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Disparities in age and gender-specific SARS-CoV-2 diagnostic testing trends: a retrospective study from Pakistan

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Abstract

Background Pakistan reported 1.57 million COVID-19 cases between 2020 and 2022, based on approximately 30.6 million SARS-CoV-2 RT-PCR (reverse-transcription polymerase chain reaction) tests conducted. This study utilized data from one of the largest in-country testing facilities, Aga Khan University Hospital (AKUH) in Karachi, Pakistan, to explore gender and age-related in RT-PCR testing patterns.

Methods We conducted a retrospective review of SARS-CoV-2 RT-PCR test data extracted from AKUH clinical laboratory records between February 2020 and February 2022. Gender and age distributions were examined in the context of testing patterns across the period. Multivariate regression models assessed independent associations between COVID-19 positivity and key variables.

Results We reviewed 470,249 RT-PCR tests, finding that most tests were in those aged 21–40 years (48.1%). Overall, COVID-19 test positivity was 20.6%. In all, 57.7% were performed for males, predominant amongst those tested across all age groups and waves. Females had significantly lower odds of testing positive for COVID-19 (OR: 0.9; 95% CI: 0.9-1.0). However, when adjusted for gender, age and pandemic phases, the positivity rates between males and females were the same. The odds of a positive result increased significantly with age; individuals aged > 80 years had 2.5 times higher odds of testing positive than those aged 0–10 years (aOR 2.5, 95% CI 2.3–2.7).

Conclusions The analysis indicates a consistent male dominance in COVID-19 testing, with higher positivity rates in older age groups. Our study highlight the importance of examining demographic characteristics in disease associated data especially, representation of females amongst cohorts.

Keywords Gender, Age, RT-PCR, COVID-19, Positivity

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Introduction

The COVID-19 pandemic has caused over 765 million cases leading to 6.7 million deaths worldwide [1]. Pakistan experienced five successive waves from 2020 to 2022: March to July 2020, October 2020 to January 2021, April to May 2021, July to September 2021, and December 2021 to February 2022, Supplementary Table S1 [2]. During this time approximately 1.57 million COVID-19 cases were detected through 30.6 million severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RT-PCR (reverse-transcription polymerase chain reaction) tests conducted across Pakistan [3].

It is necessary to stratify the risk of contracting SARS-CoV-2 for targeted prevention of disease, as certain population sub-groups showed heightened COVID-19 susceptibility linked to their ethnicity, social determinants, behaviors, healthcare-seeking practices, and comorbidities [4]. However, many studies separate age-related risk factors from age itself, potentially limiting a comprehensive understanding of the health risks such as aging, in the context of COVID-19. Previously males are reported to have a greater likelihood of increased COVID-19 severity than females [5, 6]. Scientific literature describing COVID-19 in Europe and China speculated on the biological pathways (sex-related factors) influencing COVID-19 severity [7, 8]. These views are supported by evidence highlighting differential immune responses to SARS-CoV-2 between males and females, and attributable age [9].

Separately, both gender and age-related risk factors unequally affect men's and women's health and access to healthcare. These would subsequently impact the understanding of gender and age representations in disease associated data. Here we examined the gender and age-wise trends of SARS-CoV-2 testing in Pakistan, a low-middle income country (LMIC). We analyzed data from the Aga Khan University Hospital (AKUH) in Pakistan, which is a single-center but with a nation-wide clinical laboratory network that receives specimens for testing from across the country. AKUH was at the forefront of SARS-CoV-2 diagnostic testing during the pandemic and worked closely with public health authorities to manage the pandemic response, providing key data on COVID-19 testing and viral genomics [10, 11]. We examined SARS-CoV-2 RT-PCR test data in 6-month periods during the first two years of the pandemic (2020 to 2022) to investigate the association between demographics and COVID-19 positivity rates in Pakistan.

Materials and methods

Study setting and participants

We conducted a retrospective analysis of laboratory data of specimens received for SARS-CoV-2 RT-PCR at the AKUH Clinical Laboratories between February 2020 and February 2022. The AKUH Clinical laboratories have an extensive network of more than 250 phlebotomy stations and stat laboratories, facilitating an average of 16,000 routine and specialized test requests received daily from all provinces of the country. The laboratory adheres to the highest standards of quality and holds accreditations from Joint Commission International Accreditation (JCIA) and the College of American Pathologists (CAP). AKUH was the first to initiate diagnostic testing for suspected COVID-19 cases in Pakistan [12].

We included anonymized data of all samples received from RT-PCR testing regardless of sex, age and ethnicities. Data was not segregated based on either hospital inpatient or outpatient specimens.

Data collection

All nasal/ nasopharyngeal (NP) swab specimens were tested for SARS-CoV-2 by RT-PCR assays. A positive case was identified as an individual whose SARS-CoV-2 RT-PCR test result was positive, indicating the presence of viral RNA. Demographic data available at the time of specimen submission including, age, gender and date of testing as well as the test result (Positive or Negative) were electronically captured from the laboratory records through a custom-built android application by the research officers. The data were continually assessed for quality, consistency, and validity. All identifications were removed before the final data were transferred to AKUH servers.

Statistical analysis

RT-PCR testing data was analyzed in five to six monthly intervals; P-I, February to July 2020; P-II, August 2020 to January 2021; P-III, February to July 2021 and August 2021 until February 2022. Results of RT-PCR tests were regarded as the primary outcome. Only the first test submitted for a particular individual was considered. Repeat tests conducted for the same individual were excluded.

A descriptive account of self-identified biological sex, and age at the time of testing or hospital admission were presented as proportions. We stratified age as: 0–10 years, 11–20 years, 21–30 years, 31–40 years, 41–50 years, 51–60 years, 61–70 years, 71–80 years, and >80 years. Univariable analyses were conducted using chi-square tests to compare the frequencies of positive tests with respect to age groups (in 10-year intervals) and gender. We used multivariable regression models to assess for independent association between COVID-19 positivity and exposure variables (age, gender, and time of testing). The model was adjusted for the following co-variates: age, gender, and time of testing. All analyses were conducted with STATA (version16.0 STAT Corp Austin, TX) and the level of significance was set at 0.05.

Table 1 Characteristics of individuals tested for SARS-CoV-2 by RT-PCR, 2020–2022, Pakistan

Characteristics	Total RT-PCR tests *N* = 470,249
Gender	
Male	271,281 (57.7%)
Female	198,968 (42.3%)
Age, years, *n* = 470,001	
0–10 years	20,688 (4.4%)
11–20 years	40,264 (8.6%)
21–30 years	118,306 (25.2%)
31–40 years	107,429 (22.9%)
41–50 years	70,901 (15.1%)
51–60 years	55,261 (11.8%)
61–70 years	36,627 (7.8%)
71–80 years	16,036 (3.4%)
>80 years	4,489 (1.0%)
COVID-19 wave (period)	
P-I, Feb- Jul 20	53,586 (11.4%)
P-II, Aug 20 – Jan 21	133,795 (28.5%)
P-III, Feb – Jul 21	135,606 (28.8%)
P-IV, Aug 21 – Feb 22	147,262 (31.3%)

Results

Age and gender-wise distribution of individuals tested by RT-PCR

A total of 470,249 RT-PCR tests for SARS-CoV-2 were conducted, with males and females constituting 57.7% (271,281) and 42.3% (198,968) of those tested respectively (Table 1). Individuals aged 21–30 years comprised the largest proportion of the tested population at 25.2% (118,306), followed by those aged 31–40 years at 22.9% (107,429) (Fig. 1). The least testing was performed in age groups of 70 years and above, 5.4% (20,525), and the pediatric population and young individuals; 4.4% (20,688) and 8.6% (40,264) among the 0–10 year and 11–20 years age groups, respectively. During the initial period, P-I (Feb-Jul 20), 11.4% (n=53,586) of the total tests were conducted. Subsequently, during P-II (Aug 20 – Jan 21), an increasing number (28.5%, 133,795) of tests were conducted (Table 1). The momentum was sustained in P-III (Feb-Jul 21), with a 28.8%, equivalent to 135,606 tests conducted within this period. Whilst, the last 6 month interval considered, P-IV (Aug 21 – Feb 22), witnessed the highest proportion of tests, constituting 31.3% (147,262).

In the pediatric age group (0–10 years), 57.9% and 42.1% tests were conducted in males and females respectively. Further, in all older age groups, a consistent male predominance was observed. Compared across periods;

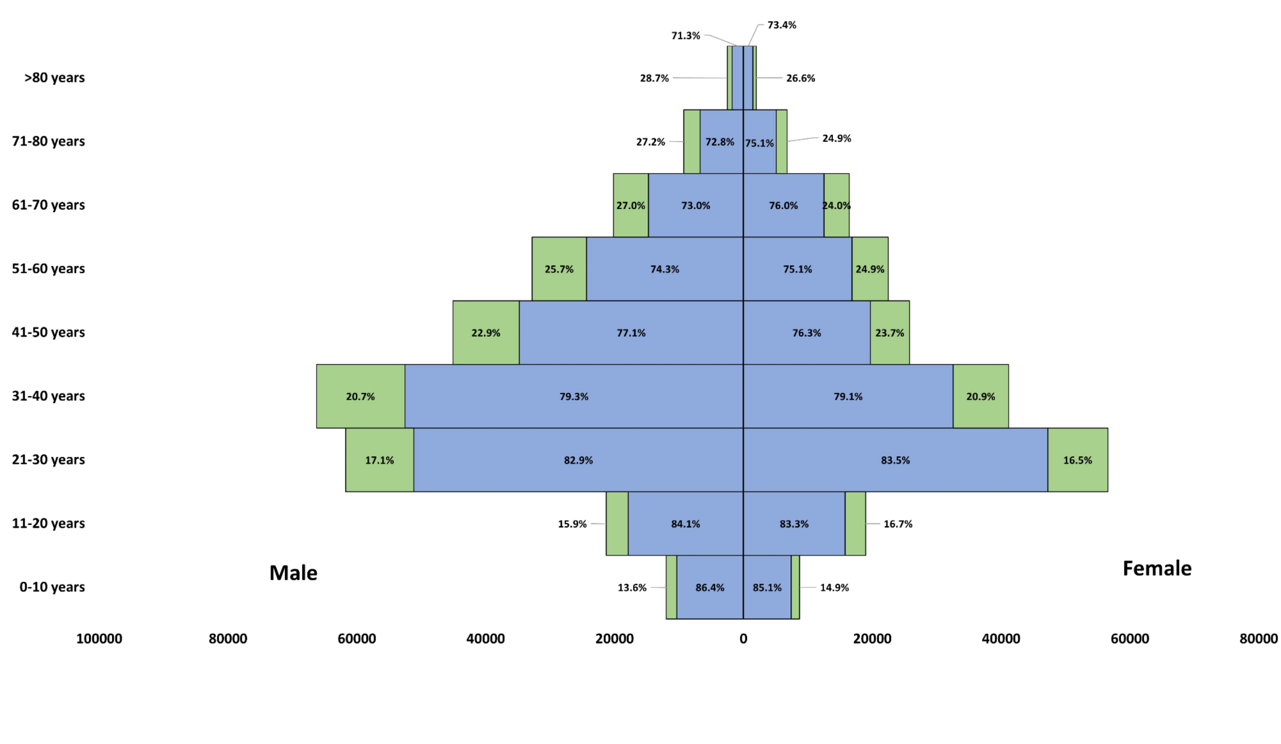

Fig. 1 Gender and age-disaggregated data for SARS-CoV-2 RT-PCR tests conducted February 2020 – February 2022

Table 2 Sex and age-wise distribution of SARS-CoV-2 RT-PCR testing, 2020–2022, Pakistan

Characteristics (*N* = 470,249)	SARS-CoV-2 RT-PCR tests conducted			*P*-value
	Male	Female	Total	
Age, years, *n* = 470,001	***N* = 271,281**	***N* = 198,968**		< 0.001
0–10 years	11,984 (57.9%)	8,704 (42.1%)	20,688 (4.4%)	
11–20 years	21,307 (52.9%)	18,957 (47.1%)	40,264 (8.6%)	
21–30 years	61,756 (52.2%)	56,550 (47.8%)	118,306 (25.2%)	
31–40 years	66,283 (61.7%)	41,146 (38.3%)	107,429 (22.9%)	
41–50 years	45,118 (63.6%)	25,783 (36.4%)	70,901 (15.1%)	
51–60 years	32,802 (59.4%)	22,459 (40.6%)	55,261 (11.8%)	
61–70 years	20,203 (55.2%)	16,424 (44.8%)	36,627 (7.8%)	
71–80 years	9,273 (57.8%)	6,763 (42.2%)	16,036 (3.4%)	
>80 years	2,519 (56.1%)	1,970 (43.9%)	4,489 (1.0%)	
COVID-19 wave (period)				< 0.001
P-I, Feb- Jul 20	35,026 (65.4%)	18,560 (34.6%)	53,586 (11.4%)	
P-II, Aug 20 – Jan 21	80,716 (60.3%)	53,079 (39.7%)	133,795 (28.5%)	
P-III, Feb – Jul 21	76,511 (56.4%)	59,095 (43.6%)	135,606 (28.8%)	
P-IV, Aug 21 – Feb 22	79,028 (53.7%)	68,234 (46.3%)	147,262 (31.3%)	

Table 3 SARS-CoV-2 RT-PCR positivity, unadjusted and adjusted odds ratios by age group and COVID-19 waves

Characteristics (*N* = 470,249)	SARS-CoV-2 RT-PCR result		OR (95% CI)	*P*-value	aOR (95% CI)	*P*-value
	Positive, *N* (%)	Negative, *N* (%)				
Total tests	96,980 (20.6%)	373,269 (79.4%)				
Sex						
Male	56,736 (58.5%)	214,545 (57.5%)	Ref		Ref	
Female	40,244 (41.5%)	158,724 (42.5%)	0.9(0.9,1.0)	< 0.001	-	
Age, years, *n* = 470,001						
0–10 years	2,927 (3.0%)	17,761 (4.8%)	Ref		Ref	
11–20 years	6,568 (6.8%)	33,696 (9.0%)	1.2(1.1,1.2)	< 0.001	1.2(1.2,1.3)	< 0.001
21–30 years	19,894 (20.5%)	98,412 (26.4%)	1.2(1.2,1.3)	< 0.001	1.3(1.2,1.3)	< 0.001
31–40 years	22,299 (23.0%)	85,130 (22.8%)	1.6(1.5,1.7)	< 0.001	1.7(1.6,1.7)	< 0.001
41–50 years	16,414 (16.9%)	54,487 (14.6%)	1.8(1.8,1.9)	< 0.001	1.9(1.8,2)	< 0.001
51–60 years	14,015 (14.5%)	41,246 (11.1%)	2.1(2,2.2)	< 0.001	2.2(2.1,2.3)	< 0.001
61–70 years	9,395 (9.7%)	27,232 (7.3%)	2.1(2,2.2)	< 0.001	2.3(2.2,2.4)	< 0.001
71–80 years	4,210 (4.3%)	11,826 (3.2%)	2.2(2,2.3)	< 0.001	2.3(2.2,2.5)	< 0.001
>80 years	1,247 (1.3%)	3,242 (0.9%)	2.3(2.2,2.5)	< 0.001	2.5(2.3,2.7)	< 0.001
COVID-19 wave (period)						
P-I, Feb- Jul 20	17,865 (18.4%)	35,721 (9.6%)	Ref		Ref	
P-II, Aug 20 – Jan 21	23,582 (24.3%)	110,213 (29.5%)	0.4(0.4,0.4)	< 0.001	0.4(0.4,0.4)	< 0.001
P-III, Feb – Jul 21	24,753 (25.5%)	110,853 (29.7%)	0.4(0.4,0.5)	< 0.001	0.4(0.4,0.4)	< 0.001
P-IV, Aug 21 – Feb 22	30,780 (31.7%)	116,482 (31.2%)	0.5(0.5,0.5)	< 0.001	0.5(0.5,0.5)	< 0.001

OR, odds ratio; aOR, adjusted odds ratio; CI, 95% confidence interval; RT-PCR, reverse-transcriptase polymerase chain reaction. The model was adjusted for sex, age and COVID-19 phases

during P-I, 65.4% of tests were conducted in males, compared with 34.6% in females. Of note, across subsequent waves, there was a gradual decrease in the proportion of tests conducted in males and a corresponding increase in female testing. Hence, in the last wave studied (P-IV, Aug 21 – Feb 22), 53.7% of tests were carried out in males and 46.3% in females (Table 2).

Trends in SARS-CoV-2 test positivity

Of the 470,249 SARS-CoV-2 RT-PCR tests performed, 20.6% (96,980) were positive. The proportion of cases positive was comparable in males (26.4%) and females (25.3%), Table 3. The youngest age group of 0–10 years, comprised 3.0% of all positive cases, increasing to 6.8% in those aged 11–20 years, with the highest number of positive individuals in those aged 31–40 years (23%). Of note, the fewest tests were conducted in those aged > 80 years (*n* = 4489), resulting the fewest proportion of total COVID-19 cases (1.3%). However, of those tested, a progressive rise in positivity was observed in older age groups, reaching 31.7% in individuals aged over 80 years.

Overall, for the 2- year pandemic period studied between 2020 and 2022, we observed an increasing COVID-19 positivity from 18.4% during the first 6 month period P-I, then 24.3% in P-II, followed by 25.5% in P-III, and then 31.7% in P-IV, Table 3.

Predictors for SARS-CoV-2 positivity

Using a multivariable model we observed that females had lower odds of testing positive as compared to males (OR: 0.9; 95% CI: 0.9-1.0; $P<0.001$). Older age groups had progressively higher odds of testing positive; these increased from 1.2 (95% CI: 1.2–1.3; $p<0.001$) for the 21–30 years group to 2.3 (95% CI: 2.2–2.5; $p<0.001$) for those over 80 years old. During the four successive 6 month periods examined, the odds of testing positive varied, with the lower odds of testing positive in latter as compared to the primary reference period of February to July 2020.

After adjusting for potential confounders, there was no significant difference in the odds of testing positive between males and females. The odds of a positive test increased significantly across different age groups. Individuals aged > 80 years had 2.5 times higher odds of testing positive than those aged 0–10 years (aOR 2.5, 95% CI 2.3–2.7, $p<0.001$). In addition, we observed lower odds of a positive result over successive waves (aOR 0.5, 95% CI 0.5–0.5, $p<0.001$). (Table 3).

Discussion

We describe the trends in SARS-CoV-2 RT-PCR testing as observed from the AKUH, Pakistan clinical laboratory network which receives diagnostic specimens from all over the country and was at the forefront of COVID-19 diagnostics especially, during the early pandemic period when limited diagnostic testing was available nationwide. We performed an age and sex-disaggregated analysis on a dataset of 470,249 SARS-CoV-2 RT-PCR tests which allowed us to study trends through the first two years of the pandemic. A male predominance was observed amongst individuals tested, and also across all age groups tested. The highest proportion of tests were conducted in those aged 21–30 years old, with the fewest in the older age groups. COVID-19 diagnostic testing increased across the pandemic period, as depicted here in 6-monthly intervals between 2020 and 2022. The overall COVID-19 RT-PCR positivity rate was 20.6%. Males comprised greater cases (58.5%), most likely due to higher proportion of testing in this group.

In our study, females had lower odds of testing positive for COVID-19, however, when adjusted for gender, age and COVID-19 waves, SARS-CoV-2 positivity rates tested between females and males did not differ. Previous studies have shown that biological differences in immunity or comorbidities between men and women may contribute to more severe COVID-19 in men [9]. Our data did not address COVID-19 severity and only focused on demographic data analysis of those for whom SARS-CoV-2 RT-PCR testing was conducted. There may be an interplay of cultural, societal, individual as well as, sociocultural factors that created a disparity between those able to seek healthcare and access testing. Sex-based disparity between COVID-19 cases was evident at the start of the pandemic, when Provincial and National data on COVID-19 in Pakistan showed 70% of cases to be males [13, 14].

Our findings are consistent with COVID-19 testing trends reported from other South Asian countries. In Afghanistan, women made up only 27% of the roughly 30,000 confirmed cases. Cultural restraints and a centralized testing system with limited capacity and accessibility were key factors associated with these estimates [15]. A study from Bangladesh, reported 23% of men and 17% of women tested positive for COVID-19, but only 33% of tests were conducted in women [16]. In Nepal, 92% of confirmed cases were reported to be young males, reflecting the higher testing rates among this population [17]. In a report from India, 6.4% of males and 5.7% of females were found to be positive, with 14,642 males versus 10,392 females tested per 100,000 population. Further, a higher COVID-19 incidence among the elderly was observed likely due to their aging immunological status and co-morbidities [18].

Pakistan reports one of the highest gender inequality rates in the world and the pandemic further widened these disparities. Similar, trends of lower COVID-19 testing rates in females have been reported from other countries with higher gender inequality rates such as, India, Nepal, Bangladesh and Afghanistan [13, 19]. Males had better access to testing facilities due to higher mobility and economic independence. However, they faced higher transmission risks at work, reinforced by societal expectations to engage in health-related activities. Other factors associated with increased increased awareness for males include public health messaging, which may reach males more effectively due to their greater access outside the home. Conversely, the majority of females in Pakistan spend most of their time at home, primarily engaged in caregiving and household maintenance tasks, and they often rely on their husbands and in-laws for the decision to seek care [20]. Another study from Pakistan reported that women's symptoms were often dismissed by their family members as being unimportant or, assumed to be psychosomatic [21]. Limited access to healthcare facilities, aggravated by geographical barriers and transportation issues, can disproportionately affect the ability of females to reach health facilities. A report cited that only 55% of Pakistani women have access to adequate healthcare and 34% of them had not consulted a doctor for health-related issues [20]. Factors associated with health care utilization in Pakistan include, the socioeconomic status and education level of females which impacts their access to diagnostic testing [22, 23]. Concerns around personal modesty as well as the absence of female staff at health facilities have been shown to discourage women from seeking COVID-19 testing in Pakistan [21]. In addition, the societal stigma related to COVID-19 testing was seen to disproportionately affect women, a gender-bias previously observed with the diagnosis of other diseases such as, tuberculosis and breast cancer in Pakistan [24, 25].

Our observation of a difference in COVID-19 testing patterns across age groups, with lesser testing in pediatric cases may reflect parental decisions which in turn are guided by healthcare beliefs, and the perceived severity of symptoms in children. A study from Pakistan reported that a majority of the parents did not consider COVID-19 to be a serious issue or, think their children to be at risk of infection [26]. In contrast, Saqlain *et* al. reported higher odds of COVID-19-related awareness and practices among the population above 50 years of age in Pakistan [27].

We performed an age-and sex-adjusted analysis to control for sampling and survivor biases in the older age group. We found that elderly individuals were the least represented amongst those who were tested, but had higher odds of testing positive possibly, as they may have been symptomatic leading to their seeking care. COVID-19 impact due to their age-related vulnerability has been reported previously [28] however, an analysis of disease severity is not within the scope of our current study. Limited mobility and accessibility issues may have impacted testing rates in the older group, explaining why in our study we observed the fewest tests to be conducted in those aged 80 years and older. In contrast, individuals aged 20–40 years constitute a majority of the Pakistan population [29]. As this is also likely to be the predominant age group in the work force, the would have greater mobility, autonomy, financial resources and therefore, more likely to be tested for COVID-19 during the pandemic.

We examined COVID-19 diagnostic trends between 2020 and 2022 in four periods which are coincident with but do not exactly follow the pandemic waves witnessed in Pakistan (Supplementary figure S1). The lowest proportion of RT-PCR tests we conducted in the first period between February and July 2020 may be explained both by, limited availability of COVID-19 diagnostics as well as stigma related to the disease in Pakistan [30]. Later in 2020, COVID-19 diagnostic testing increased with availability of diagnostics worldwide, local government mandates for testing, combined with policies that allowed people came back to work when lock-downs were removed. The decrease in COVID-19 positivity we observed between P-I and P-IV waves may be due to the greater numbers of tests conducted. Additional factors associated with reduced COVID-19 in the population would be the increased protective immunity observed as reflected by rising antibody seroprevalence in the community during the early pandemic period [31].

Recommendations for raising awareness of females and the elderly in the context of health care access and diagnostic testing include public campaigns targeting these groups. The establishment of mobile testing units and home testing facilities in rural areas can improve access. Deployment of female healthcare workers can help alleviate sociocultural barriers. Expanding healthcare infrastructure, having flexible timings, as well as advocating for inclusive policies can ensure equitable healthcare access for all demographic groups.

Our study had limitations as we used anonymized laboratory records and did not have clinical details related to COVID-19 disease severity or, outcomes of individuals who were tested. The data were collected from a single tertiary care center, however as AKUH was integral to the COVID-19 diagnostic effort in Pakistan especially in the early pandemic period, data from the center has been key in identifying disease trends and also SARS-CoV-2 epidemiology from the country [11, 32]. We are unable to account for patient bias in selecting their testing facility, this is a private facility however discounted rates were available. We could not investigate the effect of socioeconomic factors, service availability, and educational status, which significantly impact treatment-seeking behavior. While we observed a general increase in testing over successive waves, we could not explore temporal factors influencing these trends, such as changes in testing strategies or public awareness campaigns.

Conclusions

Our findings indicate that increased reporting of COVID-19 cases in males and also in 21–40 year old age groups from Pakistan may have been due to underrepresentation of females, children and the elderly in the RT-PCR tests conducted. Our work highlights the importance of having representative testing for accurate surveillance during disease epidemics. Further, it points to considerations of socioeconomic factors and gender-specific barriers when describing COVID-19 trends in the country.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12889-024-19958-w.

Supplementary Material 1

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Author contributions
The study was conceptualized and designed by principal investigators ZH, MIN, and NKG. All authors conducted the acquisition of data. MFQ, SS, and KIM were involved in data analysis and preparation of the first draft of the manuscript under the supervision of ZH, NKG, MIN, ZA, AN and SFM. All authors read and approved the final manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Data availability
Data pertaining to the study will be available upon reasonable request after the publication of this manuscript.

Declarations

Ethics approval and consent to participate
This study received ethical approval as an exemption study from the Ethical Review Committee of the Aga Khan University. Informed written consent was obtained from all eligible participants prior to recruitment.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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