

Original Research Paper**SARS CoV-2 sero-prevalence amongst cohort of Health Care Workers (HCW) in Yavatmal****Authors:**

¹Durgesh G Deshmukh, MD, DNB, ²Meshrum Prashant P, MD, ³Gujar Vivek M, MD, ⁴Mankar Shital N, DNB, ⁵Qazi M.S.,MD, ⁶Joshi Sanjeev L, MD, ⁷Domple Vijay K, MD, ⁸Bachewar Naren, MD, ⁹Lunge Ajitesh H, MBBS, ^{1,2,3,5,7,8,9}Shri Vasantnao Naik Government Medical College, Yavatmal

⁴Datta Hospital, Yavatmal

⁶Joshi Hospital, Yavatmal

Department of Microbiology, Shri Vasantnao Naik Government Medical College, Yavatmal.

Corresponding authors

Lunge Ajitesh H, MBBS, Shri Vasantnao Naik Government Medical College, Yavatmal.

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ABSTRACT

World is currently engulfed by the catastrophic pandemic of SARS CoV-2. The virus-driven protective response to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) remains cryptic with respect to the role of protective and specific antibodies in circulation. Such protective antibody response is vital, particularly in Health Care Workers (HCW), as they are the cardinal barrier and driver of the ever evolving pandemic. Hence, we performed Seroprevalence study to retrospectively identify the rate of asymptomatic infections in HCW not caring for COVID-19 patients. Peripheral blood serum was collected from five different tertiary care hospitals in Yavatmal hospital employees with no apparent COVID-19 symptoms and called the cohort as **COMAPS-Y (Cohort of Medical And Paramedical Staff- Yavatmal)**. Spike (S) specific IgG were analyzed in the peripheral blood samples. We observed that 34% of the study candidates were found to harbor IgG antibodies against COVID-19 with no significant bias to a particular gender. We believe determination of asymptomatic but immune HCW (seropositive) can be vital for understanding the dynamics of nosocomial transmission and be pivotal in determining the necessary step for protection of HCW in the future.

Keywords: Seroprevalence, SARS CoV-2, HCW

INTRODUCTION:

Recent catastrophic COVID-19 pandemic has caused major desecration of human society on multiple levels with still unknown long term implications (1). We have suffered, lost and endured physiological, psychological and professional calamity one after another to have realized our fallacies and feebleness of our existence (2). Our primary response to this pandemic was majorly dependent on the selfless, brave and prompt actions of the Health Care workers (HCW) and various frontline workers along with groundbreaking work from the scientific community (3-7). While everyone contributed, the HCW were primarily treating and caring for the active COVID-19 patients, which continuously exposed them to the infection (8). This resulted into a large population of HCW getting infected and suffering morbid sequel (9). More importantly pre-immunization stage of the pandemic resulted into huge number of population getting infected and subsequently overtaxing the health care system (10,11). As the symptomatic and

severely ill patients overwhelmed our caregivers, rampant surge of the active infections never declined as the asymptomatic individuals facilitated the unrestrained transmission (12). Multiple studies have now discovered that as much as four fifths of all the infections were asymptomatic (13) and hence we faced such unprecedented epidemiological crisis. One of the most important aspects of this pandemic was the unaccounted rate and degree of nosocomial transmission of SARS CoV-2 infection by virtue of lack of ignored functional dynamics of tertiary care health establishments. Our study was planned to estimate the rate of Seroprevalence in asymptomatic HCW working in Non-COVID multi-specialty hospitals in central India during pre-immunization period of the pandemic. Such retrospective assessment of exposure to COVID-19 will help us gain novel insights into dynamics of nosocomial transmission and aid us in devising better preventive strategies.

MATERIAL AND METHODS

Duration: 1st January 2021 to 21st March 2021

Setting

Five prominent multispecialty clinics from Yavatmal city NOT managing COVID-19 patients were selected for the study. Standard operative procedures during the study period warranted immediate reporting, referral and transfer of “Confirmed Positive” patients to the nearest State Government run Tertiary care hospital. Hence all the Hospitals chosen for the study were not designated to treat COVID-19 and hence can be assumed that the HCW’s never attended patient of COVID-19 for extensive duration at In Patient Department (IPD). Names and details of the Multispecialty clinics are mentioned in the sheet attached as supplementary information. Ethical approval was taken after due diligence from Institutional Ethics committee at Shri Vasant Rao Naik Government Medical College, Yavatmal.

Participants Exclusion criteria

Health Care Workers (HCW) who were tested positive for SARS CoV-2 anytime 14 days or before the beginning of study period were not considered for the participation. Anyone with close and prolonged (>30 mins.) contact with confirmed COVID-19 infection was also excluded.

Inclusion criteria

Consenting HCW who passed the scrutiny of exclusion criteria were enrolled and followed up for the study.

Study design

Aim of the study was to determine the exposure and subsequent categorical immune response against SARS CoV-2 in front line HCW who are not actively caring for confirmed COVID-19 patients. Hence after stringent application of various exclusion criteria, we selected various groups of HCW’s from the above mentioned NON-COVID -19 clinics and named the cohort as **COMAPS-Y (Cohort Of Medical And Paramedical Staff-Yavatmal)**. We subsequently obtained the written consent from each participant after individually explaining the scope and implications of the study. 5 ml of venous blood was collected from each participant in plain bulb by trained technician using standard clinical protocol. About 3 ml of serum was finally obtained from every collected sample and processed subsequently for IgG ELISA as per the manufacturer’s protocol. Every collected sample was processed within permitted window of 24-36 hours in order to prevent any obvious technical failures. Total of 185 samples were collected at the end of study, out of which 2 were hemolysed and hence were not processed further, resulting into technical failure rate of 1.08%. Finally 183 samples were successfully processed for Anti SARS CoV-2 IgG

ELISA and individual antibody titer index were estimated using the manufacturer recommended protocol.

IgG ELISA Kit: ICMR approved ErbaLisa COVID-19 IgG Kit (Erba Mannheim, IME00136) was used for detection of IgG antibodies against SARS-CoV-2 from serum samples of various HCW. ErbaLisa is semi quantitative Indirect Enzyme Linked ImmunoSorbent Assay (ELISA). It uses recombinant SARS CoV-2 spike protein subunit as primary antigen and HRP-labeled Anti-Human IgG as tracer for final detection. 96-well ELISA takes about 50 minutes to complete with sensitivity and specificity of 98.3% and 98.1% respectively. Reagents used show no interference with common clinical molecules such as Hemoglobin, Bilirubin, Biotin or IgM and the test has precision CV of 6.37%.

Data analysis

Data analysis was performed using Microsoft Excel 2015 for Windows.

RESULTS:

COMAPS-Y cohort characteristics

During 1st January 2021 to 21st March 2021, **COMAPS-Y (Cohort Of Medical And Paramedical Staff-Yavatmal)** study group comprising of 185 participants was sampled and questioned for the study (for details, kindly refer to M&M). Cohort consisted of 97 female HCW (53%) and 86 male HCW participants (47%) as evident in **Fig. 1.A**. Doctors and nursing staff were the major contributors (65%) while Lab technicians, Laboratory attendants and Human resource personnel made up the remaining 35% (**Supplementary Fig. 1**). Age of the participants varied from 21 to 68 years (**Supplementary Fig. 2**), with median age of the group being 31. While the median age of Female and Male HCW participants was 28 and 32 respectively. As seen in **Supplementary Fig. 3 & 4**, the respective gender wise histogram for age was more skewed towards left in case of Females when compared to males, indicating predominantly younger female participants. Finally 183 total participants were confirmed and further analyzed for presence of Spike protein specific IgG antibodies as described in M&M.

Retrospective assessment of SARS CoV-2 infection in asymptomatic HCW

Asymptomatic infections of COVID-19 were predominant during the epidemic and caused unrestrained transmission of the deadly pathogen (REF). Communal as well as nosocomial transmission was characteristically driven by such asymptomatic individuals and hence we aimed to determine the

prevalence of such asymptomatic HCW by retrospectively determining the IgG against SARS CoV-2. As per the manufacturers guidelines, results less than 0.9 were considered negative, 0.9 to 1.09 were considered borderline positive and more than or equal to 1.1 were annotated as positive. Out of 185 participants, 49 (27%) were positive, 13 (7%) were borderline positive and 121 (66%) were negative. For simplified inference, both Positive and Borderline positive results were considered as Positive. As seen **Fig.1 B & C**, the resultant serum seropositivity was similar in both male (32%) and female (34%). participants. Observed IgG titer in the participants ranges from 0.02 to 9.6 after normalization, with most values tending towards the lower end with resultant median of 0.7 (**Fig.2 A**) While **Fig. 2.B.** show significantly left skewed scatter plot indicating predominant and robust immune response in participants below the age of 40 years. More importantly, when the outliers are compared for each age group, we observe that the respective antibody titer values peak at 7.72 at 22 years, 9.6 at 26 years, 9.56 at 32 years and 4.69 at 38 years. We did not observe any difference in the rate of seroconversion in asymptomatic HCW based on the gender. Hence we compared the rate of seropositivity according to participant's respective job title to find a declining trend in seropositivity as shown in **Fig. 3**. Doctors and Nursing staff that were involved in direct patient care were found to be the most seropositive individuals in the COMAPS-Y cohort (36% in both groups). While as the relative contact and exposure of the HCW to the patient widened the seropositivity rate also declined (**Fig.4**). Positivity rate for HR, Lab technicians and lab attendants was 31%, 27% and 20% respectively (Supplementary excel sheet).

DISCUSSION:

Multi-center, retrospective Seroprevalence study was conducted in cohort of HCW in Non- COVID tertiary health care referral center during pre-immunization presence of neutralizing antibodies in HCW is vital to decide the time of administrating the booster dose of vaccination as well as development of novel vaccine modalities (23). One more important factor that can drive the pandemic is rate and dynamics of nosocomial transmission of SARS-CoV-2. Complex drivers of such transmission are novel sites of viral reservoir such as Salivary gland, unrestricted movement of the asymptomatic individuals etc. (4,19,24,25). Use of such screening programme can not only prevent nosocomial infections but also boost the morale of HCW and avert issues like medical burnouts, prolonged absenteeism etc. (17,26). Antibody generation is complex biological

period between 1st January 2021 and 21st March 2021. Aim of the study was to determine the prevalence of asymptomatic infections in group of individuals who are High risk of exposure to infection. We found that 34 % of the participants (N=62) (**Fig.1**) were harboring anti SARS CoV-2 spike protein IgG but remained asymptomatic. Presence of antibodies indicates that not only these individuals were exposed to the pathogen (SARS CoV-2) but were also actively infected to generate IgG antibody response. The seropositivity rate observed in the current study is very similar to the rate observed by the ICMR Serosurvey group during duration of December 2020 & January 2021. SARS CoV-2 IgG antibody prevalence in India varied from 0.7% (May-June 2020), 7.1% (August –September 2020), 24.1% (December 2020-January 2021) to 67.6 % (June-July 2021)(14–16). Similar level of prevalence of antibodies in general population and HCW who are incidentally exposed more than the general population suggest equal possibility of transmission dynamics in community as well as the hospitals. But more importantly presence of similar seropositivity warrants more emphasis on use of stringent safety procedures and protocols in order to prevent the possibility of enhanced nosocomial transmission (17– 19). Screening of HCW during the pandemic is vital and was conducted in various ways such as RT- PCR screening for active infection and antibody detection for identification of exposure in cases of asymptomatic infections (8,9,20). Through the establishment of HCW SARS-CoV-2 screening, we discovered that 62/183 (34%) of HCWs tested positive for Anti SARS-CoV-2 IgG in the absence of symptoms. This observation suggests that such seroprevalence studies are vital both in pre- and post-immunization period as evidenced by the rapid decline in neutralizing antibodies particularly in HCW (21,22). Hence it can be inferred that continuous monitoring for

process and involves intricate mechanisms which are not yet completely understood. India has very successfully navigated the challenge of vaccination and has reached state where communal transmission is minimal(27). But this robustness of immune function can be attributed to various factors such as continuous exposure to environmental antigens and pathogens due to sub-par and accepted hygienic conditions, extensive and successful implementation of Universal Immunization Programme (UIP) etc.(28,29). Also we interestingly observed decreasing trend in the rate of seropositivity according to the professional title as the possibility of direct exposure to the infected patient decreased

(Fig.4). More importantly such varied rate of transmission probability can be used to calculate the Practical Ro which can hold substantial importance in disease epidemiology and policy making. To conclude, our study highlights the importance of seroprevalence studies, especially in asymptomatic HCW in order to determine the continual use of stringent safety protocols and practices and also will be crucial in vaccine policy making and implementation of novel vaccine strategies such as nasal vaccines (30).

Limitations of the study:

Demographic and clinical categorization could have been better as the participants are primarily HCW and understand the importance of any such questionnaire better than the general population. Follow up of the participants for future disease incidence and sequel as well as the rate of seroconversion and durability would have significantly enhanced the impact of the current study. Also rigorous documentation of the all previous vaccinations of candidate HCW`s would have helped in predicting novel associations between the immune exposure and future protection from COVID-19/better antibody response against COVID-19.

Conflict of Interest: Authors declare no competing interest related to this manuscript.

Contributions: DGD conceptualized and supervised the study. VG, AL, PM, MSQ, SNM, SJ, DGD performed the experiments, analyzed the data and wrote the manuscript.

Supplementary Information:

Supplementary sheet containing all the collected demographic, clinical as well as biochemical data is submitted for reference. Supplementary figures are attached in PDF file separately.

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Figure legends

Figure 1: Gender based distribution of the participants

- A) Pie diagram depicting the distribution of Males and Females in the current study.
- B) Distribution of the Seroprevalence among males.
- C) Distribution of the Seroprevalence among females.

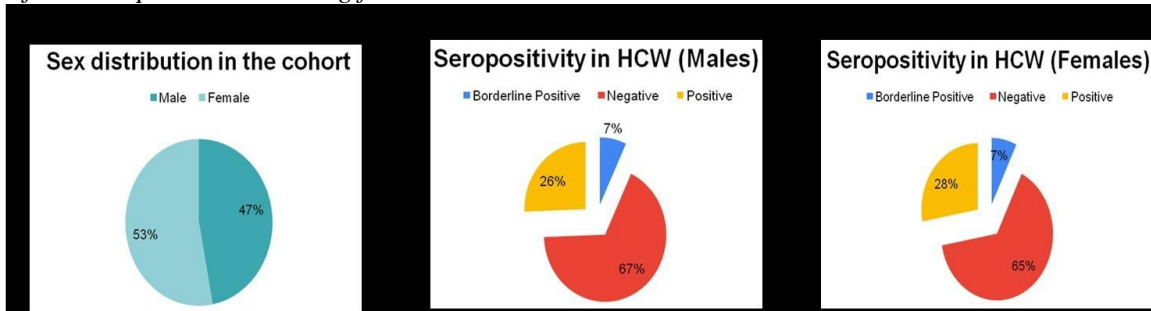


Figure 2: Cluster diagram depicting the distribution of the antibody titer among different groups of individuals.

- A) Plot showing the distribution of antibody titer observed among various participating HCW.
- B) Scatter plot depicting the trend of antibody titer vs. Age of the HCW showing significant antibody titer values below the age of 40 years.

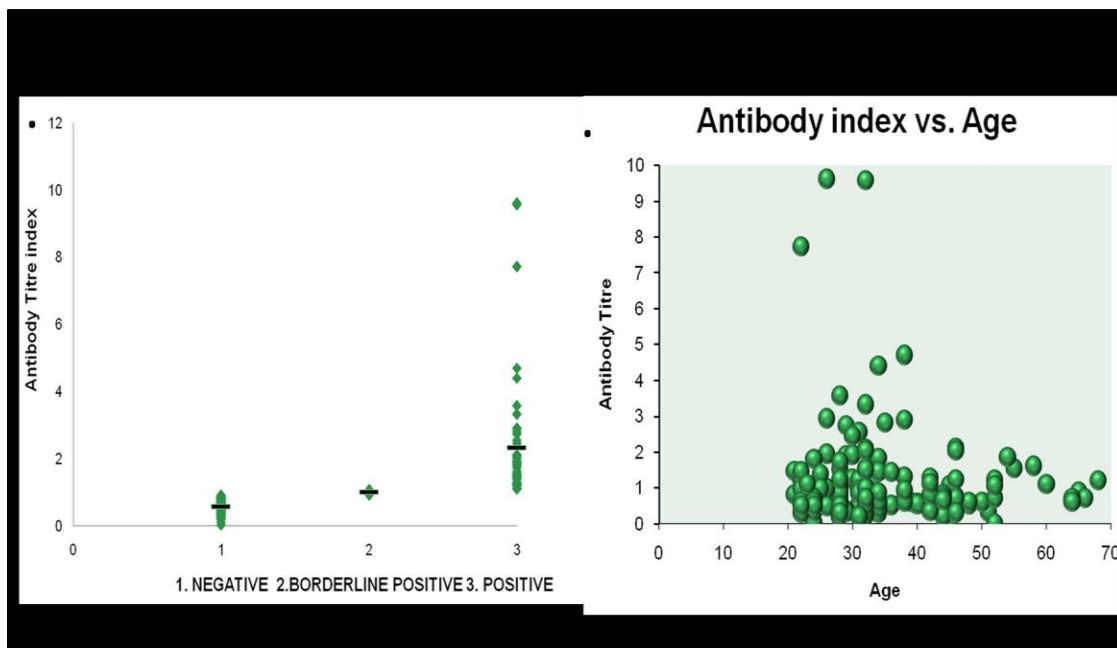
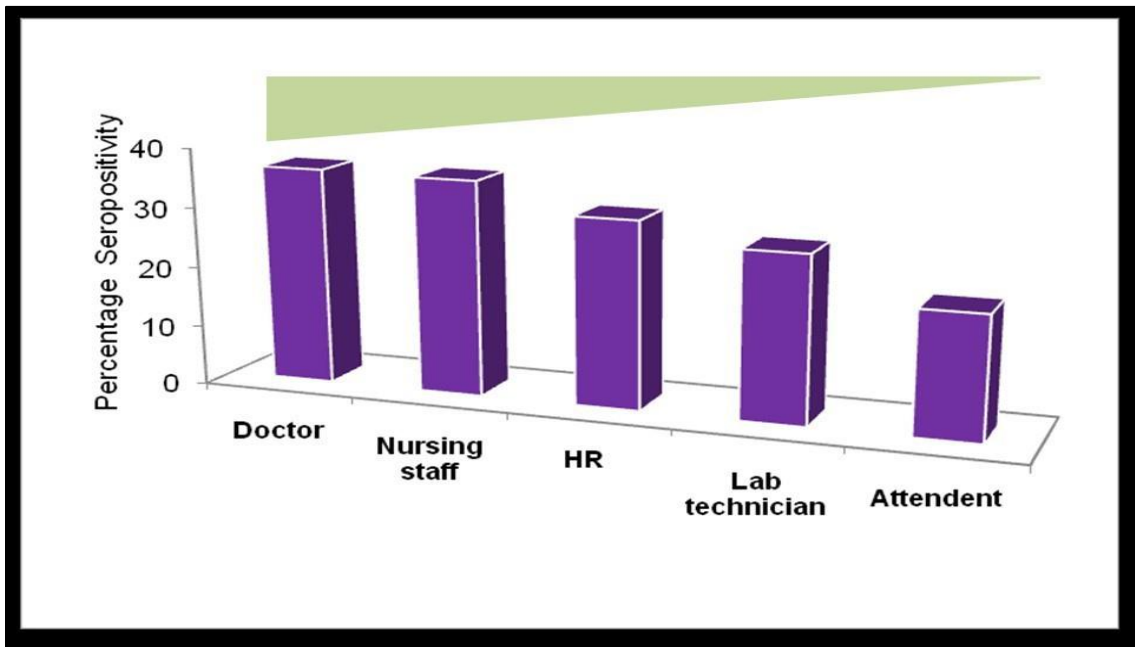


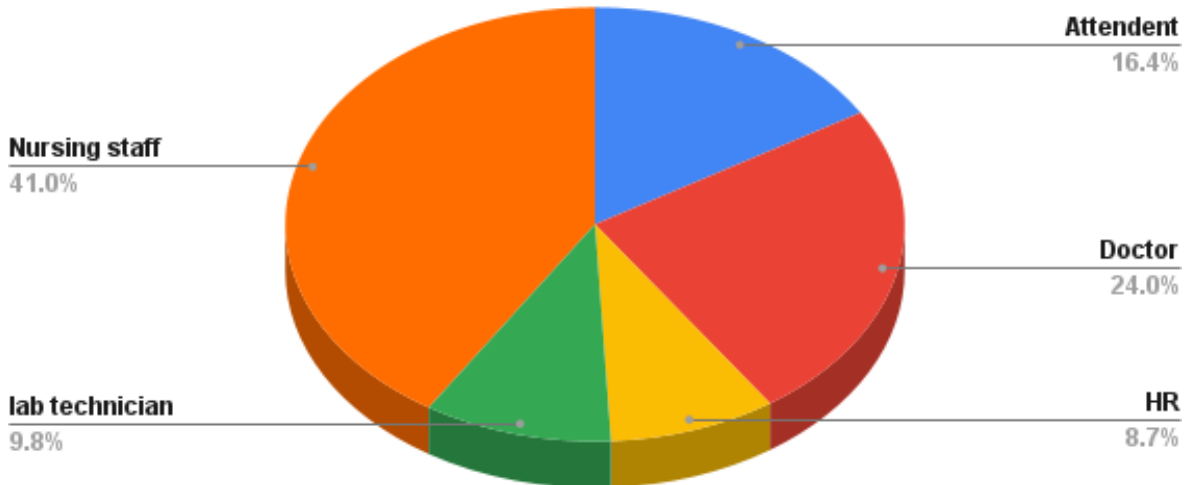
Figure 3: Cumulative rate of seropositivity amongst various members of Hospital staff.

Doctors and Primary caregivers' i.e. immediate Nursing staff were found to be the most seropositive individuals in the COMAPS-Y cohort.

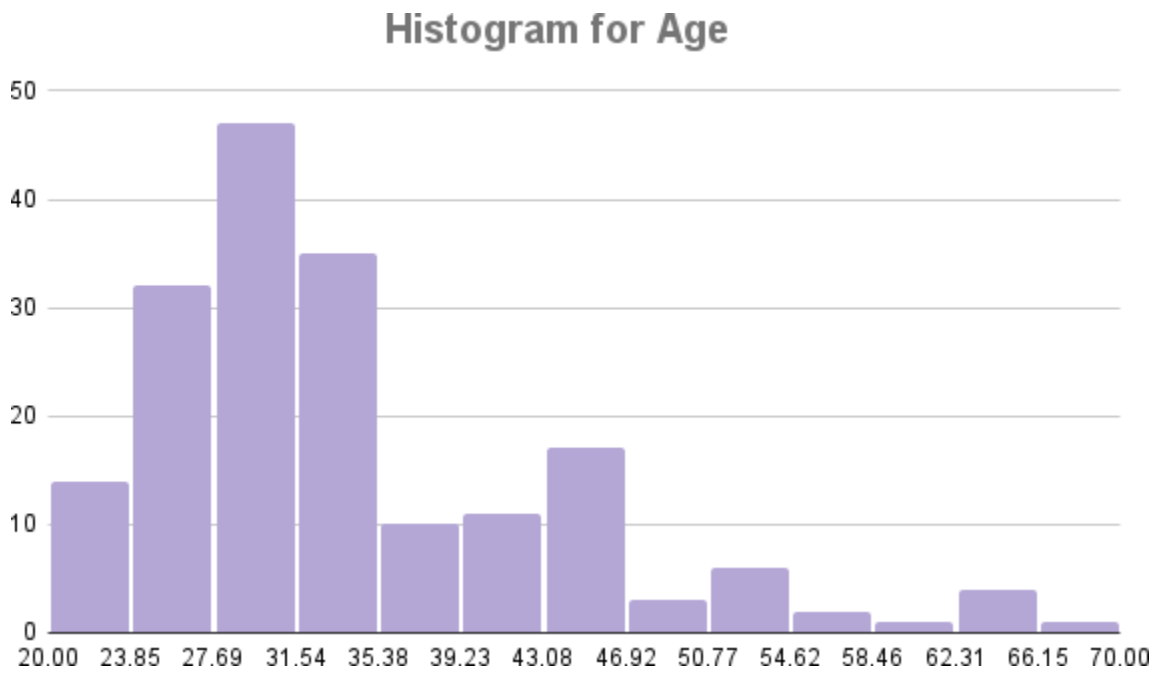


Supplementary figures

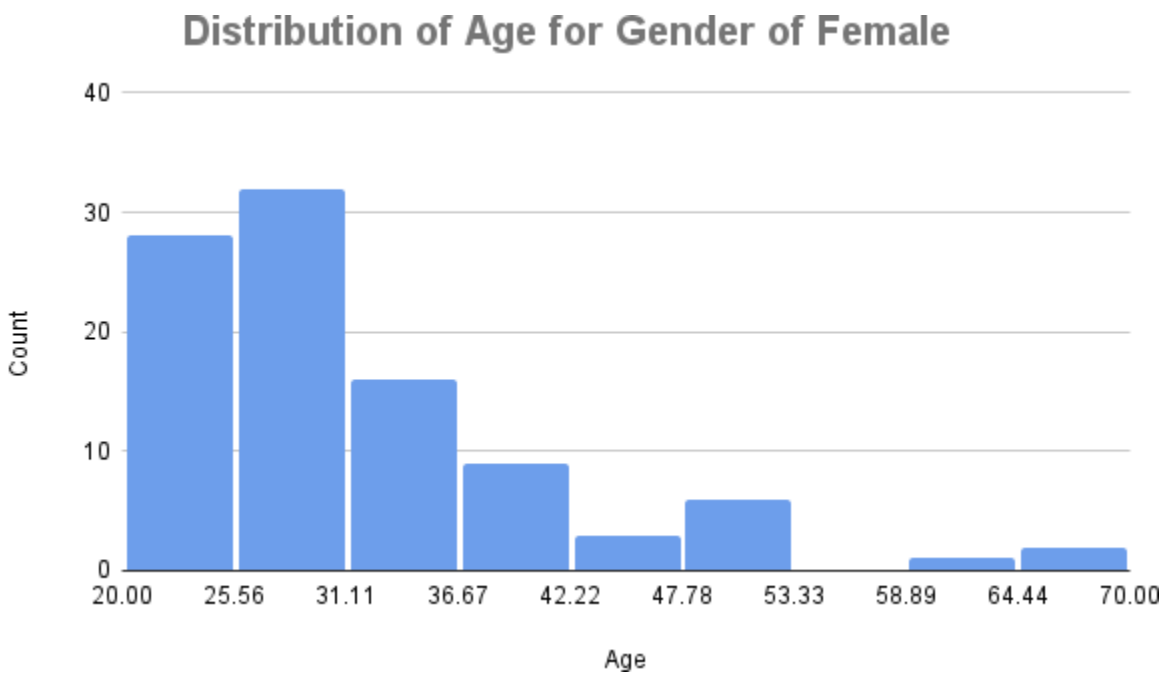
Count of Profession



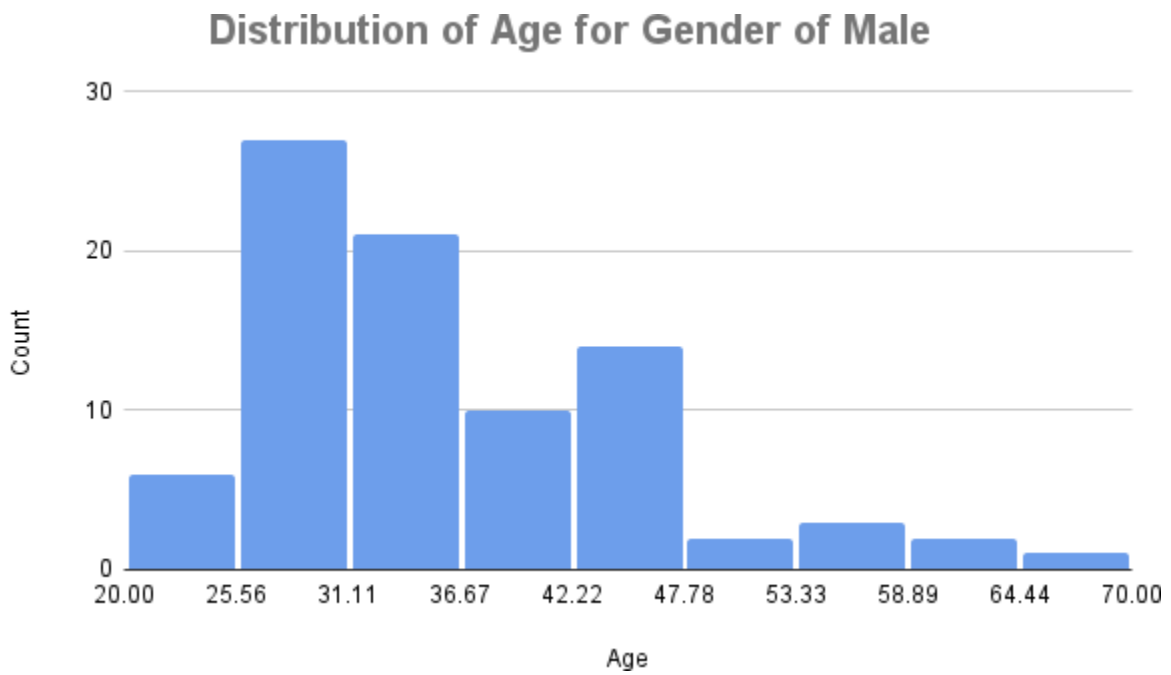
Supplementary figure 1: Distribution of participants according to their respective professional job titles.



Supplementary figure 2: Age specific distribution of the participating all HCW of COMAPS-Y cohort.



Supplementary figure 3: Histogram showing age wise categorization of all the participating females.



Supplementary figure 4: Histogram showing age wise categorization of all the participating males.

Figure 4: Schematics of functional working hierarchy in Tertiary care centers in India.

