

Estimating SARS-CoV-2 Spike Antibody Levels Among Sputnik V First Dose Vaccinated People in Pakistan: Formulation of Anti-COVID-19 National Mass Vaccination Strategy.

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Short report

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Abstract

Rapid ramp up of immune responses against SARS-CoV-2 during pandemic enables adequate prevention and treatment for COVID-19. Estimating levels of SARS-CoV-2 spike protein antibodies post vaccination is crucial for designing mass-vaccination strategies. The aim of this study was to evaluate effectiveness of Sputnik V first dose in Pakistan. A cross-sectional study of 2000 participants was conducted for examining Gam-COVID-Vac or Sputnik V first dose effects at 21st days post administration at Islamabad Diagnostic Center, Islamabad, Pakistan. From 100 real-time PCR negative (SARS-CoV-2 RNA) individuals, samples were collected and analyzed for antibodies to the SARS-CoV-2 spike protein using Electro-chemiluminescence immunoassay (ECLIA) (Elecsys # 09289267190 Roche, USA). 85% of the participants showed strong positive results with SARS-CoV-2 spike protein antibodies >1.5 AU/ml. The individuals with antibody titer >250 AU/ml were 34.9%. Among those with antibody levels >250 AU/ml 52% had previous history of COVID-19 infection. While participants with >100 AU/ml of antibodies were 12.7%. However 9.5% showed antibody titer of >25 AU/ml. 27% of participants had antibody titers of >1.5-2.5 AU/ml. While antibody titers of <1.5 AU/ml were observed among 15.9% of participants. Majority of the individuals represented significantly high antibody titers against SARS-CoV-2 even before second booster dose of Ad5 based Sputnik V vaccine. Continuous monitoring of antibody levels among COVID-19 vaccinated populations are deemed to assess humoral immunity status against SARS-CoV-2 infections.

Introduction:

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), positive-strand RNA coronavirus of *Coronaviridae* family, is responsible for causing devastating COVID-19 pandemic with millions of deaths worldwide (1). The genome consists of non-structural and structural proteins including envelope (E), membrane (M), and nucleocapsid (N) and spike (S). During SARS-CoV-2 course of infection, the spike glycoproteins interact with angiotensin converting enzyme 2 (ACE2) across the epithelial Alveolar Type 2 (AT2) progenitor cells and regulate several biochemical mechanisms or pathways to favor viral pathogenesis (2).

To date, majority of COVID-19 vaccine strategies aim to induce neutralizing antibodies against spike region, with ultimate goal of blocking SARS-CoV-2 early-stage infection (3). According to World Health Organization, 63 candidate vaccines are in human clinical trials, and > 172 vaccine candidates are at preclinical stages (4). Among SARS-CoV-2 vaccines at developmental stage, only Gam-COVID-Vac or Sputnik V utilized heterologous (adenovirus 5 and adenovirus 26) prime-boost recombinant adenovirus approach given 21 days apart (to overcome any pre-existing adenovirus immunity) responsibly provoked humoral and cellular immune response among 98% and 100% volunteers, respectively (5, 6).

Post-intramuscular injection of Sputnik V, the replication deficient Ad26 and Ad5 penetrate into host cells and deliver recombinant DNA into the nucleus to stimulate transcription *via* synthesizing mRNAs translating spike proteins which migrate towards the cell surface. The vaccinated cells may break spike proteins into fragments. Afterwards, protruding spikes, recognized by the immune system induces strong immune responses to kill the vaccinated cell. However, the cell debris containing spike proteins or

fragments are taken up by antigen-presenting cell (APC), which presents fragments of the spike protein on cell surface. Upon encounter with helper T cells, the B cells are activated and start proliferation to generate antibodies against spike protein. The APC can also activate killer T lymphocytes to destroy SARS-CoV-2 infected cells displaying spike protein fragments on surfaces (7).

A Phase III clinical trial conducted by Gamaleya Research Institute Russia on 19,866 volunteers who received Sputnik V revealed strong efficacy, immunogenicity and safety results. The efficacy results were 91.6% with no serious adverse events (8, 9). Among vaccinated volunteers with history of wild type SARS-CoV-2 infection, the virus neutralizing antibodies level were 1.3–1.5 times higher (6). The levels of neutralizing antibodies are significantly important in context of SARS-CoV-2 vaccines. Reliable quantification of the antibody responses is critical for estimating the time of protection or possible vaccine related failures.

Depending upon SARS-CoV-2 global burden of continuously increasing COVID-19, there is a dire need for mass vaccination worldwide. In Pakistan due to several healthcare challenges, identification of neutralizing antibodies against SARS-CoV-2 might be crucial in formulating new vaccination strategies to curtail unprecedented COVID-19 epidemics. To date, 0.8 million people have been infected with COVID-19 in Pakistan and caused deaths in 16,999 individuals. As of 21 April 2021, 1,548,714 vaccine doses have been administered among general public (10). We aimed to evaluate SARS-CoV-2 spike antibody levels among Sputnik V vaccinated group of individuals in Pakistani population.

Material And Methods:

To investigate SARS-CoV-2 spike antibody response to vaccination in Pakistani populations, a cross-sectional study was conducted on 2000 participants enrolled for Sputnik V (Gamaleya National Research Center, Russia) vaccination after first dose of Ad26-based vaccine. From 100 anti-SARS-CoV-2 real-time PCR negative cases, respective clinical samples were obtained at 21st day by medical experts at Islamabad Diagnostic Center Islamabad, Pakistan. Patients written consent was obtained prior to examination.

Quantitative determination of antibodies to the SARS-CoV-2 spike protein was based upon double-antigen sandwich assay principle using Electro-chemiluminescence immunoassay (ECLIA) (Elecsys # 09289267190 Roche, USA). The samples were incubated with biotinylated and ruthenylated RBD antigen, followed by addition of streptavidincoated microparticles, and transferred to the measuring cell, where microparticles are magnetically captured onto the surface of the electrode. The Electrochemiluminescence is later on induced by providing voltage and measured *via* photomultiplier. Test kits were placed according to manufacturer's instructions. Current study was approved by institutional review board of IDC Pakistan.

Results:

A total of 100 participants were enrolled in the study. Of these, real-time PCR negative (for SARS-CoV-2 RNA) cases were pre-selected for evaluation of SARS-CoV-2 spike protein antibody levels on 21 days after 1st dose administration. Among selected subjects, 58.8 % were males and 41.2 % were females. The mean

age was 45 years (range 18–60). The ECLIA analysis revealed SARS-CoV-2 spike antibody titers among enrolled participants.

85% of the participants showed strong positive results of SARS-CoV-2 spike protein antibodies > 1.5 AU/ml. It was shown that > 250 (in arbitrary units, AU/ml) antibody titer occurred among 34.9% of the participants. Among individuals with > 250 AU/ml antibody levels, 52% individuals had previous history of COVID-19 infection. The individuals with antibody titer > 100 AU/ml were 12.7%. And 9.5% showed antibody titer of > 25 AU/ml. However, 27% of the individuals constituted antibody titers of > 1.5–2.5 AU/ml. While antibody titers of < 1.5 AU/ml were observed among 15.9% participants (As shown in Fig. 1).

Overall, it can be inferred that majority of the individuals represented significantly higher antibody titers against SARS-CoV-2 even before second booster dose of Ad5 based Sputnik V vaccine. The sensitivity of ECLIA was 98.8% (95% CI: 98.1–99.3%). And the diagnostic assay showed no cross reactivity against MERS-CoV, common cold, coronavirus HKU1, NL63, 229E, OC43, or other potentially cross-reactive samples.

Discussion:

In Pakistan, the population exceeds 223.9 million and literacy rate is relatively lower (11, 12). Viral infections are on its surge (13–18). The vaccine coverage is not successful in Pakistan, due to two major reasons; including limited procurement or supply of vaccine and other might be generalized conspiracy theories, which are also prevalent across the globe. In Pakistan, majority of the people are willing to get benefit from the vaccine preventable COVID-19. However, huge demand of anti-SARS-CoV-2 vaccine supply from manufacturing companies has put a large gap across under-developed countries.

Vaccine hesitancy is integral, underlying factor linked with sporadic proliferation of viruses worldwide. Although education level is lower but awareness about vaccination is shown by the fact that people are willing to pay the actual price of vaccine (which is approximately 70 \$). Due to utmost support of Pakistan Armed Forces, efficient smart lock down policies and implementation of standard operating procedures by National Command Operation Center (NCOC) of Pakistan, the expected number of deaths (80,000 per day), predicted by Medical Research Council Centre for Global Infectious Disease Analysis, Imperial College London, were efficiently reduced during first wave of COVID-19 in Pakistan (19). Several countries across the globe and WHO also appreciated smart policies implemented by Government of Pakistan to control COVID-19 first wave in Pakistan. Currently there are several multi-variant strains of SARS-CoV-2 spreading across the nation, and several deaths are being reported from neighbor country India. During the need of hour, China and Russia outreached and joined hands in support of vaccine supply under Government-Government (G/G) or Government-Business (G/B) schemes, to facilitate mass vaccination in Pakistan.

The immune responses to Sputnik V vaccine were analyzed among 602 healthcare personnel volunteers from Tucumán-Argentina. It was interesting to know that among subjects with prior COVID-19 history of infection significantly strong immune response after a single vaccine dose were elicited (20). Similarly another study conducted in United States of America among 110 study participants with or without pre-existing SARS-CoV-2 immunity, vaccinated with Pfizer and Moderna COVID-19 vaccines, revealed that after

the first dose of administration the antibody titers with preexisting immunity were around 10 to 45 times as high as those of vaccines without preexisting immunity (21).

In contrast to other vaccines with two doses, the Sputnik V is adenoviral (serotypes 5 and 26) based recombinant DNA vaccine, in which antigen insert is an unmodified full-length S-protein (22). In current study among volunteers from Pakistan origin, the SARS-CoV-2 real time PCR negative volunteers were pre-selected for the analysis. Majority of the individuals presented notably higher anti-SARS-CoV-2 antibody levels of > 250 AU/ml. Among those with antibody levels > 250 AU/ml 52% had previous history of COVID-19 infection. Generally among two dosage vaccines, the ramp up of immunity after first dose is rare. However, the Sputnik V has interestingly and amazingly aroused ramp up immunity among majority of enrolled participants even after the first dose of administration, which can further affirm the efficacy of the vaccine in Pakistani population. One of the limitations of our study is that cellular immunity was not evaluated in clinical laboratory due to lack of facilities.

Current study is important not only for policy making strategic organizations at national level but also demonstrate need for accurate determination of SARS-CoV-2 spike antibody levels among vaccinated group of individuals across the world.

Conclusion:

Evaluation of neutralizing antibodies levels post vaccination is very helpful to determine level of humoral immunity, after vaccination. Herein, we demonstrated for the first time that among Sputnik V vaccinated group, prior to second dose administration, the SARS-CoV-2 spike antibodies levels were significantly high which indicates promising results for immunization against COVID-19. Based upon this study, it can be speculated that after the second dose administration of Sputnik V, the SARS-CoV-2 spike antibody levels will further ramp up significantly to immunize against COVID-19. Also quick immunity with 1st dose gives Sputnik V an edge over other vaccines, which can be useful at a time when virus spread is fast and many patients get infected before second dose of other vaccines.

Abbreviations:

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

WHO: World Health Organization

MERS-CoV: Middle East Respiratory Syndrome Coronavirus

ECLIA: Electro-chemiluminescence immunoassay

APC: antigen-presenting cell

NCOC: National Command Operation Center

ACE2: Angiotensin-Converting Enzyme 2

AT2: Alveolar type 2 progenitor

PCR: Polymerase Chain Reaction

Declarations:

Ethics approval and consent to participate:

The study has been approved by ethical review board of Islamabad Diagnostic Center Pakistan, and informed patients concern was obtained.

Consent to publication:

All authors approved the submission of the manuscript for publication

Availability of data and material:

The data is available and can be used for the academic or research purposes.

Competing interests:

The authors have no conflict of interest.

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Authors Contribution:

RiU is principal investigator of the study. RiU and US conceived the study, US wrote manuscript and analyzed data and Co-PI of the study; ZZP assisted in manuscript writing and analysis, SRU, AAK, AR, AW, and ReU performed the experiments and helped in data analysis.

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Figures

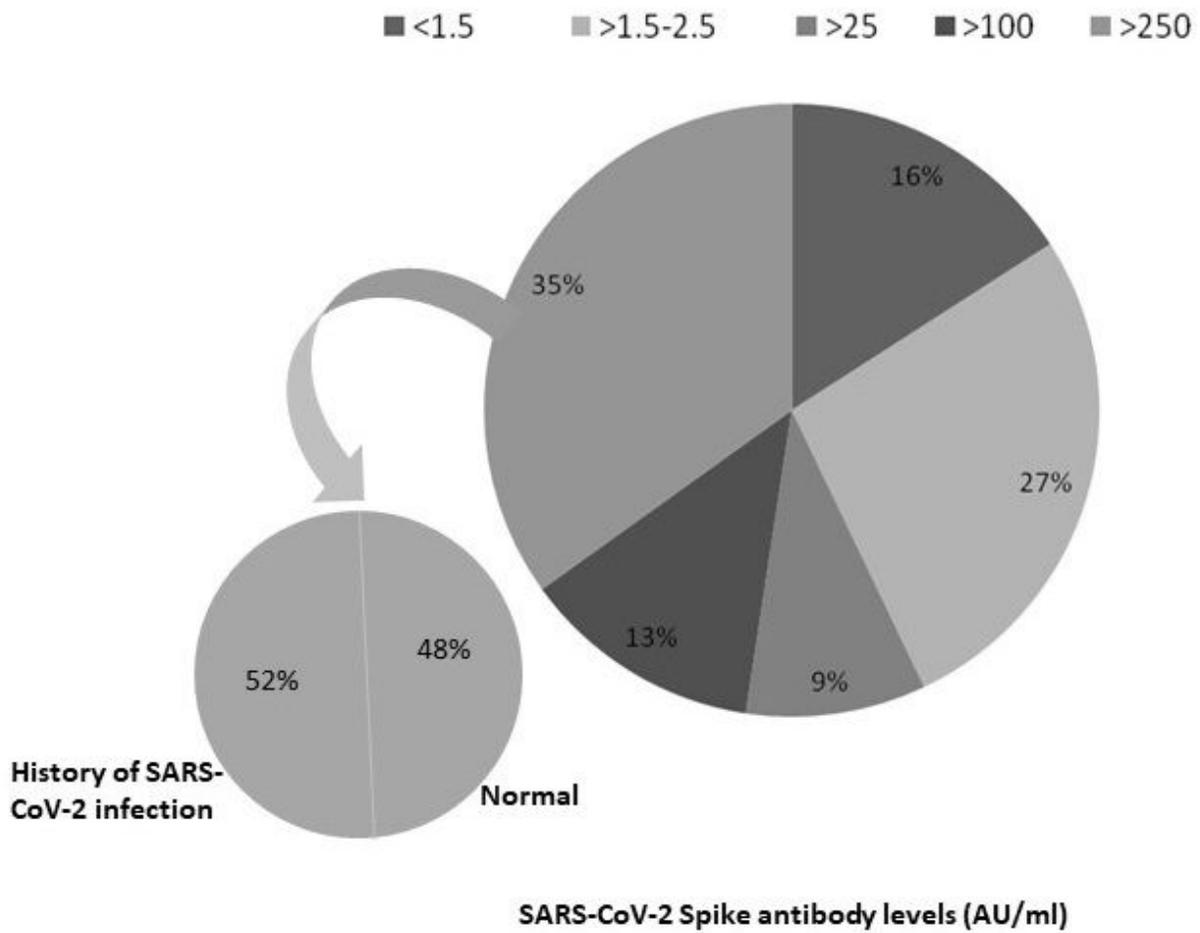


Figure 1

Prevalence of neutralizing antibodies against SARS-COV-2 spike protein among Sputnik V first dose vaccinated.