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ORIGINAL ARTICLE

Immunoglobulin G Responses to SARS-Cov-2 and Patterns of Adverse Vaccination Effects Among Health Care Workers in North-Eastern Tanzania: A Retrospective Longitudinal Study

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ABSTRACT

Background: The global response to the COVID-19 has been largely successful due to widespread vaccination programs, which have resulted in significant clinical and socioeconomic achievements. Nonetheless, there is a significant information gap on the efficacy and safety of COVID-19 vaccines. Scientific data on basic immunological attributes of COVID-19 vaccines such as; duration of protection, and potential side effects associated with vaccination is inadequate, leading to high hesitancy rates towards vaccination. This study aimed at bridging these knowledge gaps and addressing these crucial issues.

Methods: This was a retrospective longitudinal study involving 273 health care workers (HCWs) from Kilimanjaro Christian Medical Centre, a referral zonal hospital in northern Tanzania, between August 2022 and February 2023. Immunoglobulin G concentrations were measured over a 21 months period post- COVID-19 vaccination using an indirect Enzyme Linked Immunosorbent Assay (ELISA). Data was analysed using STATA software version 15 (College Station, TX). Descriptive statistics was used to summarise the study participant's characteristics and prevalence of antibodies against SARS-CoV-2. Kruskal-Wallis and Mann–Whitney U test were used to assess the differences between exposure variables and median SARS COV-2 IgG concentration. Logistic regression was used to determine association of independent variables with seroprevalence, using a p-value of .05 as the cut off for statistical significance.

Results: The study population of HCWs at the KCMC is strongly seropositive to COVID-19. Vaccinated individuals had a significantly higher median IgG concentration (137.5 IU/ml) than unvaccinated individuals (122.12 IU/ml) ($p<.01$). Individuals who received a booster vaccination dose showed a higher median IgG concentration (145.7 IU/ml) compared to those who received a single dose (137.5 IU/ml). Our findings identified two IgG concentration peaks at 5 months (136.17U/ml) and 17 months (146.4 IU/ml) post vaccination. These peaks align with the peaks in immune response following vaccination and natural exposure during the second COVID-19 wave, respectively. Regarding adverse effects, only a few HCWs reported side effects after vaccination, and these were not found to be associated with any specific host factors.

Conclusions: Majority of HCWs at KCMC were seropositive to COVID-19 during the study period. The combination of vaccination and natural exposure to SARS-CoV-2 contributed to the high seropositivity rate among HCWs in the study site. Vaccine related adverse effects were rare among recipients indicating a high degree of safety of the vaccines. Further studies are warranted to better understand and characterise immune responses in terms of longevity and the level of protection conferred by vaccination and natural exposure.

BACKGROUND

The COVID-19 epidemic has caused significant losses to the world's population.[1] Following its declaration as a pandemic, variuos strategies were put in place to provide an overarching framework for the global response to the COVID-19 across different levels; individual, community, and health care facilities. At individual level, strategies like; social distancing, hand sanitisation and wearing face masks and in several countries, physical lock-down were adopted.[2–4] At the beginning of the COVID-19 outbreak, the Tanzanian Government swiftly implemented several World Health Organization (WHO)-recommended measures, as outlined in 15 guidelines, although a complete lock-down was not imposed due to concerns about severe adverse economic consequences. Tanzania collaborated with diverse stakeholders in the health sector, and became part of the COVID-19 Vaccine Global Access (COVAX) initiative program in mid-2021. This participation led to about 9.2 million Tanzanians receiving COVID-19

vaccinations between October 2021 and September 2022.[4]

While millions of people globally have been staying at home to minimise SARS-CoV-2 transmission, health-care workers (HCWs) have been doing the exact opposite. They have been at the forefront of managing COVID-19 patients, exposing themselves to a higher risk of contracting and transmitting the disease to the vulnerable population under their care.[5,6] Vaccination has been named as the most effective means to end the pandemic. Vaccination against COVID-19 has not only prevented the spread of the virus, but also mitigated the severe health consequences of the pandemic. HCWs have been given priority in receiving COVID-19 vaccination due to their classification as a high-risk group.[6] However, despite global recognition of the efficacy of COVID-19 vaccines, a significant scientific hurdle persists regarding various aspects of the vaccines. These include; the duration of vaccine-induced protection, variations in immune responses across different demographic groups, and the range of side effects associated with different vaccine types.[6,7] This study was therefore, designed to assess the persistence and trends of COVID-19 seropositivity after vaccination, using IgG concentrations as the dependent variable. Further, this study aimed at investigating the prevalence and pattern of the most common adverse side effects of COVID-19 vaccination among HCWs, and the factors associated with such side effects.

METHODS

Study Site, Design and Population

This retrospective longitudinal cohort study was conducted among HCWs at the KCMC, a large tertiary, northern zone medical centre in Tanzania. The Kilimanjaro region, located in the North Eastern part of Tanzania was selected as the study site due to its status as a tourist destination, leading to high interactions among persons from various parts of the world. In addition, having a Zonal Referral Hospital authorised to handle COVID-19 cases, Kilimanjaro region was one of the most COVID-19 hit regions of Tanzania. The study was conducted between August 2022 and February 2023. The study recruited 300 HCWs, comprising; medical professionals, students, administrative staff, volunteers, and retired personnel on contracts. Participants were selected regardless of their prior COVID-19 infection or vaccination status. Only participants who consented were enrolled into the study.

Sample Size and sampling Procedures

Sample size was calculated using the formula:
$n = (Z^2 \times P \times (1 - P))/e^2$;

Where: 'Z' = value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI),

'P' is expected true proportion

and 'e' is the desired precision (half desired CI width).

The minimum sample size was estimated at 246 participants taking P to be .4 with assumption that 40% of the studied population was seropositive to SARS-COV-2 antibodies, a desired precision of .05, and a confidence level of .95. To increase statistical power, the study enrolled 273 participants. In order to ensure representation and avoid selection bias, the population of HCWs in KCMC hospital was divided into strata. The strata represented the different hospital departments; paediatric, surgical, obstetrics and gynaecology, internal medicine, emergency, pharmacy, oncology, urology, housekeeping, pathology, and laboratory departments, covering both inpatient and outpatient HCWs. A convenience sampling approach was employed to select HCWs from each stratum, with the sample size not exceeding 38 participants per stratum.

Data Collection tools and Procedures

Data Collection Tool

The study questionnaire was derived from the WHO-AFRO Guidance document for Cohort studies, specifically designed to assess the effectiveness of the COVID-19 vaccine among healthcare professionals.[6] This tool has been validated by the WHO Regional Office for Africa (AFRO) for use with HCWs. The adapted questionnaire included demographic and clinical characteristics, information about COVID-19 vaccination history, and COVID-19 sickness, occupation and community-related behaviour during the pandemic. Baseline demographic and clinical characteristics included; age, sex, resident area, height, weight, blood group, smoking history, regular medication use, and chronic illnesses (Diabetes, Hypertension, Heart disease, Immunodeficiency and organ transplant, Asthma, Lung Disease, Cancer, Renal disease, Liver disease, and Joint disease). Additionally, it also gathered data regarding the history of COVID-19 vaccinations, including the type of vaccine received, vaccination date, doses administered, and whether a booster dose was received. In the part addressing occupation and community-related behaviour during the pandemic, questions were asked about the application of infection prevention and control (IPC) measures, household size, and occupation category, providing a comprehensive overview of participants' experiences and exposures during the COVID-19 pandemic.

Participant Recruitment

Participants were recruited between September and November 2022. Prior to recruitment of study subjects, permission was obtained from respective departments and hospital directors. A sensitisation meeting was held to introduce the study objectives to all potential participants. In addition, department-specific sensitisation meetings were conducted to enable administrators grasp the project's rationale. To protect participant privacy, interviews and sample collection took place in a private room where only one investigator and one participant were present. All HCWs who voluntarily consented and were not experiencing critical health emergencies were eligible for inclusion in the study. Participants were interviewed using a digital questionnaire installed on tablets with the Research Electronic Data Capture (REDCap) data management system.

Weight and Height Measurement

Weight and height measurement were performed following previously established procedures with minor adjustments. [9] The weight of each participant was measured in kilograms using a newly calibrated digital weighing scale. The height of each participant was measured by using a calibrated portable stadiometer.

During height measurement, participants were asked to stand on the base plate of the stadiometer without shoes, with feet flat, heels close together, knees straight, arms hanging naturally on the sides, and buttocks and shoulders touching the stadiometer. Participants were also asked to relax their shoulders, look straight ahead and take a deep breath. Subsequently, the headboard of the stadiometer was lowered gently but firmly onto the top of the head with sufficient pressure to compress the hair so that it rests on the crown of the head. Some participants were asked to undo or adjust hairstyles and remove hair accessories that could interfered with the accuracy of the measurement. To maintain consistence throughout the study, all measurements were recorded in centimetres for height and kilograms for weight.

Sample Collection

Collection of blood samples was conducted between 15th October 2022 and 30th November 2022. From each participant, 3ml of blood were collected using a 5ml syringe. In most participants, the median cubical superficial vein, which is located in the ante-cubital region of the upper limbs, was used. The risk of bleeding was minimised by providing extra caution to those participants who are using oral anticoagulants, using a small needle gauge but also blood was collected by a qualified and experienced phlebotomist. All contaminated needles and other items were disposed according to standard Operation Procedures (SOPs) for sharps disposal. Collected blood was placed in the labelled red-cap tubes with clot activator, and stored in a cool box containing ice blocks before they were transferred to the Biotechnology Laboratory at Kilimanjaro Clinical Research Institute for processing.

Sample Processing and Detection of SARS-COV 2 Antibodies

The clotted blood samples were centrifuged at 1000 g for 15 minutes using a refrigerated centrifuge. After centrifugation, the serum was carefully transferred to labelled plain tubes and stored at 20°C. Detection of SARS-COV-2 Antibodies was done using Generic Assays (GA) Enzyme-Linked Immuno-Sorbent assay (ELISA) kit, designed for SARS-CoV-2 IgG Screening (MedipanGmbHGA Generic Assays GmbH, Ludwig-Erhard-Ring 3, 15827 Blankenfelde-Mahlow OT Dahlewitz, Germany). Antibodies (IgG) against SARS-CoV-2 were detected. This indirect ELISA protocol involved two stages that targeted the Spike and Nucleocapsid antigens of the SARS-CoV-2 virus. All procedures were performed according to the manufacturer's instructions. The kit comprised of a microtiter plate with 96 wells coated with spike (S) and nucleocapsid (N) antigens, along with concentrated wash buffer, sample diluents, conjugate, substrate, stop solution, positive and negative controls. To conduct the assay, 200µl of both the negative and positive controls were dispensed into duplicate wells, with the first well left blank. In order to ensure that any background pre-COVOD-19 IgG is accounted for in the results, 2 more wells were used for polled serum samples collected from HCWs before the COVID-19 outbreak. The IgG concentration from these pre-COVID-19 samples was subtracted from all readings during data analysis to ensure accurate interpretation of results.

In all the remaining wells, 200µl of sample diluent was dispensed. Subsequently, 10µl of patient serum samples were added to each well, followed by 50µl of start reagent in all wells except the blank well. The plate was then covered and incubated at 37°C for 45 minutes. Using an automated ELISA washer, each well was washed 5 times using 350µl of wash solution preceded by a 20-second soak time in between washes. The wash solution was prepared by diluting the provided wash buffer in 1140mls of distilled water. Following the completion of the first wash stage, a 100µl of the conjugate solution containing anti-human-IgG coupled with HRP was added to all wells except the blank. Each plate was again incubated at 37°C for 45 minutes before undergoing another round of washing for 5 cycles as described earlier. Subsequently, 100µl of substrate solution (3',3',5',5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide) was added to all wells except the blank, followed by incubation at room temperature while protected from light. Finally, 100µl of stop solution was added to each well to stop the reaction process.

Interpretation of Optical Density Readings

Optical Densities were measured using an ELISA plate reader at 450nm versus 620nm. The validity of the test was checked and confirmed based on the manufacturer's criteria. The sample's optical density measurements were quantified as SARS-COV-2 IgG antibody concentration in IU/ml using a standard curve. The value of IU/ml corresponds to the value of BAU/ml (binding antibody Units). The concentration results were interpreted as either strong positive, positive, weakly positive, borderline, or negative results according to the manufacturer's cut-off value for titers (Table 1).

TABLE 1: Interpretation of GA ELISA Test Results Table

IU/ml	SARS-CoV-2 IgG
< 10	Negative
10 - < 12	Borderline
12 - < 50	Weak positive
50 – < 250	Positive
≥ 250	Strong positive

Legend: Table 1 presents the ranges of IgG concentration in IU/ml, and their interpretation in terms of seropositivity

Data Management and Analysis

Data Management

In this study, REDCap (Vanderbilt University, Nashville, Tennessee-USA) data management system was used for capturing data from participants' responses. After collection, data was exported from REDCap into excel format for further analysis. Using the study ID assigned to each participant, the results obtained from the ELISA assays were integrated into the Excel data set, alongside the respective patient IDs. This approach allowed for efficient organization and correlation of participant-specific ELISA assay results with their corresponding data

in the Excel dataset, facilitating comprehensive analysis and interpretation of the study findings

Statistical Analysis

Before analyses, the titer values of SARS-CoV-2 IgG antibodies were quantified as a continuous variable. However, the final presentation was categorised as negative, borderline, and weak or strong positive. All data from the created spreadsheet was imported to STATA software version 15 (College Station, TX) for statistical analyses. Descriptive statistics were employed to summarise the study participant's baseline socio-demographic and clinical features, as well as the seroprevalence of antibodies against SARS-CoV-2. To assess significant differences between exposure variables and median SARS-CoV-2 IgG concentrations, Kruskal-Wallis and Mann–Whitney U tests were utilised. Logistic regression analysis was used to compute odds ratios and 95% confidence intervals when examining the association of independent variables with seroprevalence. Significant parameters related to seropositivity in bivariate analysis were independently tested for their association with seropositivity using multivariable logistic regression, correcting for all significant variables as covariates. A p-value of .05 was regarded as cut off for statistical significance.

Ethics

Ethical clearance for conducting this study was obtained from the College Research Ethical Review Committee (CRERC) of Kilimanjaro Christian Medical University College (KCMUCo), with ethical clearance number PG61/2022. Prior to commencing the study, permissions were also secured from the Regional and District Medical Officers, as well as administrative secretaries. Written consent in both Swahili and English languages was obtained from all participants. Sample collection was conducted in a private room within the participant's department to ensure privacy. To maintain confidentiality and conceal participants' identities, numerical labels were used for both the questionnaire and blood samples. Confidentiality was ensured through anonymisation of participants' information.

RESULTS

Baseline participant information

The study enrolled a total of 273 participants, out of which 108 (39.6%) were males. The median age of participants was 32(19-64). More than three quarters (78.0%) had college education. Of the 273 participants, 103 (37.7%) had received COVID-19 vaccines (Table 2). Most HCWs (99.00%) were seropositive to SARS-COV-2 IgG antibodies (Figures 1 & 2).

Dynamics of IgG Concentration among Recipients and Non-recipients of COVID-19 Vaccines

Ninety of the participants (87.4%) received the Johnson &Johnson vaccine. Out of the 103 participants who received COVID-19 vaccines and tested for presence of IgG antibodies, 83 (81.5%) received a single dose whereas 20 (18.5%) received two doses. The study's results show that vaccinated individuals had a significantly higher median IgG concentration than those who were unvaccinated (Figure 3). Furthermore, our results showed that participants who were vaccinated 17 months prior to the study had the highest mean IgG concentration (146.4026 IU/ml), whereas those vaccinated 5 months before the study had the lowest IgG concentration (177.8IU/ml). (Figure 4). Individuals who received a booster vaccination dose displayed an overall higher IgG concentration compared to those who received only a single dose (Figure 5). When comparing vaccinated individuals against unvaccinated individuals in terms of trends of antibody concentrations over 21 months since vaccination, it was observed that vaccinated individuals, regardless of whether they received a single or double vaccination dose, had consistently maintained higher IgG concentrations (Figure 6). Interestingly, the study also found that participants who received AstraZeneca vaccine had the highest median IgG response (197. 83IU. mL) where as those vaccinated with Sinopharm exhibited the lowest IgG response (97.02IU/mL) (Figure 7). These findings highlight the differential immune responses elicited by different COVID-19 vaccine formulations.

FIGURE 1: Seroprevalence of SARS-COV-2 IgG Antibody Titers among Vaccinated Health Care Workers (N=169)

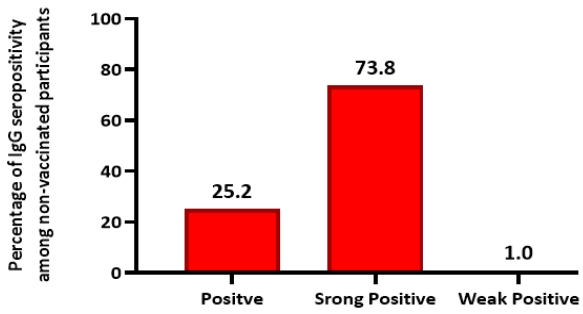


FIGURE 2: Seroprevalence of SARS-COV-2 IgG Antibody titers among Non-Vaccinated HCWs (N=103))

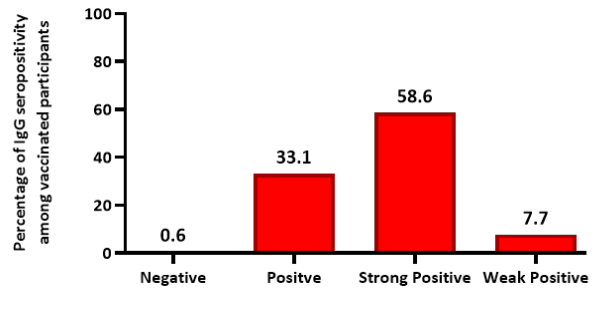


FIGURE 3: A Comparison of Median IgG Concentrations Between Vaccinated and Unvaccinated Health Care Workers (P<0.01)

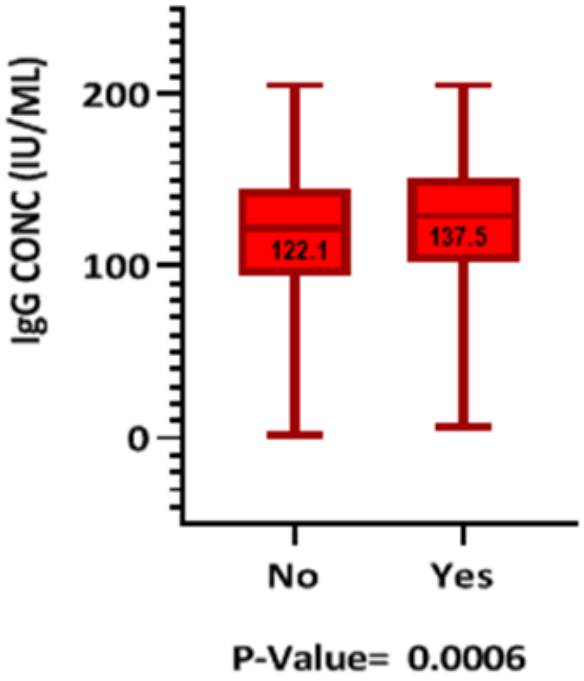


TABLE 2: Descriptive Information of Study Participants

Variable	Frequency	Percentage
Age Category		
19-29	109	39.8
30-39	65	23.7
40-49	49	17.9
50-59	47	17.2
60-65	3	1.1
Sex		
Female	168	61.5
Male	105	38.5
Education		
Primary School	33	12.1
Secondary School	23	8.4
College	217	79.5
Marital Status		
Single	134	48.9
Married	126	46
Widowed/Divorced	12	4.4
BMI		
Normal weight	115	42.1
Obese	64	23.4
Overweight	86	31.5

Continue

TABLE 2: Continued

Variable	Frequency	Percentage
Underweight	8	2.9
Chronic conditions		
No	224	82.1
Unknown	11	4
Yes	38	13.9
Smoking status		
Stopped smoking	8	2.9
Never smoked	257	94.1
Currently smoke	8	2.9
Alcohol consumption		
Stopped taking alcohol	17	6.2
Never took alcohol	158	57.9
Currently taking alcohol	98	35.9
Blood group		
A	45	16.5
AB	16	5.9
B	39	14.3
O	122	44.7
Unknown	51	18.7
Vaccination status		
Yes	103	37.7
No	170	62.3
Vaccine type		
Oxford-AstraZeneca	2	1.9
Johnson & Johnson	90	87.4
Sinopharm-BIBP	3	2.9
Pfizer-BioNTech	7	6.8
Sputnik-V	1	1

Legend: Table 2 summarises various social demographic characteristics, vaccination parameters, and life style characteristics of the study participants

FIGURE 5: A Comparison of Median IgG Concentrations Between Vaccinated and Unvaccinated Health Care Workers

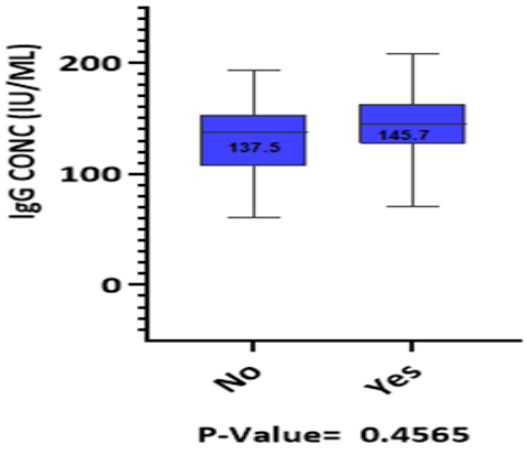


FIGURE 4: Combined Temporal Trend of Median IgG Concentrations among Recipients of COVID-19 Vaccines, 21 Months Post-vaccination

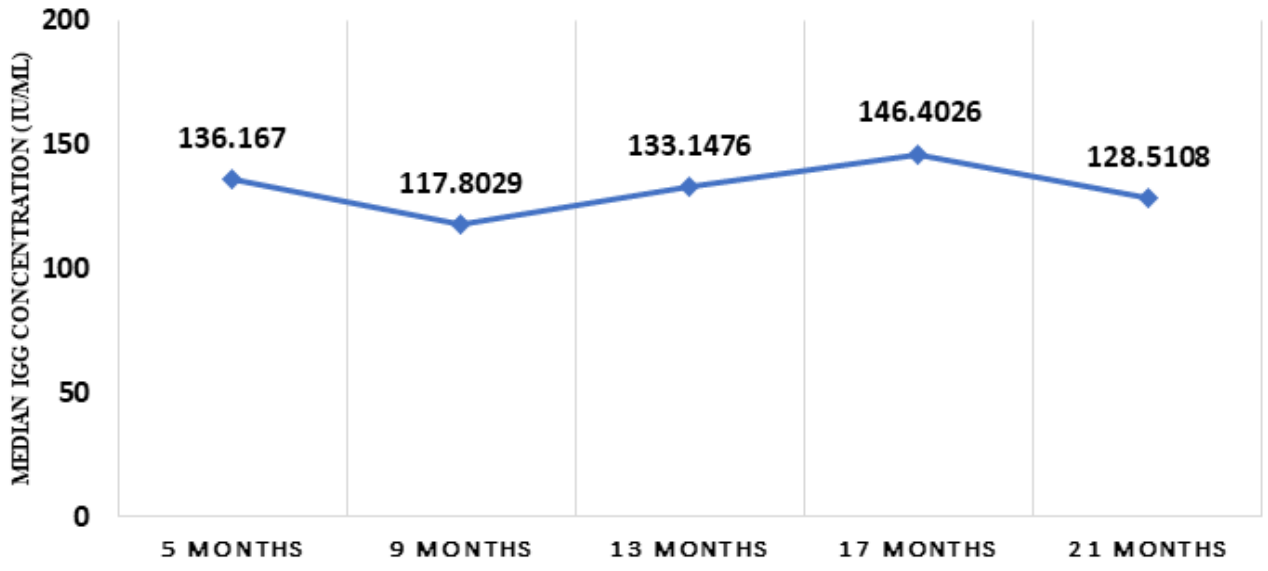
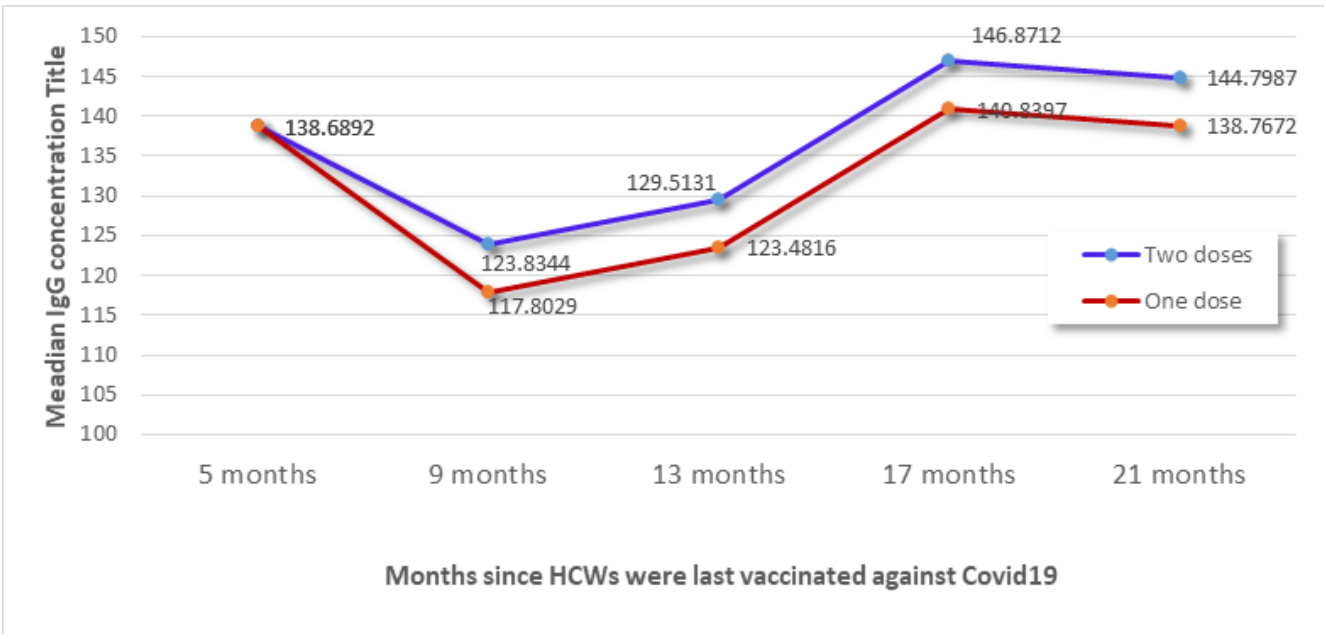
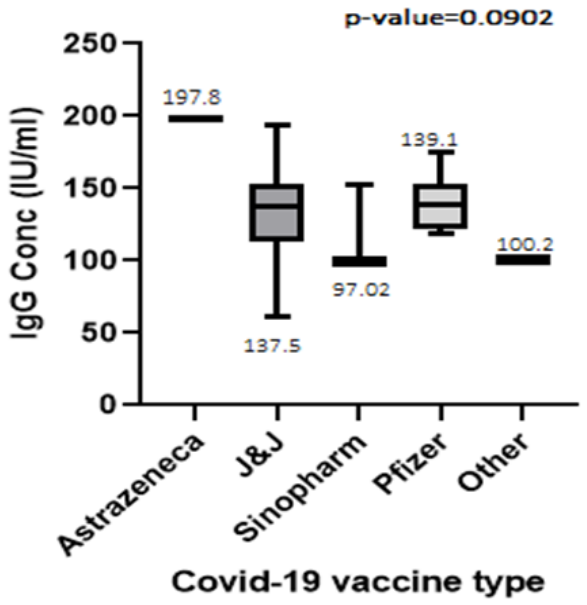


FIGURE 6: A Comparison of the Trends of Median IgG Concentrations Between Participants Receiving a Single Vaccine Dose of COVID-19 and Participants Receiving a Second (Booster) Vaccine Dose Over a Period of 21 Months from the Vaccination Date



The Booster Dose was given 2 Months after the First Dose.

FIGURE 7: Median IgG Concentrations among Individuals Vaccinated by Different COVID-19 Vaccine Types



Patterns of Adverse effects of COVID-19 vaccines among Vaccine recipients

The Distribution of adverse effects related to different vaccines is presented in Figure 8. Generally, adverse effects were recorded among only a few vaccine recipients. Specifically, all individuals who received AstraZeneca and Sputnik 5 reported adverse effects, whereas Sinopharm was associated with the lowest rate of adverse effects. However, none of these factors were statistically associated with the occurrence of adverse effects after vaccination (Table 3).

TABLE 3: Associations between Demographic/ Exposure Factors and Adverse Effects Across

Variables	n (%)	Adverse effects n (%)	(95% CI)	X2	*P-value*
Age					
19-35	33(32.0)	21(63.6)	32.4-53.7	2.76	.367
36-52	55(53.4)	22(40.0)			
53-69	26(25.2)	8(30.8)			
BMI					
Normal	24 (23.3)	9 (37.5)	21.3-60.4	3.701	.157
Overweight	35 (34.0)	15 (42.9)	29.2-62.8		
Obese	44 (42.7)	26 (59.1)	46.2-75.5		
Sex					
Female	77 (74.8)	35 (45.5)	35.0-57.5	1.853	.173
Male	26 (25.2)	16 (61.5)	41.4-78.3		
Vaccine Brand					
AstraZeneca	2 (1.9)	2 (100.0)	-	4.899	.300
J&J	90(87.4)	42 (46.7)	36.9-57.7		
Sinopharm	3 (2.9)	1 (33.3)	2.5-90.7		
Pfizer BioNTech	7 (6.8)	5 (71.4)	29.4-93.8		
Sputnik 5	1 (1.0)	1 (100.0)	-		
Blood group+					
A	12 (11.7)	8 (66.7)	35.9-87.7	1.211	.774
AB	8 (7.8)	4 (50.0)	18.2-81.8		
B	15 (14.6)	10 (66.7)	39.3-86.1		
0	50 (48.5)	27 (54.0)	40.8-68.6		
Chronic conditions					
No	74 (71.8)	37 (50.0)	40.5-63.4	0.611	.737
Unknown	3 (2.9)	1 (33.3)	2.5-90.7		
Yes	26 (25.5)	12 (46.2)	27.9-65.5		

Legend: Table 3 presents Chi Squared analysis for association between selected demographic and exposure factors with adverse effects after vaccination against COVID-19

DISCUSSION

The emergence of the COVID-19 pandemic in early 2020 led to the rapid development of various types of vaccines. In Tanzania, mass vaccination campaigns started in late July 2021, with widely administered vaccines including; Johnson & Johnson, Sinopharm, Pfizer, AstraZeneca and sputnik-five Vaccines. Despite these advancements, there remains limited clarity on the dynamics of IgG antibodies following COVID-19 vaccination. Studies conducted in Europe, US and Israel have shown antibody levels to wane after 4 to 6 months post infection and vaccination.[8–11]

Results from our study indicate that COVID-19 vaccines administered in Tanzania resulted in seroconversion in most individuals, irrespective of their age and other demographic factors. Antibody levels were found to sharply rise 5 months after vaccination, followed by a brief drop of median IgG concentrations 9 months after vaccination. Interestingly, our findings indicate that IgG levels among vaccinated individuals reached their highest concentrations 17 months after vaccination, which coincided with Tanzania's second wave of COVID-19 in early 2021. This observation suggests that natural SARS-CoV-2 infection significantly impacted seroconversion and humoral immune response levels in vaccinated individuals.[2,12] Notably, individuals who received a booster dose of any vaccine type exhibited a higher concentration of IgG antibodies compared to those who only received only a single dose. This finding suggests that receiving a booster dose confers an immunological advantage. Despite varying degrees of prior exposure to COVID-19 among the majority of participants, our study underscores the beneficial role of prior vaccination in inducing a state of "hybrid immunity" among recipients of COVID-19 vaccines.[13]

Our research findings indicate a robust seroconversion rate exceeding 95% across all four vaccines studied, namely; Janssen Ad26.COV2.S COVID-19, Pfizer-BioNTech BNT162b2, Sinopharm BBIBP-CorV, and Moderna COVID-19 (mRNA-1273). In terms of immunogenicity, the highest immunogenic vaccine was found to be Astrazeneca (197.8 IU/ml), whereas the least immunogenic was Sinopharm. The highest rate (99%) was observed in individuals who received Pfizer-BioNTech BNT162b2 vaccine, while the lowest rate (94%) was observed in those who received the inactivated virus Sinopharm vaccine (97.02IU/ml). Notably, majority of participants who received mRNA-based vaccines displayed enhanced humoral responses, consistent with previous reports, with a few reports of strong immune responses in individuals who received an adenovirus-based vaccine.[14]

In the present study, adverse reactions were observed solely in a limited number of individuals who received the vaccine. This indicates that the COVID-19 vaccines available for vaccination did not cause significant adverse effects in recipients, contrary to initial concerns. The absence of association between host factors and the occurrence of adverse effects suggests that reported side effects post- vaccination may not be primarily influenced by recipient factors, but instead may stem from the inherent mechanism of action of the vaccines.

CONCLUSIONS

COVID-19 vaccination among HCWs in Tanzania resulted in seroconversion in most individuals, with antibody levels peaking 17 months after vaccination. Those who received a booster dose of any vaccine type exhibited higher concentrations of IgG antibodies. Vaccination proved beneficial in inducing "hybrid immunity." Adverse reactions to the vaccines were minimal and not associated with recipient factors.

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