Determination of Ki67 after SARS-CoV-2 infection by enzyme linked immunoassay

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Research note

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Abstract

Objectives

The immunity status after the sever acute respiratory syndrome coronavirus-2 (SARS-CoV-2) can determine the protection against reinfection and efficacy of vaccination. Although the mechanisms of SARS-CoV-2 induced immune response need further investigation to provide more data on innate and adaptive immune responses. It can be expected that in case of fail of SARS-CoV-2 induced humoral immunity the adaptive one is developed. The NovaLisa SARS-CoV-2 (COVID-19) IgG ELISA diagnostical kit (NovaTec Immunodiagnostica GmbH, Germany) for in vitro diagnostic and Human Ki67 ELISA kit (MyBioSource Inc, USA) were used. The study has been performed according to the ELISA kits specific protocols; the absorbance was measured at 450 nm wavelength. For the calculation of results we used the manufacturers' guidelines.

Results

It has been revealed that: a) 16 (32.7%) samples are negative; b) 5 (10.2%) samples are equivocal; and c) 28 (57.1%) samples are positive for IgG for nucleocapsid protein of SARS-CoV-2. Median levels of Ki67 in: a) negative for IgG of SARS-CoV-2 nucleocapsid protein samples is 14.95 ng/ml; b) equivocal samples is 10.6 ng/ml; and c) positive - 8.3 ng/ml. Increased level of Ki67 correlates with the negative status for IgG of SARS-CoV-2 nucleocapsid protein.

Introduction

The sever acute respiratory syndrome coronavirus-2 (SARS-CoV-2) causes Corona Virus Disease-2019 (COVID-19), which changed our daily habits [1] and is considered as a major global health challenge. The peculiarities of SARS-CoV-2 infection and its induced immune response are currently the top research priority. It has been reported that the protective immunity after infection is the basis of the immunological memory. It can determine the protection against reinfection of SARS-CoV-2 and efficacy of vaccination as well [2]. Although the mechanisms of SARS-CoV-2 induced immune response need further investigation to provide more data on innate and adaptive immune responses [3]. It has been reported, that immunoglobulin G (IgG), IgM, and IgA antibodies against SARS-CoV-2 spike (S) and nucleocapsid (N) proteins develop within 2 weeks after the COVID-19 clinical symptoms onset. Although, IgG may be not detected in some cases after infection [4]. The development of SARS-CoV-2 specific T-cell immune memory in SARS-CoV-2 infection recovered cases have been reported [5]. We postulated that in IgG negative cases the T-cell immune memory is developed. To address this question, we initiated the study aimed determination of Ki67 expression after SARS-CoV-2 infection by enzyme linked immunoassay (ELISA). It has been reported that Ki67 expression is a specific, quantitative and reproducible indicator of antigen-specific T lymphocytes proliferation [6].

Methods

In this study were used blood samples of the students and staff members of the Petre Shotadze Tbilisi Medical Academy with clinically documented episodes of SARS-CoV-2 infection. The study participants registered after being informed about the details of the study. 35 participants were females, and 14 were males; the age distribution of participants was between 17 and 55. Blood samples were collected and analysed at the university facilities.

The NovaLisa SARS-CoV-2 (COVID-19) IgG ELISA diagnostical kit (NovaTec Immunodiagnostica GmbH, Germany) for in vitro diagnostic and Human Ki67 ELISA kit (MyBioSource Inc, USA) were used. The study has been performed according to the ELISA kits specific protocols; the absorbance was measured at 450 nm wavelength. For the calculation of results we used the manufacturers' guidelines: a) sample absorbance value x 10 / cut-off control absorbance value (NovaLisa SARS-CoV-2 (COVID-19) IgG ELISA diagnostical kit), and b) samples concentrations interpolation from the standard curve (Human Ki67 ELISA kit). The results were interpreted as positive (> 11), equivocal (9-11), and negative (< 9) in case of IgG for nucleocapsid protein of SARS-CoV-2. The detection range of Human Ki67 ELISA kit is 0,016-10 ng/ml. It has been reported that the median serum levels of Ki67 in healthy population is 3.92 (2.72-7.29) ng/ml [7].

Results

It has been revealed that: a) 16 (32.7 %) out of 49 samples are interpreted as negative for IgG for nucleocapsid protein of SARS-CoV-2; b) 5 (10.2 %) out of 49 samples are interpreted as equivocal for IgG for nucleocapsid protein of SARS-CoV-2; and c) 28 (57.1 %) out of 49 samples are interpreted as positive for IgG for nucleocapsid protein of SARS-CoV-2. Furthermore, it has been revealed that: a) median levels of Ki67 in negative for IgG of SARS-CoV-2 nucleocapsid protein samples is 14.95 (11.8-18.9) ng/ml; b) median levels of Ki67 in equivocal for IgG of SARS-CoV-2 nucleocapsid protein samples is 10.6 (8.2-14.2) ng/ml; and c) median levels of Ki67 in positive for IgG of SARS-CoV-2 nucleocapsid protein samples is 8.3 (5.7-10.9) ng/ml.

Findings suggest that increased level of Ki67 correlates with the negative status for IgG of SARS-CoV-2 nucleocapsid protein. Therefore, we assume that in cases negative for antibodies T-cell immune memory is developed. Although study with larger number of participants should be performed.

Table 1. Correlation between Ki67 levels and status of IgG for nucleocapsid protein of SARS-CoV-2

	IgG	Ki67
Value	Positive	8.3 ng/ml
Value	Equivocal	10.6 ng/ml
Value	Negative	14.95 ng/ml

Limitations

- The study has been performed on a small number of participants.
- The Ki67 expression on the background of SARS-CoV-2 infection should be further investigated.

Declarations

Ethics approval and consent to participate

The present study has been approved by the Bioethics Committee of the Petre Shotadze Tbilisi Medical Academy. All procedures performed in the present study were in accordance with the Helsinki Declaration of 1975, as revised in 2013. The participants were informed about the design and objectives of the study; all participants provided informed consent for inclusion by their registration for participation in the study.

Consent for publication

Not applicable.

Availability of data and material

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare that they have no competing interests.

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Author’s contributions

SI performed experimental activities; TS organized and performed samples collection; ML organized technical issues of experimental activities; SR performed data analysis; EK contributes study performance and manuscript preparation. All authors read and approved the final manuscript.

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