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COMPARISON OF COVID19(SARS-COV-2) INFECTION, BASED ON GENDER, AGE GROUP AND SEQUENTIAL WAVES BY RT-PCR ANALYSIS AMONG THE POPULATION OF WESTERN DISTRICTS OF ODISHA

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ABSTRACT
As SARS-CoV-2 has rapidly spread all over the world, it is evident that patient's gender, age, and comorbidity might render these patients more vulnerable to either increased mortality or increased risk of infection. In the first wave of pandemic, percentage of infection was 5% in May 2020, which rose to 23% in August. Towards the end of the year, it was around 1.8%. In second wave, infection gradually increases to 3.4% in the later part of March 2021, reaching maximum of 31% in April. of the recorded positive cases, infection rate in male population was comparatively higher them the female. In September 2020 where the rate of infection was highest in the first wave the male infectivity rate is 16% compared to 7.13% in females. In April 2021, male infection rate was at 21% compared to 9.7% in the female population. The positivity rate in male population was at 3.7% in third wave of infection. During this period the positivity rate in female population was at 1.87%. Our study shows the positivity rare was maximum in the age group of 18-29(25.5%) followed by 30-39(23.5%) and 40-49(17.2%) in 2020. Similar trend was observed in the year 2021 where the infection rate was 24.2%,23.6%,18.46% in the age group of 18-29,30-39 and 40-49 respectively. However, in 2022 maximum infection was recorded in the age group of 18-29 at around 29%, followed by age group of 30-39(20.9%) and 6-17(15%).

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected more than 611 million people and claimed 65,12438 lives worldwide as of September, 2022[1]. At the start of pandemic, the regions of the America and Europe were affected badly, contributed 85% of new cases and 86% of new deaths globally. The USA and India are top two Countries with highest number of infection and deaths related to COVID-19 and related complications. The coronavirus genome is the largest among RNA viruses. SARS-CoV-2 is classified together with SARS-CoV-1, MERS-CoV, HCoV-OC43 and HCoV-HKU1 within the genus *Beta coronavirus* of the *Coronaviridae* family. It has a positive sense, single-strand RNA (~29.9 kb) genome. The viral RNA is 5'capped and consists of 13 active open reading

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frames (ORFs) that encode a total of 27 proteins, i.e., 16 non-structural, 4 structural and 7 accessory proteins [2,3].

Clinical manifestations of COVID-19 include fever, dry cough, sore throat, and fatigue. However, other symptoms may develop such as headache, nasal obstruction, myalgia, loss of smell and taste, and diarrhoea etc. Also, acute respiratory distress syndrome (ARDS) may rapidly develop in seriously ill patients [4,5]. The virus mainly affects the respiratory system leading to flu-like symptoms, including high temperature, coughing, and in more severe cases, breathing difficulty [6]. Transmission of SARS-CoV-2 was initiated first from infected animals to humans and then spread rapidly throughout the world by human-to-human interaction. It spreads via contact to respiratory droplets or aerosols through nosocomial transmission from an infected to uninfected [7].

Nucleic acid detection is an important and sensitivity diagnostic tools for correct and accurate identification of causative agents like microbes in microbial diseases, in genetic diseases and at protein levels are very essential for the management of patients, making specificity in clinical diagnosis, effective treatment, rehabilitation and discharge of patients [8]. For quick diagnosis of Covid-19, before the onset of symptoms, a specific viral test using real time reverse transcription polymerase chain reaction (qRT-PCR) was quickly developed to confirm the infection. QRT-PCR is considered the gold standard test for diagnosis of SARS Covid-19[5]. As SARS-CoV-2 has rapidly spread all overthe world, it is evident that patient's sex, age, and co-morbidity might render these patients more vulnerable to either increased mortality or increased risk of infection. Studies showed that COVID-19 infection was higher in males than females [9]. Influence of sex and gender differences are observed in the frequency, symptoms, and severity of several diseases, in addition to the response against treatments and adverse drug interactions. A sex and gender-based approach to clinical practice can significantly contribute to health promotion by improving the appropriateness of care and, therefore, providing benefits for patients and the National Health System's sustainability. This is important in the context of the infectious Coronavirus 2019 disease (COVID-19) [10]. Previous studies have revealed that sex has considerable effect on the outcome of infection and has been associated with underlying differences in immune responses leading to physiological and anatomical variances which may influence exposure, receptor recognition and even transmission of microorganisms. The X-linked nature of immune response proteins deeply marks the difference as women mount a stronger immune response to infections and vaccinations to outlive against men [11]. Several assumptions were made for the divergence impact of COVID-19 on age and gender [1]. The susceptibility to external pathogen differs due to biological differences among the population of different age and between the genders [12]. Patients of different ages may have distinct physiological characteristics, susceptibility, clinical presentations and response to medical treatment [13]**.** Therefore, age specific risk factors for disease severity are very be useful for clinical management.

The present study attempted to explore the association between age and gender in different waves of infection among positive COVID-19 infected population in the western districts of Odisha state. This study has its own significant as the western region of the Odisha is one of the poorest and backward regions compared to rest of the state. Moreover, a large population in this region are from the backward community. In addition, substantial mass of the population from this region goes as migrant labour to different part of the country annually. The present analysis is expected to provide evidence for framing age and gender-specific public health policies and treatment of COVID-19 and other similar infectious diseases in the socio-economically lagging regions of Odisha.

MATERIALS AND METHODS

Sample Collection:

Nasopharyngeal and Nasal swab samples were collected from individuals belonging to each of the three groups mentioned above. Sterile nylon, dacron or rayon swabs with flexible plastic shafts were used to collect nasopharyngeal specimens (NPS) from symptomatic patients. After collection, swabs were placed in sterile Virus Transport Medium (VTM). The samples were processed immediately or stored at 4ºC overnight. For long term storage the samples were aliquoted in screw cap vials and stored at -80 ºC [14,15]. As the data was compiled from a retrospective study and there was no direct involvement of patents so ethical committee approval was not required.

RNA Extraction

Before testing, samples were vortexed for 10-15 Seconds and a calibrated pipette was used to transfer the required volume specified by the kit manufacturer for Ribose nucleic acid (RNA)

extraction in the BSL 2 plus facility. RNA extraction from the collected samples was carried out using different Viral RNA purification kits as per the instructions. The RNA purification kits provided by OSMCL, Govt of Odisha. The extracted viral RNA was stored at −80 ◦C till further use.

RT-PCR assay

Laboratory confirmation of SARS-CoV-2 infection SARS-CoV-2 genome from nasal swabs and respiratory samples was detected. After the purification of viral RNA from clinical samples, the detection of RdRp, ORF1ab, S, E and N viral genes was obtained by real time Polymerase Chain Reaction (RT-PCR) according to WHO protocol [16]. The volume of extracted RNA sample, specified by the respective kit manufacturers, was subjected to diagnosis using 2019-nCoV Kits approved by ICMR- New Delhi and supplied through OSMCL, Govt. of Odisha. Real time PCR was carried out in Rotorgene Rt-PCR(Qiagen) and Quant Studio 5Dx (Thermos Scientific).

As recommended by the manufacturers, sigmoidal curve with specific Ct value was considered as the criterion for considering a sample as positive for SARS-CoV-2. The criteria provided by the manufacturers of the respective kits were used for interpretation of the results. All the required controls namely no-template control, extraction control and positive control were tested simultaneously with every set of samples, as part of the quality control of the procedure. Month wise infection in different waves among gender and age groups are recorded. Age groups are divided in to 07 categories as 0-5,6-17,18-29,30-39,40-49,50-59 and 60 above. The data were analysed in MS Excel and with one way ANOVA using Origin 8 software.

RESULT AND DISCUSSION

As of August 10, 2022, COVID-19 has claimed more than 6.4 million lives worldwide [1]. The spread and the impact of the disease since early 2020 have not been uniform over time [17]. The SARS-CoV-2 virus being a novel virus can infect human race irrespective of age categories and gender [18]. However, there exist individual variations in physiological functions, immune responses and risk factors across gender and age. Therefore, the chances of getting infected might vary among gender and different age categories [19]. A retrospective secondary data analysis was performed for COVID-19 cases in the western districts of Odisha. We collected the data of all qRT-PCR positive individuals in all the months starting from May 2020 till September 2022. Month wise infection rate is presented in Table 1. In the first wave of the pandemic the percentage of infection is 5.1±0.3% in the month of May 2020 which rose to peak 23.1±0.5% during the month of August. The infection rate takes a downward trend after September 2020 and towards the end of the year it was around 1.8%. The situation is similar to earlier reports of Covid 19 infection in Indian context [20]. In the state of Odisha, the first wave of started in June 2020 lasting until November 2020 with maximum peak in the month of September. The first COVID-19 wave in Odisha, declined by early December 2020[21].

Table 1- Year wise Infection rate of the tested population in % by qRT-PCR for Covid 19 expressed in mean±SD

Months	**2020**	**2021**	**2022**
January	00	1.13±0.2	5.6±0.17
February	00	1.0±0.2	2.0±0.15
March	00	3.36±0.5	0.83±11
April	00	32.1±3.5	0.91±0.07
May	5.1±0.3	15.9±0.8	0
June	3.33±0.15	1.6±0.26	0.19±0.01
July	6.17±0.45	0.73±0.2	0.8±0.1
August	9.26±0.85	0.36±0.04	1.8±0.17
September	23.11±0.53	0.1±0.01	0.33±0.26
October	15.26±0.49	0.21±0.01	0
November	4.46±0.51	0.07±0.01	0
December	1.8±0.43	0.06±0.01	0

The infection again set to rise towards the end of March 2021 till it starts declining towards the end of June 2021, which is the second wave of infection of Covid 19 [20]. The third wave of Covid 19 Fuelled by Omicron variant which is very short lived started during the month of 2021 and ends by March 2022 [22]. Our observation from the data of qRT-PCR positive rate of infection in the western districts of Odisha from Table-1 was very much and in corroboration with the results reported earlier for the first second and third waves of infection in India. In our study the rate of infection in the second wave gradually increases to 3.36±0.5% in the later part of March 2021. The infection reaches maximum in the month of April at around 32.1±3.5% (Table 1). The infection rate decreases to 15.9±0.8 in the month of May and to 1.6±0.26% in June 2021. The pandemic might impact very differently on men and women, both for social and biological reasons [23].

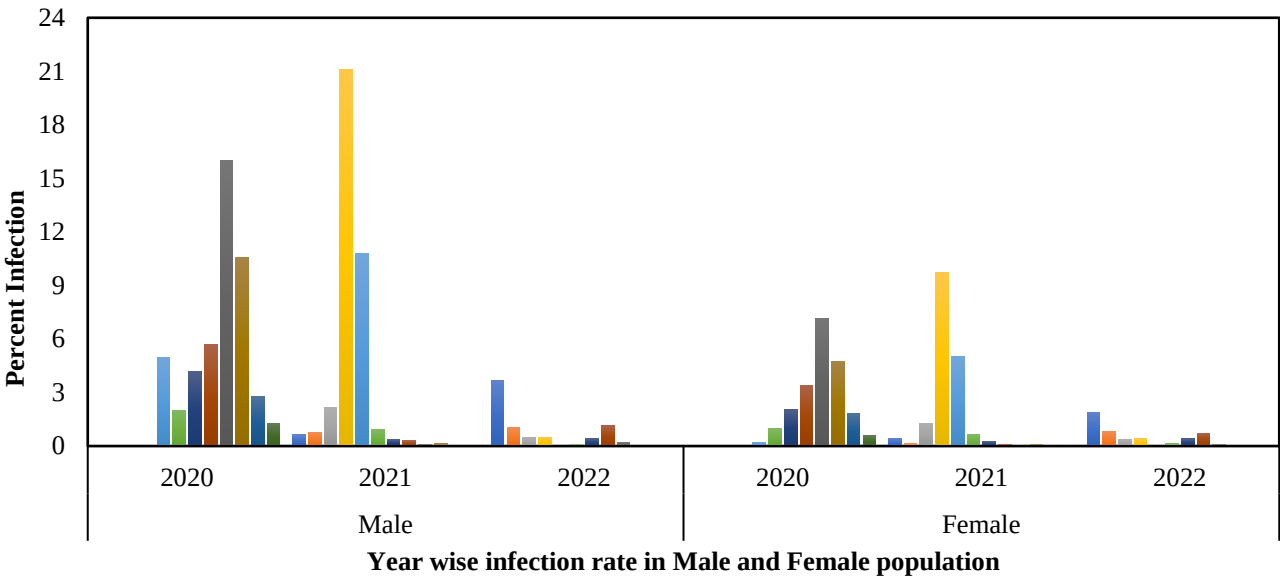

Figure 1: Year wise infection rate in different waves in Male and Female population studied

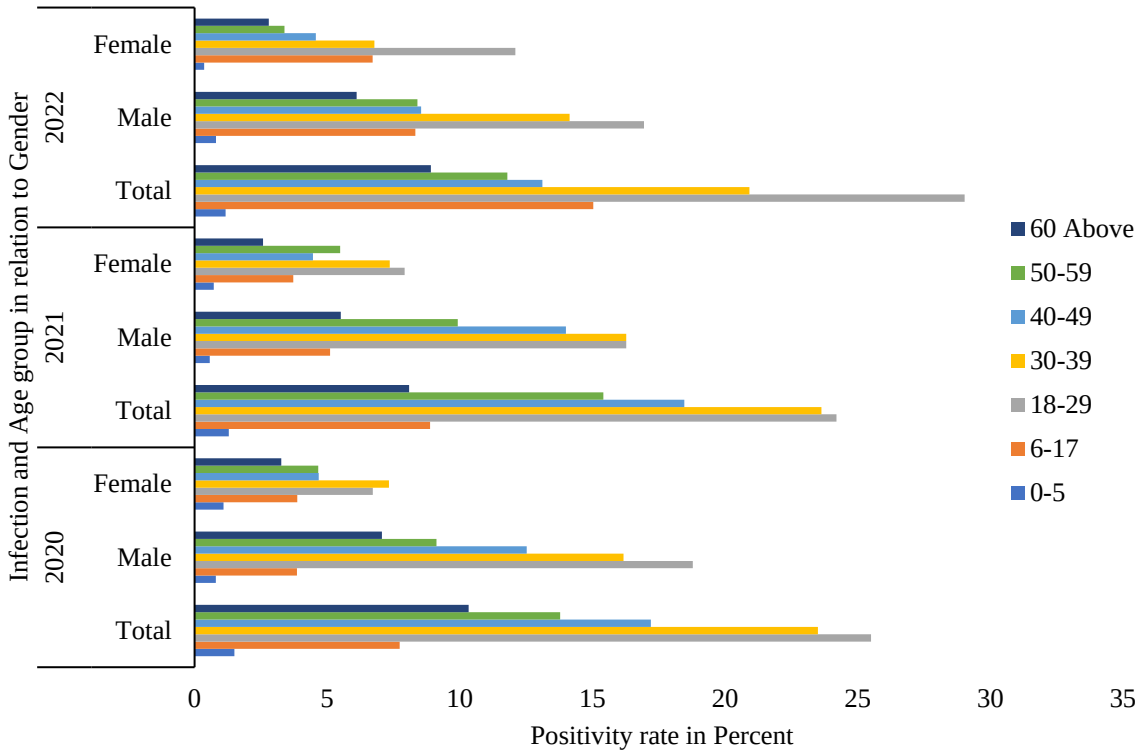

Figure 2: Rate of Infection in different age group in different waves of infection

Healthcare workers are mostly women in several regions of the world, i.e in the Americas, Europe, South-East Asia, the Western Pacific, and Eastern Mediterranean areas. They play a crucial role in family caring in most of the countries [24]. SARS-CoV-2 has spread rapidly all over the world, it is presumed that patient's sex, age, and co-morbidity might render them more vulnerable to either increased mortality or greater risk of infection [25]. From the observation of the recorded positive cases the infection rate in male population is comparatively higher them the female. In the month of September 2020 where the rate of infection was highest in the first wave (Table 1) the male infectivity rate is 16% as compared to 7.13% in case of females (Figure 1). Similarly, the rate of infection in second wave was highest in the month of April 2021. In this month the

male infection rate was at 21% compared to 9.7% in the female population. In the third wave of infection the maximum infection was recorded in January 2022. The positivity rate in male population was at 3.7%. In the same period the positivity rate in the female population was at 1.87% (Figure 1). The observed data from all the months of the year's show the positivity rate in males was clearly higher than the female population tested (Figure 1). In the Odisha the male and male percentage was 50.54%,49.46% ratio [26]. It is obvious that from the 50% male population the percentage infection was quite high related to females. Kushwaha *et al* highlighted the infection rate in mele population was higher against the females [19]. In earlier studies the mail reason behind more infection in male was attributes to higher rates of smoking, lower handwashing rates, prior respiratory conditions, biological difference between sexes as a driving force for higher infection and mortality among males [27]. It has been reported that male patients had higher expression of angiotensin-converting enzyme 2 (ACE2), which may be regulated by male sex hormones rendering them to more risk for SARS-CoV-2 infection [28].

The role of age in transmission of disease is critical for designing interventions aiming to decrease transmission in the community as a whole and for projecting the expected global burden [29]. In this study the positivity rare is maximum in the age group of 18-29(25.5%) followed by 30-39(23.5%) and 40-49(17.2%) in the year 2020. Similar trend was observed in the year 2021 where the infection rate was 24.2%,23.6%,18.46% in the age group of 18-29,30-39 and 40-49 respectively. However, in the year 2022 maximum infection was recorded in the age group of 18-29 at around 29%, followed by age group of 30-39(20.9%) and 6-17(15%). The age group of 18-29 includes population of early adolescence, college going, job seekers and early career professional. This group was maximum active in the day-to-day life and are more socially connected then other group. Second most infected population is of the age group of 30-39 which comprise the mostly the working class and economically active group. In this age bracket both the male and female populations have maximum percentage of infection. The age group of 18-29,30-39 and 40-49 comprises of more than 60% of positivity rate. Lowest rate of infection was observed in the 0-5 age group followed by above 60years of population. In a similar retrospective study reported maximum number of confirmed cases of COVID19 in the age group of 20-49[30]. The age-specific susceptibility profile suggested that those aged under 20 years were half as susceptible to SARS-CoV-2 infection as those aged over 20 years [29]. Least infected are the infants and children's group (0-5) followed by the school group (6-17). The possible cause of low infection may be attributed to several factors as inherent high immunity among children, vaccinations against other seasonal flu, school closure which reduces the social gathering [29].

CONCLUSION

The data presented from the positivity rate of COVID19 from the western districts of Odisha with particular reference to Balangir. The infectivity rate was high in the month of September 2020 for the first wave. In the second wave there was a rapid surge in the infection rate and was highest in the month of April 2021. The third wave was shot lived and maximum positive cases were detected in the month of January 2022.

The data show there is strong co-relation between the infection rate with the gender of the population studies as male population comprises twice then the female population tested positive. Similar trends were observed in all the third waves of infection. The age group of 18-29 was most infected compared to other age group. Though the study highlights COVID19 positivity rate along the gender and age specifics manner in different waves of infection, the comorbidity and clinical could not be carried out.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

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AUTHOR CONTRIBUTION

Bhuban Mohan Panda and Sanghamitra Padhi contributed in conception of work and study design. Bhuban Mohan Panda, Lopamudra and Satish Dalai performed experimental work, collected data and performed statistical analysis and interpretation. All the authors helped in proofreading and reviewing the final manuscript

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