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Use of a Non-Invasive Method for Detection of Nasal and Pharyngeal Neutralizing Antibodies in Immunized Patients against Sars-CoV-2

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ABSTRACT

Nowadays, several vaccines have been approved by the FDA for prevention of severe COVID-19. Mexico is one of the countries with the highest SARS-Cov-2 infection rate and associated deaths, so determining the post-vaccination immunity status is essential. Multiple studies have evaluated immune response to SARS-CoV-2 in plasma or serum of infected and immunized patients, meanwhile there are fewer studies in saliva although it can be obtained by non-invasive methods. On the other hand, local nasal and pharyngeal immune response is even lesser known. Because the nasal cavity has been identified as the primary site for virus replication and transmission, we decided to investigate the presence of neutralizing antibodies in swabs (nasal and pharyngeal) and saliva of 5 young immunized individuals. Results showed the presence of neutralizing antibodies in nasal and pharyngeal swab supernatants in all samples analyzed, suggesting that nasal and pharyngeal surfaces may be clinically important and relevant for the identification of individuals with early infection, reinfection, or that have been immunized against SARS-CoV-2. We propose the use of swabs as a non-invasive method for dual identification of SARS-CoV-2 and immune mediators.

INTRODUCTION

Mexico is one of the most affected countries by the COVID-19 pandemic [1]. To date, vaccination campaigns against SARS-CoV-2 have been implemented in our country using different FDA-approved vaccines, Pfizer/BioNtech vaccine among them. Plasma and serum have been used to assess immune response to SARS-CoV-2 following vaccination (memory antibodies) [2]. However, nasal cavity has been reported as the primary site for virus replication and transmission [3], and relevant to evaluate local antibody, so studying the immune response to SARS-CoV-2 on these surfaces could provide useful information for the development of therapeutic targets focused on virus early entry [4]. Recently the use of saliva has been proposed for quantization of local antibodies [5,6] but, this fluid has proteolytic enzymes that degrade the sought antibodies.

Nasal and pharyngeal swabs are routinely used for COVID-19 diagnosis and such sampling method could be used to evaluate local immune response. This communication aims to assess the use of swabbing for the non-invasive identification of antibodies and immune mediators.

SAMPLE COLLECTION AND PROCESSING

Nasal swabbing was performed on five asymptomatic 25-year-old males 14 ± 2 days after receiving a second dose of Pfizer/BioNtech comirnaty vaccine. Subjects were previously informed about the study and signed informed consent, according to ethical and safety guidelines, also in agreement to Federal Health Law.

Nasopharyngeal Specimen Collection Swab (Nest Biotechnology, USA): Patient were asked to sit with head back-tilted at 70 degrees. Swab was then gently inserted into the nostril following the nose septum floor and straight back until posterior nasopharynx was reached. Swab was rotated several times while in contact with the nasopharyngeal wall. Oropharyngeal Specimen Collection Swab: Patient were asked to sit with head back-tilted at 70 degrees. Swab was then gently inserted into the back of the throat and tonsillar area and rubbed over both tonsillar pillars and posterior oropharynx, avoiding contact with tongue, teeth, and gums. Samples nasopharyngeal and oropharyngeal swab was them placed into tube 15 ml (Sarstedt, USA) with 2 ml. transport media (medium MEM and antibiotic) were kept 4°C.

Saliva was collected by gently brushing the gum line for 2 min and collecting into an Oracol S14 device (Malvern Medical Developments, UK) or by drooling into a simple plastic tube. For debris removal, Oracol S14 saliva collection device was placed into eppendorf tube was containing 50µl of protease inhibitors (Thermo Fisher, USA) and 1 ml of Hartmann's solution, were centrifuged 5 min. at 4000 rpm. Supernatant was then transferred into a 2ml microtube and samples were stored at 20°C for a week until use.

PROCESSING

All samples (nasal and pharyngeal swabs and saliva) were them centrifuged 5 min at 4000 rpm.

TOTAL NEUTRALIZING ANTIBODIES BY ELISA

Presence of total neutralizing antibodies in samples from swabs (nasal and pharyngeal) and saliva were obtained using the AcroBiosystems kit (#cat. TAS-K022, lot #TA22-211P-V4) following the manufacturer's instructions.

RESULTS AND DISCUSSION

In this preliminary study, the presence of total neutralizing antibodies was detected in all nasal and pharyngeal swab samples as well as in saliva of immunized individuals; pharyngeal swab could also be used to detect antibodies. Total neutralizing antibodies quantization is shown in figure 1, and presence of IgA antibodies against the RBD region was detected in two immunized individuals. Detection of neutralizing IgG antibodies in the plasma of immunized individuals suggests that they could reach the lumen of lower respiratory tract by transudation/extravasation. However, a limited number of studies have evaluated the immune response in the mucosa of the upper respiratory tract, mainly on Nasal strip and Epithelial Lining Fluid (NELF) through Leukosorb medium method [2].

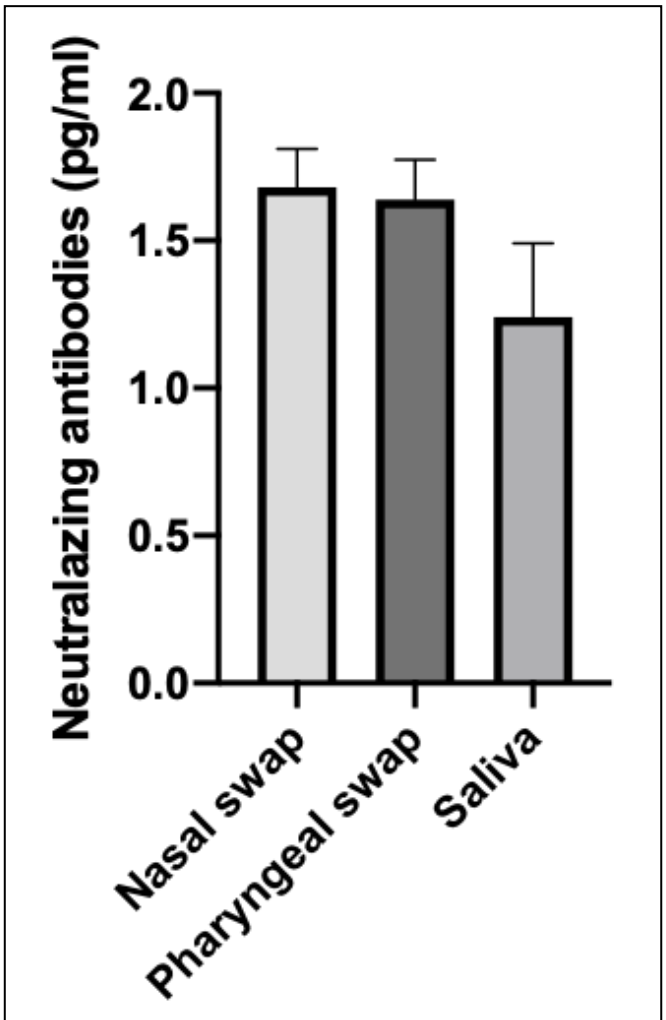

Figure 1: Total neutralizing antibodies compared (pg/ml) SARS-CoV-2 in the 3 study groups.

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Nevertheless, a foreign object such as a nasal strips does not reach the back of the nasal cavity with ease. Swabs are used routinely to identify SARS-CoV-2 and is an inexpensive and available method for many hospitals. The induction of nasal antibodies would indicate a quick protection response to viral infection by aerosols, on the other hand, using a nasal mask as we previously reported reduces the risk of virus transmission [7,8]. Additionally, we observed the presence of chemokines such as MCP-1 in low concentration in the same group of individuals (data not shown), hence we propose that swabbing (nasal and pharyngeal) could be used simultaneously to identify SARS-CoV-2 by PCR and to identify neutralizing antibodies and cytokines within the same sample. Moreover, swabs may be used in a broad range of age groups, in epidemiological field sampling and while evaluating intranasally administered vaccines. In the future, the use of swabs could be useful for epidemiological evaluation in the nasal mucosa and the response of nasal vaccines in pediatric and geriatric groups [9,10].

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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