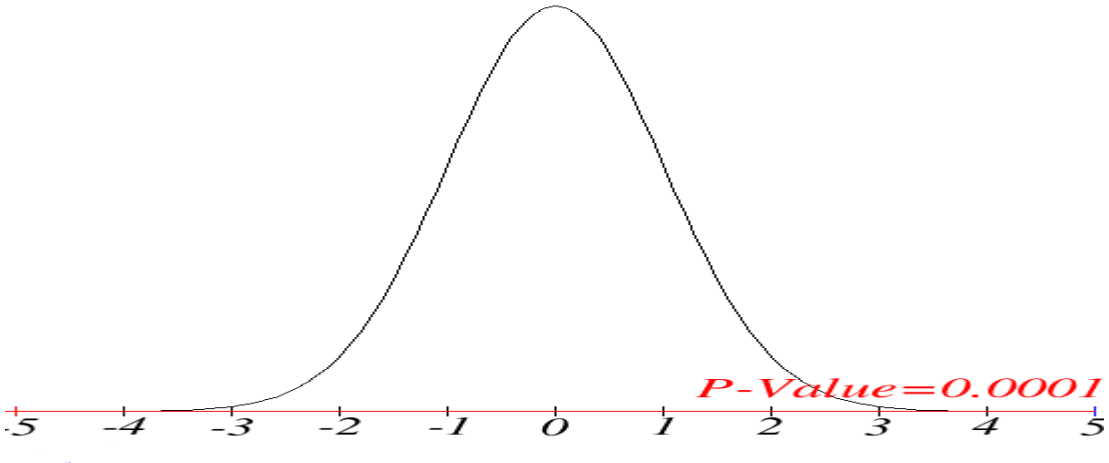


Figure 4.4.1 P value of antibody titers among male in Lahore city

4.4.2 Lahore city (females)

Average antibody titer of 442 patients (null hypothesis H_o) = μ = 74.669

No. of female patients n = 238

Average antibody titer of female patients $\bar{x}$ = 8.653

Standard deviation σ = 19.457

Confidence = 0.95

Significance level α = 1-C (1-0.95) = 0.05

Z value (Z_c) = $\bar{x}-\mu_o / S/\sqrt{n}$

Z = - 52.341

P value <0.0001 which is significant value, and we reject null hypothesis that average antibody titer in females of Lahore city is 74.669 but its more than that.

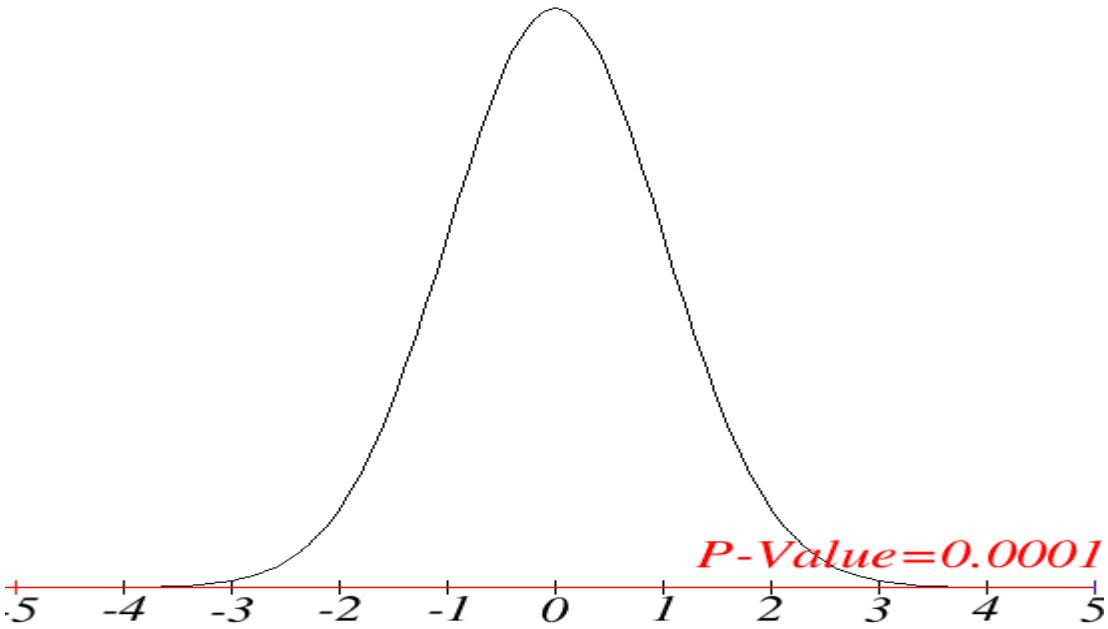


Figure 4.4.2 P value of antibody titers among female in Lahore city

4.4.3 Sialkot city (males)

Average antibody titer of 194 patients (null hypothesis H_o) = μ = 9.995

No. of male patients n = 90

Average antibody titer of male patients $\bar{x}$ = 7.372

Standard deviation σ = 18.687

Confidence = 0.95

Significance level α = 1-C (1-0.95) = 0.05

Z value (Z_c) = $\bar{x}-\mu_o / S / \sqrt{n}$

Z = - 1.332

P value is 0.182 which is greater than .05 and result is not significant, and we accept null hypothesis that at confidence level of 95% average antibody titer in males of Sialkot city is 9.995.

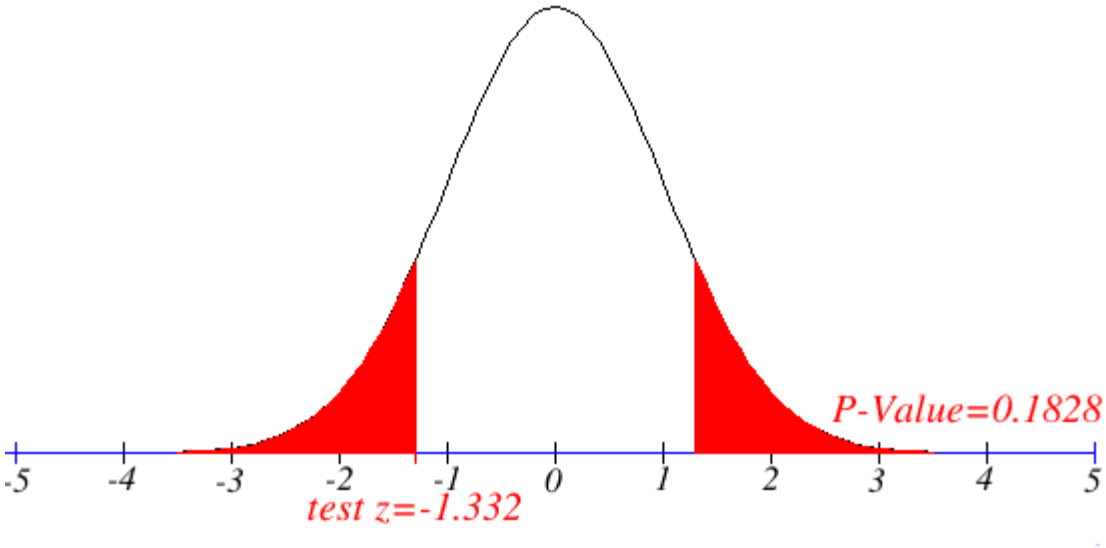


Figure 4.4.3 P value of antibody titers among male in Sialkot city

4.4.4 Sialkot city (females)

Average antibody titer of 194 patients (null hypothesis H_o) = μ = 9.995

No. of female patients n = 104

Average antibody titer of female patients $\bar{x}$ = 9.989

Standard deviation σ = 22.560

Confidence = 0.95

Significance level α = 1-C (1-0.95) = 0.05

Z value (Z_c)= $\bar{x}-\mu_o / S/\sqrt{n}$

Z = - 0.00262

P value = 0.998 which is not significant, and we can say that at 95% confidence level average antibody titer of females of Sialkot city is 9.995 accepting the null hypothesis.

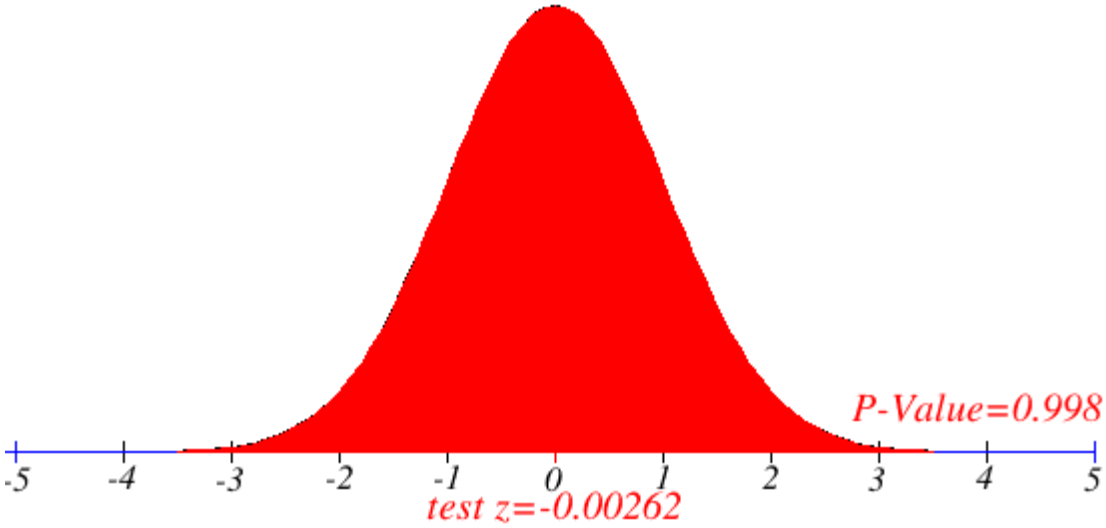


Figure 4.4.4 P value of antibody titers among female in Sialkot city

4.4.5 Gujrat city (males)

Average antibody titer of 135 patients (null hypothesis H_o) = μ = 8,283

No. of male patients n = 80

Average antibody titer of male patients $\bar{x}$ = 5.482

Standard deviation σ = 17.0992

Confidence = 0.95

Significance level α = 1-C (1-0.95) = 0.05

Z value (Z_c) = $\bar{x}-\mu_o / S / \sqrt{n}$

Z = - 1.465

P value = 0.143 which is not significant, and we can say that at 95% confidence level average antibody titer of males of Gujrat city is 8.283 accepting the null hypothesis.

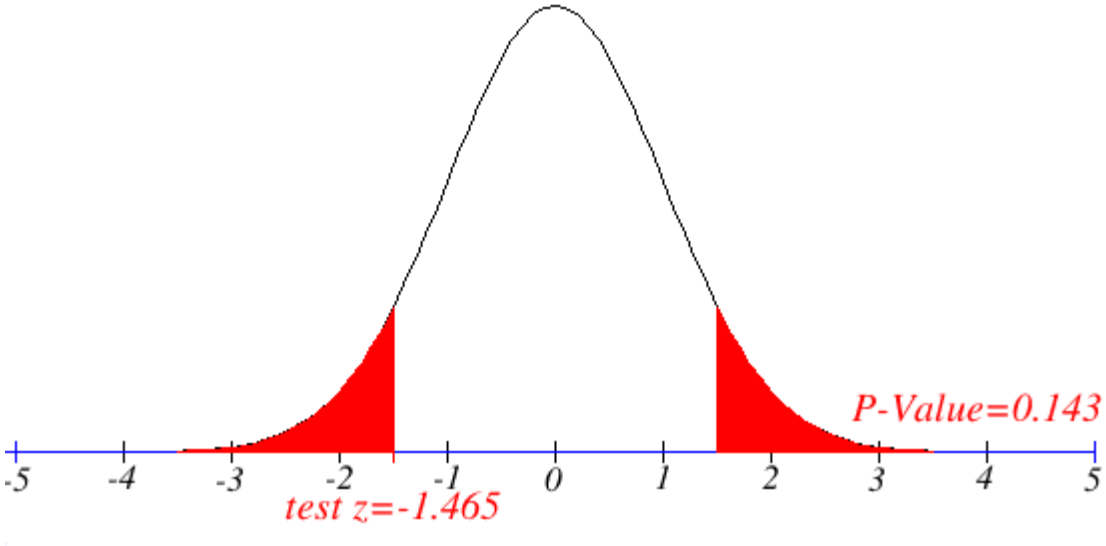


Figure 4.4.5 P value of antibody titers among male in Gujrat city

4.4.6 Gujrat city (females)

Average antibody titer of 135 patients (null hypothesis H_o) = μ = 8.283

No. of female patients n = 55

Average antibody titer of female patients $\bar{x}$ = 2.244

Standard deviation σ = 2.7996

Confidence = 0.95

Significance level α = 1-C (1-0.95) = 0.05

Z value = $Z_c = \bar{x}-\mu_o / S/\sqrt{n}$

Z = - 15.995

P value < 0.0001 which is significant value, and we reject null hypothesis that average antibody titer in females of Gujrat city is 8.283 but its more than that.

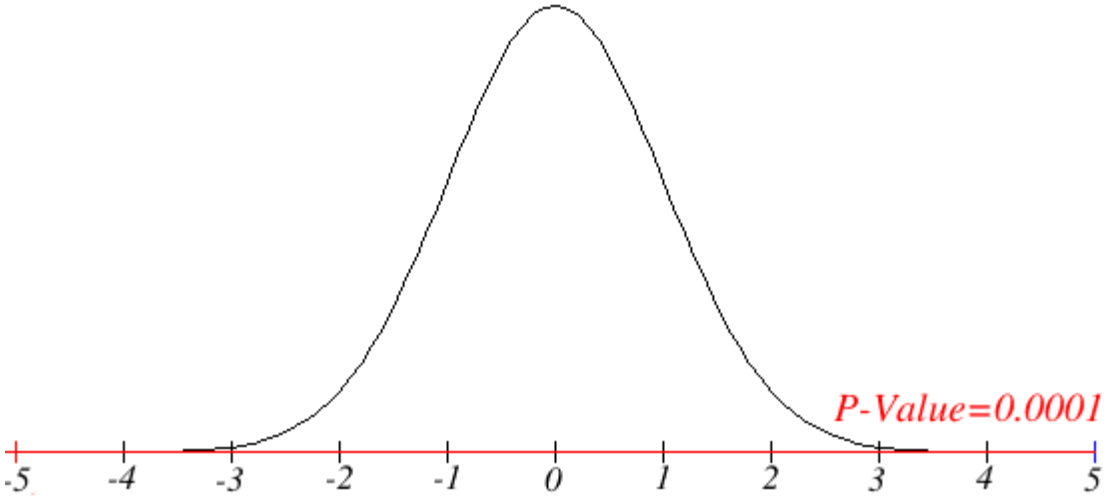


Figure 4.4.6 P value of antibody titers among female in Gujrat city

P values of antibody titer according to city and gender wise are compiled in following table.

Cities	Total reactive patients	Males reactive	P value	Females reactive	P value
Lahore	442	204	0.00001	238	0.00001
Sialkot	194	90	0.1835	104	0.998
Gujrat	135	80	0.141	55	0.00001

Table 4.4 P values of antibody titers in male and females in cities

4.5. Gender Wise Distribution of Reactive Subjects

We made a gender wise distribution according to which out of 84% (n=771) positive reacted participants 40% (n=313) of adult males and 43% (n=337) adult females were reactive against SARS CoV2. Among children 15% (n=121) were reactive against SARS CoV2. Results are declared in tabular form (Table 4.4) and (Figure 4.4)

Total reactive patients	Total non-reactive patients	Male Adult patients reactive	Female Adults patients reactive	Total children reactive
771(84%)	148(16%)	313(41%)	337(43%)	121(16%)

Table 4.5 Gender wise distribution of positive SARS-CoV-2 cases

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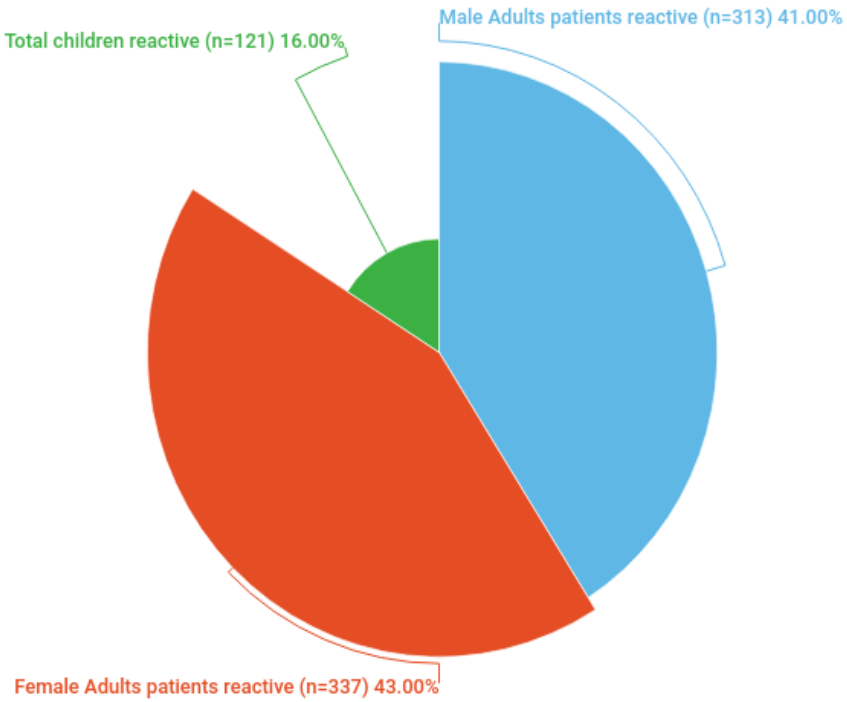

Figure 4.5 Gender wise distribution of positive SARS CoV2 cases

4.6. P value calculation gender wise

4.6.1 For Male patients

Average antibody titer of 919 patients μ (null hypothesis) = 105.243

No. of male patients n = 399

Average antibody titer of male patients $\bar{x}$ = 95.23

Standard deviation $\sigma = 212.716$

Confidence level = 0.95

Significance level $\alpha = 0.05$

Z value $(Z_c) = \bar{x}-\mu_o / S/\sqrt{n}$

Z = -0.940

P value = 0.347 which is not significant, and we can say that at 95% confidence level average antibody titer of male patients is 105.243 accepting the null hypothesis.

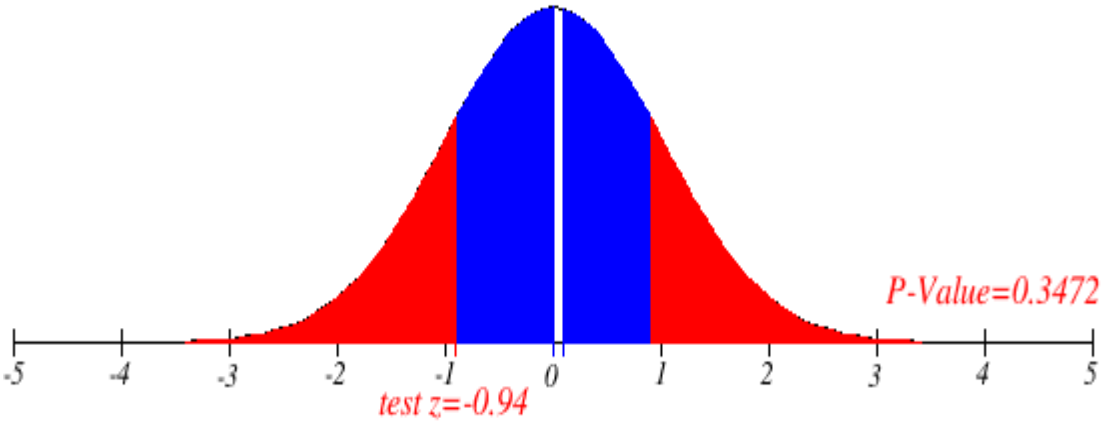

Figure 4.6.1 P value of antibody titers in males out of all patients

4.6.2 P value for female patients

Average antibody titer of 919 patients μ (null hypothesis) = 105.243

No. of female patients n = 382

Average antibody titer of male patients $\bar{x} = 95.23$

Standard deviation $\sigma = 212.716$

Confidence level = 0.95

Significance level $\alpha = 0.05$

Z value $(Z_c) = \bar{x}-\mu_o / S/\sqrt{n}$

Z = -0.940

P value = 0.3804 which is not significant, and we can say that at 95% confidence level average antibody titer of male patients is 105.243 accepting the null hypothesis.

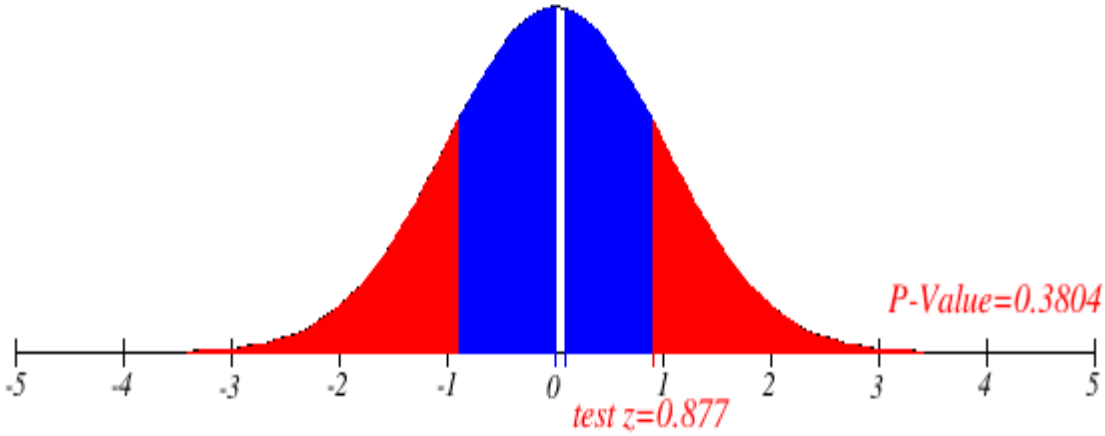

Figure 4.6.2 P value of antibody titers in females out of all patients

The P values of male and female out of total patients are compiled in the following table.

Total patients	Male patients	P value	Female patients	P value
919	399	0.347	382	0.877

Table 4.6 P values of antibody titers gender wise out of all patients

4.7. Conditional Probability among positive and negative cases

Total reactive patients	771
Total non-reactive patients	148
Male Adult reactive	313
Probability (Male reactive)	0.40
Male Adult non-reactive	86
Probability (Male non-reactive)	0.57
Female Adult reactive	337
Probability (Female Adult reactive)	0.43
Female Adult non- reactive	45
Probability (Female Adult non-reactive)	0.30
Children reactive	121
Probability (Children reactive)	0.15
Children non- reactive	17
Probability (Children non-reactive)	0.11

Table 4.7 Probability among Positive and Negative Coronavirus cases

4.7.1 Probability of Male reactive patients

P (Male | Reactive) = P (Male ⋂ Reactive) / P (Reactive) = (313/919) / (771/919) = 0.40 x 100 = 40% chance of coronavirus infection

4.7.2 Probability of Male non- reactive patients

P (Male | Non- Reactive) = P (Male ⋂ Non- Reactive) / P (Non-Reactive) = (86/919) / (148/919) = 0.57 x 100 = 57% chance of not having coronavirus

4.7.3 Probability of Female Reactive patients

P (Female | Reactive) = P (Female ⋂ Reactive) / P (Reactive) = (337/919) / (771/919) = 0.43 x 100 = 43% chance of coronavirus infection

4.7.4 Probability of Female Non- reactive patients

P (Female | Non- Reactive) = P (Female ⋂ Non- Reactive) / P (Non-Reactive) = (45/919) / (148/919) = 0.30 x 100 = 30% chance of no coronavirus infection

4.7.5 Probability of Children Reactive patients

P (Children | Reactive) = P (Children ⋂ Reactive) / P (Reactive) = (121/919) / (771/919) = 0.15 x 100 = 15% chance of coronavirus infection

4.7.5 Probability of Children Non- reactive patients:

P (Children | non- reactive) = P (Children ⋂ non- reactive) / P (non- reactive) = (17/919) / (148/919) = 0.11 x 100 = 11% chance of no coronavirus infection

4.8. Age Wise Distribution of Subjects

We also made age wise distribution among participants to find out how many cases were present in different age groups. We found 11% (n=80) reactive patients in age group (1-10), 10% (n=76) were reactive in age group (11-20), 13% (n=103) were reactive in age group (21-30), 16% (n=124) were found reactive in age group (31-40), highest no of patients 17% (n=132) were found reactive in age group (41-50), 16% (n=126) were reactive in age group (51-60), 11% (n=88) of patients were reactive in age group (61-70). In age group (>71), 6% (n=42) of patients were recorded positive. These results are declared in tabular form (Table 4.6) and (Figure 4.6) below

Age Groups	No of Patients	Reactive	Percentage %
1-10	88	80	11%
11-20	83	76	10%
21-30	137	103	13%
31-40	153	124	16%
41-50	151	132	17%
51-60	160	126	16%
61-70	103	88	11%
>71	44	42	6%

Table 4.8 Age wise distribution of subjects

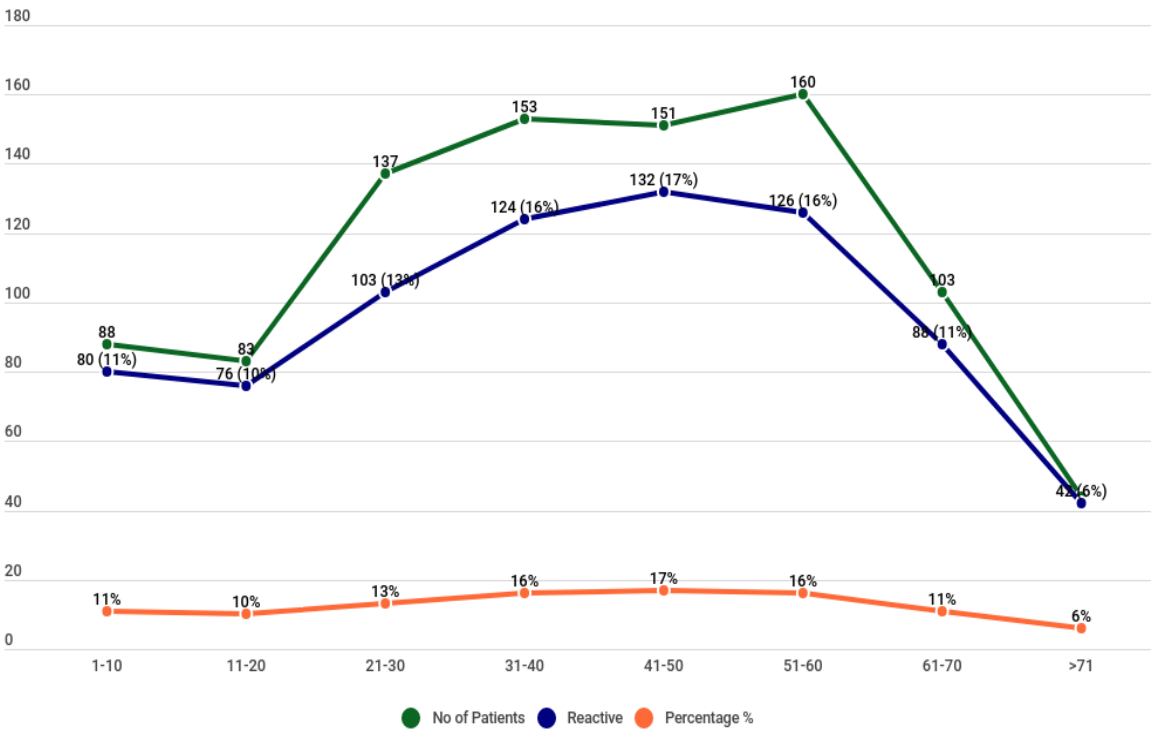

Figure 4.8 Age wise distribution of subjects

4.9. Regression Line Graph Between No. of Patients in Eight Age Groups and No. of Reactive Patients

Age Groups	No of Patients	Reactive
1-10	88	80
11-20	83	76
21-30	137	103
31-40	153	124
41-50	151	132
51-60	160	126
61-70	103	88
>71	44	42

Table 4.9 Regression line graph table

Regression line graph below tells us the estimated value of dependent variable (Y) for the values of independent variable (X) where,

Y = No. of patients in eight different age groups

X = No. of reactive patients

R^2 = Coefficient of correlation

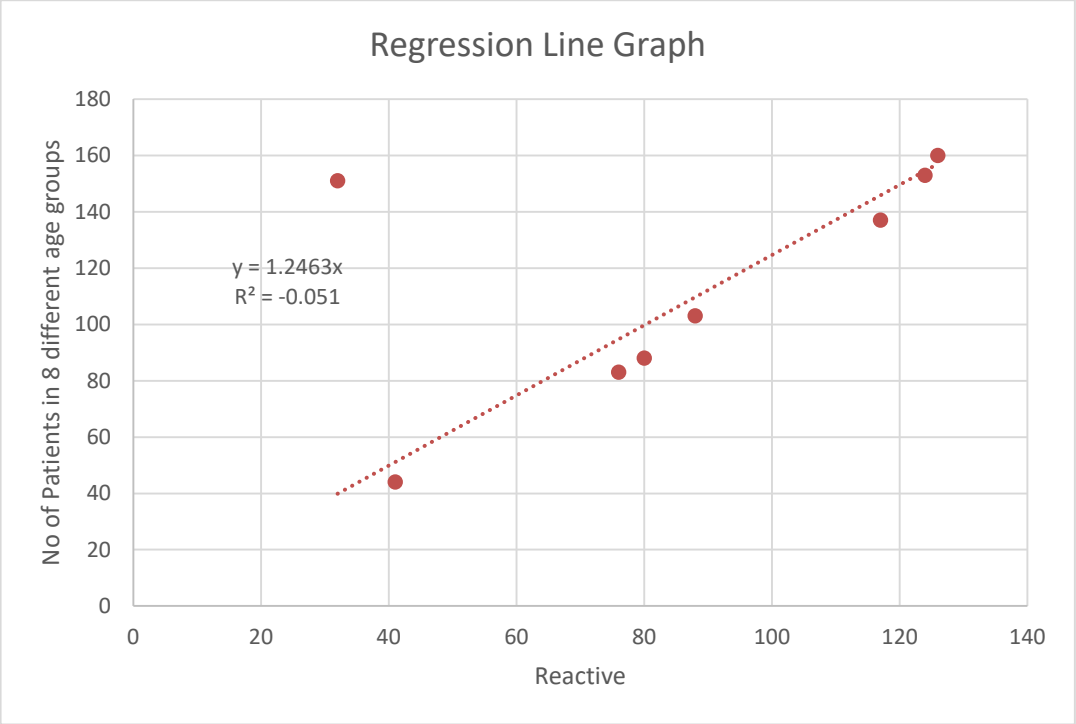

Figure 4.9 Regression line graph

Y= 1.2463x

$R^2 = 0.8913$

4.10. Correlation Graph Calculation Between Total Patients and Reactive Patients

X values represents reactive patients.

Y values represents total number of patients.

M_x represents mean of X values.

M_y represents mean of Y values.

$(X - M_x)^2$ and $(Y - M_y)^2$ represents deviation scores.

$(X - M_x)(Y - M_y)^2$ represents product of deviation scores.

For X values

$\sum$=684

Mean = 85.5

$\sum (X - M_x)^2 = SS_x = 9084$

For Y values

$\sum (Y - M_y)^2 = SS_y = 12186.875$

X and Y combined

N = 8

$\sum (X - M_x)(Y - M_y) = 5634.5$

Value of R calculation

$r = \sum ((X - M_y)(Y - M_x)) / \sqrt{((SS_x)(SS_y))}$

$r = 5634.5 / \sqrt{((9084)(12186.875))} = 0.5355$

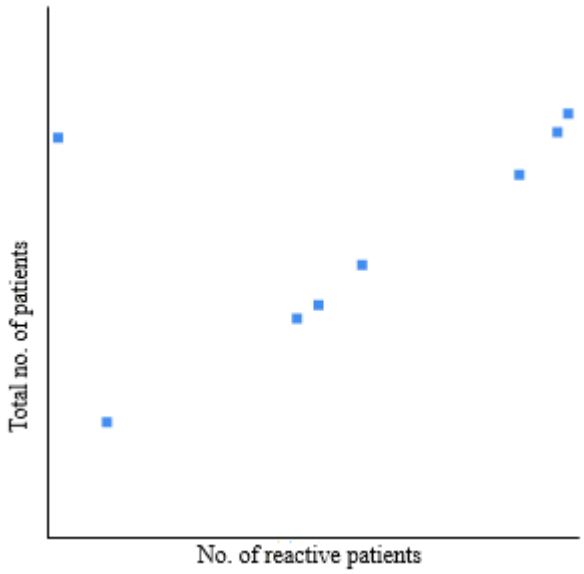

Figure 4.10 Correlation graph between total no. of patients and reactive patients

The value of R shows a positive correlation between X and Y values which means as the number of total patients increases the number of reactive patients also increases showing strong positive correlation.

4.11. Mean of Antibody Titer between Different Age Groups

We had taken the mean of antibody titer according to different age groups and we found the highest mean of antibody titer which was 171 in the age group (21-30) followed by the age group (11-20) and age group (31-40). The results are shown in (Table 4.9) and (Figure 4.9)

Age groups	**Mean of antibody titer (cut off value is 1)** **> 1 *Reactive*** **< 1 Non-reactive**
1-10	125
11-20	170
21-30	171
31-40	155
41-50	121
51-60	126
61-70	128
> 71	134

Table 4.11 Mean of antibody titer between different age groups

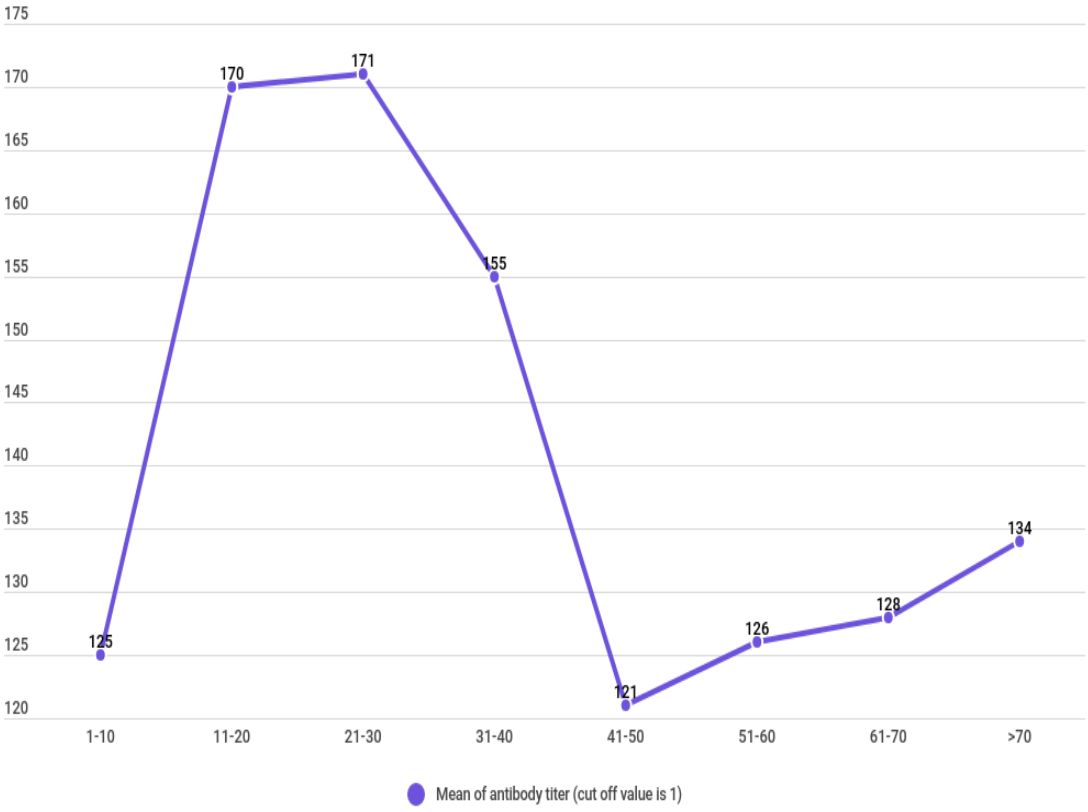

Figure 4.11 Mean of antibody titer between different age groups

4.12. Box or Whisker Plot of Antibody Titers

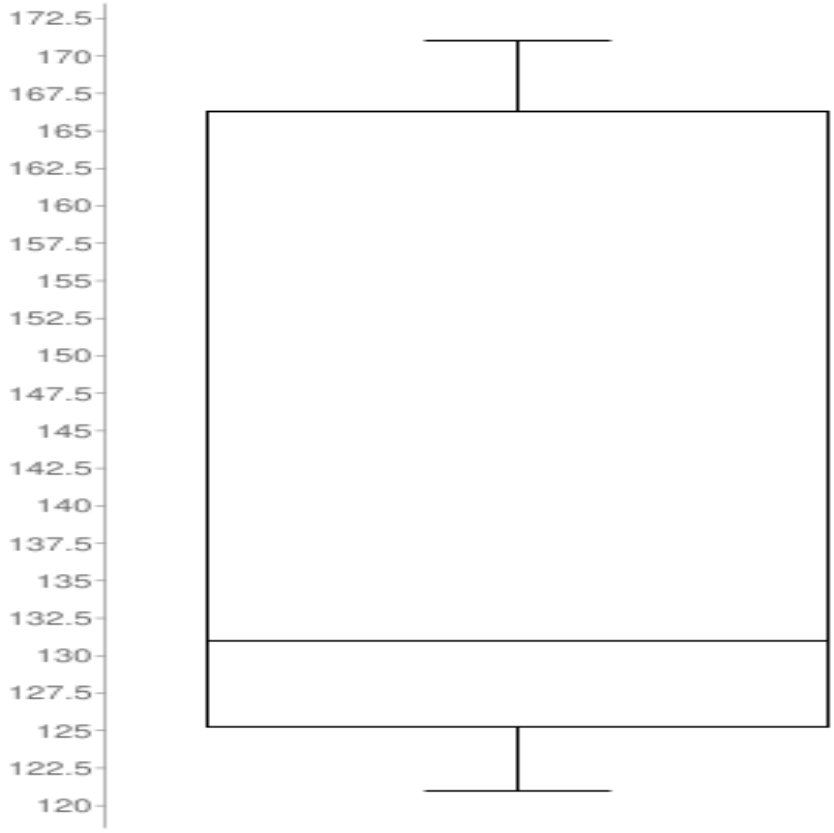

Figure 4.12 Box plot of antibody titers

Size of antibody titer	8
Median	131
Minimum	121
Maximum	171
First quartile	125.25
Third quartile	166.25

Table 4.12 Parameters of box plot

From the table 4.10 the given first quartile is 125.25 which means that 25% of antibody titers are below 125.25 and 75% of antibody titers are above than 125.25.

CHAPTER V

Discussion

Coronavirus belongs to a family of viruses which contain hundreds of variants. It may cause severe illnesses, and common cold leading to severe pneumonia, such as SARS-CoV-2 and Middle East Respiratory Syndrome (MERS) (Zaki et al., 2012). Chinese Center for Disease Control and Prevention first identified SARS-CoV-2 in Chinese Wuhan city (Zhu et al., 2020). Epidemiological (Chan et al., 2020; Herroelen et al., 2020) and clinical features of patients infected with SARS-CoV-2 have been reported recently.

Data from different laboratories of Pakistan were collected from August 30,2020 to November 30,2020, using ELISA method to found seroprevalence among the population of Punjab province, Pakistan in different age groups and it was found an approximately 83% seroprevalence of SARS CoV2 antibodies. This indicates a high prevalence among the population of Punjab province in Pakistan at the time of survey. According to the worldometer website, the country with the most confirmed COVID-19 cases is the United States with 27,444,459 almost half of which are in the state of New York. The United States is the most affected country in number of deaths as of February 10,2021 with 471,566 deaths. In Europe, the UK is at the top of the list with the highest death rate of 112,092 followed by Italy which has 91,003 total deaths. Spain is unfortunately following Italy's curve, with 2,971,914 cases and 61,386 deaths (https://www.worldometers.info/coronavirus/, accessed 10 February 2021) . In contrast to our studies, the SARS-CoV-2 report in Pakistan on February 10, 2021 showed that 559,093 of patients were tested positive for COVID-19 and 12,185 died (http://covid.gov.pk/, accessed 10 February 2021). This is a significantly very small number of cases and deaths as compared to Europe. This may be due to the fact that SARS-CoV-2 reached in Pakistan much later than Europe and other countries, so the government of Pakistan had made necessary precautionary safeguards to mitigate the outbreak and save its people in terms of imposing smart lockdown and quarantine centers. Also due to the unhealthy environment conditions in Pakistan people have perhaps very strong in-build immunity., As it started late in the country, masses living in various parts of Pakistan immediately received high doses of medicine. This factor

contributed a lot in developing strong immunity. It may have affected morbidity and mortality.

According to some U.S. studies which were accessible for comparison, used various methodology and measured seroprevalence during month of April 2020 found was Idaho; 4.7%, 1.8% in Boise, in Los Angeles, California; and 14.0% in New York (Bryan, Pepper, et al., 2020; Rosenberg et al., 2020; Sood et al., 2020). In comparison to our studies, we had estimated seroprevalence of 3 major big cities of Punjab, Pakistan. Lahore which is the capital of Punjab recorded a high prevalence of 57% which is very high as compared to New York, but we had collected samples at the peak time of the corona outbreak. Also, Lahore has not a healthy environment and it's a very highly populated city of Punjab. That's why people of Lahore were at great risk from infection with SARS-CoV-2. A study was done on seroprevalence in Faisalabad, Pakistan from April,2020 to May,2020 according to which overall prevalence was 17.18 and males were more effective than females (Raza et al., 2020). Our study suggests prevalence of Gujrat which was 18% and this is in accordance with the study done in Faisalabad. Both cities are similar in population wise as well as environmental conditions. Also, our study had done after this study at the time of peak outbreak, so it means that people of Faisalabad had developed antibodies earlier due to infection as compared to Gujrat and the outbreak in Gujrat had been less severe than Faisalabad.

According to the research article published in China, it was found that 56% of patients with SARS-CoV-2 were males in the city of Wuhan (Lu et al., 2020). Comparably, another article conducted on 140 patients in Wuhan found that males were 50.7% (Zhang et al., 2020). One study from the district of East Karachi in Pakistan defined the scenario of SARS-CoV-2 cases according to which males were found to be (64.6%) and females were (35.4%) (Tahir et al., 2020). The findings from our study are in accordance with these study results as our findings suggest that 43% of males and 41% of females were reactive against SARS-CoV-2. As per a retrospective study done by Jin et al., men developed dangerous diseases. Even though males and females had similar susceptibility, the death toll in males was higher than women by 2.4% (J. M. Jin et al., 2020). This is because of the correlation of particular organ failures depicted by clinical parameters in SARS patients with high frequency of ACE2 receptor (Yang et al., 2006; Yang et al., 2010). One study shows that circulating ACE2 levels are

higher in men than in women and in patients with diabetes or cardiovascular diseases (Patel et al., 2013)

According to the study done by Xu et al (Xu et al., 2020) on 745 children and 3174 adults who had close contact with diagnosed patients found that rate of positive cases reported in adult population was 2.7 percent higher than that in children. Another study reflected that the incidence of SARS-CoV-2 infection in children contacts was 13.2%, which was much lower than that in adults (Hua et al., 2020). Our results are in accordance with these findings and with Xu et al (Xu et al., 2020) and Lu et al (Lu et al., 2020). In our study the number of children with SARSCoV-2 was 16% which was 4 times less than adults. One research article explains about an enzyme named as angiotensin-converting enzyme 2 and transmembrane serine protease 2 (TMPRSS2) which is used by SARS-CoV-2 uses the cell receptor (ACE2) and for infecting cells. In children, the prevalence of TMPRSS2 and ACE2 receptors is very low in the upper and lower respiratory tract as compared to adults (Sharif-Askari et al., 2020). Lower expression of ACE2 and TMPRSS2 by tissues may be a possible reason that children as compared to adults have a minimal risk of infection (Grasselli et al., 2020; Guan et al., 2020).

According to one study conducted on 145 patients with SARS-CoV-2 disease suggests common symptoms in corona patients which included dry cough (81.4%), fever (75.2%), fatigue (40.7%) (Q. Chen et al., 2020). Similarly another study showed the clinical manifestations of coronavirus patients and that were fever in 87% of patients, dry cough in 65% of patients and fatigue in 425 of patients (Han et al., 2020). These results are in accordance with our findings as our study suggests 26% of patients with dry cough, 21% with fever and 3% with fatigue or tiredness.

A study based on population was recently carried out in Geneva for a time duration of five weeks between the months of April to May in 2020. This weekly study points out that there was an increase in seroprevalence from the first week to the fifth week during the survey; however, persons ranging between 20-49 years were at high risk (Perez-Saez et al., 2020). Another research report pointed out that 63% of the Pakistani population is relatively younger, e.g., 15-33 year age group and population under this age group is highly affected (Hafeez & Fasih, 2018; Zaheer Khan & Hafeez, 2017).This study is in accordance with our results as we also have 92% positive cases

of SARS-CoV-2 in age group of 11-20 years and 85% positive SARS-CoV-2 cases in age group of 21-30 years. This is due to the fact that most of Pakistani young population is linked with those professions which directly involves public dealing such as health care workers, laborers, transporters etc. so, they are at risk of developing anti-SARS CoV2.

According to one study, the highest death rate was found in the patients having the age of 60 years or more. Systematic analysis of 1.5 million of SARS-CoV-2 patients from different countries reiterates the effect of age on death rate with more relevant on age greater than 50 years especially, the age greater than 60 years (Bonanad et al., 2020). Another study revealed that data taken from Italy and China demonstrate a death rate of 2.3% in patients infected with SARS-CoV-2. Furthermore, studies indicate that the patients who are greater than the age of 50 years or older have a high death ratio (Porcheddu et al., 2020). According to the case series conducted from Northern side of Italy, patients with the age of 60 years or more have a high number of death rate which is 36% as compared to young patients which have a death rate of 15% (Grasselli et al., 2020).

Our results regarding age wise seroprevalence are in accordance with these studies in such a way that in our study age group (>71) has the highest number of seroprevalence which is 93%. It suggests that people with older age are at high risk of SARS CoV2. This may be due to the immune system of older people because as the body ages the immune system becomes weak, and it develops low levels of inflammation and SARS CoV2 could be pushing the already weakened immune system over the edge. Also, old age people have higher comorbidities such as obesity, diabetes and heart failure. That's why old age people have a higher chance of being affected by SARS CoV2 as compared to other age groups.

CHAPTER VI

Conclusion

A high seroprevalence of 57% was recorded in the city of Lahore in Punjab province, Pakistan. There was not a big difference in SARS-CoV-2 cases among males and females and both were equally found to be susceptible with SARS-CoV-2. Patients of age between 31 to 60 years had recorded a high number of cases. However, children were found to have a smaller number of cases or less susceptibility to SARS-CoV-2 infection. Highest number of antibody titers were found in patients with age between 11 to 30 showing that their immune response against the virus was very strong.

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