Antinuclear antibodies (ANA) in COVID-19 infection

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Research Article

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Abstract

We examined correlates of antinuclear antibody (ANA) positivity in individuals with COVID-19 infection. Sera from 156 patients were evaluated by indirect immunofluorescence on Hep-2 cells for ANA pattern and semi-quantitative titer. There was a significant difference in the prevalence of ANA (1:100) by gender (P = 0.0294). Male gender was correlated with ANA positivity and association persisted in multivariable analyses (OR = 0.354; CI: 0.139–0.816; P = 0.0200). Age (OR = 1.932; CI: 0.929–4.121; P = 0.0814), warm weather (OR = 1.307; CI: 0.611–2.921; p = 0.4989), geographic location (or = 0.886; CI:0.353–2.442; P = 0.8042), and diabetes mellitus (OR = 1.765; CI:0.513–5.477; P = 0.3369) were not correlated with ANA positivity. In this analysis, hospitalization was used as indicator for a more severe course. Of the 156 COVID-19 patients, 18 (11.5%) were hospitalized. Among 18 hospitalized patients, 4 (22.2%) were ANA positive (Table 2), although this did not reach statistical significance (OR = 0.841; CI: 0.227–2.527; p = 0.7725). In conclusion, although males were more prone to produce ANA during COVID-19 infection, this was not correlated with severe course.

Introduction

In all individuals there is a degree of recognition of self. A low level of autoimmunity seems to be the norm and generally does not result in pathology. If immunological tolerance fails to eliminate or control pathogenic self-reactive lymphocytes, then autoimmune disease arises. Autoimmune diseases lead to innate and adaptive immunity dysfunctions. Antinuclear antibodies (ANA) are autoantibodies directed against antigens in the cell nucleus. The presence of ANA was associated with autoimmune connective tissue diseases [1]. However, a positive ANA test may also be seen with non-autoimmune inflammatory diseases, including both acute and chronic infections. Infections may cause ANA to appear or in ANA positive patients an immunocompromised status may have resulted in infections [2].

SARS-CoV-2, the causative agent of COVID-19, is a single stranded, positive sense RNA virus belonging to the Coronaviridae family, of the beta genera. Patients infected with SARS-CoV-2 show a wide spectrum of clinical manifestations ranging from mild febrile illness and cough up to acute respiratory distress syndrome, multiple organ failure, and death [3]. Some authors believed that autoantibody positive patients are more likely to have severe disease requiring care in the intensive care unit [4]. Cytokine storm, affects adversely the prognosis of COVID-19 infection, can be triggered by autoimmune conditions [5]. Defects in the immune system may lead covid-19 infection to proceed more severely [6]. We evaluate whether ANA positivity was associated with age, gender, warm weather, geographic location, diabetes mellitus and hospitalization in patients with COVID-19 infection.

Results

Study cohort

Overall, the cohort was 35.3% (55) male and 64.7% (101) female. The mean (±SD) age of cohort was 45.2±15.1 years.

ANA positivity

Among the 156 patients studied, 40 (25.6%) were positive for ANA at 1:100. The median age of ANA positive patients was 47 (range: 18 - 78) years; 9 (22.5%) of the patients were male. Figure 1 presents the distributions of ANA by titer and pattern among the total cohort. 22.5% of positive individuals had low titers of ANA (≤1:100) in entire cohort (Fig 1). Of the 40 ANA positive samples in the cohort, 22 (55.0%) nuclear, 9 (22.5%) cytoplasmic, 5 (12.5%) mitotic and 4 (10.0%) were mixed pattern. Among the 40 ANA positive patients, no correlations were observed between hospitalization and ANA pattern (p=0.1182) or ANA titer (p=0.4482).

Correlates of ANA positivity

Table 1 presents univariable correlates of ANA positivity at ≥1:100. Age (OR=1.932; CI: 0.929 – 4.121; P=0.0814), warm weather (OR=1.307; CI: 0.611 – 2.921; p=0.4989), geographic location (or=0.886; CI:0.353 – 2.442; P=0.8042), and diabetes mellitus (OR=1.765; CI:0.513 – 5.477; P=0.3369) were not correlated with ANA positivity. Gender was significantly correlated with ANA positivity. In a multivariable model of the effect of gender on ANA positivity, controlling for age, only gender retained its significance (Table 1). Male gender was correlated with ANA positivity and association persisted in multivariable analyses (OR=0.354; CI: 0.139 – 0.816; P=0.0200).

Impact of ANA positivity on COVID-19 course

In this analysis, hospitalization was used as indicator for a more severe course. Of the 156 COVID-19 patients, 18 (11.5%) were hospitalized. Among 18 hospitalized patients, 4 (22.2%) were ANA positive (Table 2), although this did not reach statistical significance (OR=0.841; CI: 0.227 – 2.527; p=0.7725).

Discussion

Our study observed a prevalence of low levels of ANA among 25.6% COVID-19 patients. This was less than half of the normal background prevalence of ANA in patients in Kocaeli, which was approximately 57.3% [11]. We also observed a prevalence of ANA of 22.2% in hospitalized COVID-19 patients. The ANA prevalence between studies in hospitalized COVID-19 patients were divergent, ranging between 34.5% and 50%. (Table 2). A report from Greece demonstrated that the positive rate of ANA was 34.5% in hospitalized patients [10]. In Italy, 34 COVID-19 cases were analyzed, and the detection rate of ANA was 35.3% [8, 9]. Apart from those studies, a report from Japan, one of two (50%) severe covid-19 cases were found ANA positive [7]. All studies used indirect immunofluorescence (IIF) as method for serum ANA detection. The higher rates of ANA from Japan might be due to the characteristics of the two cases demographic, environmental or genetic factors. In a previous study with multiplex immunoassay the ANA was found 25.0% of covid-19 patients, three third of the cases were from intensive care unit [4]. One of the

reasons for the different results among the current and the previous studies might be related to the small study groups. The varying rates of ANA prevalence from different parts of the world might also be due to the characteristics of the study population demographic, environmental or genetic factors.

Age seems to be the most important factor associated with a more severe course of COVID-19 [12]. However, in this study, no relation was found between age and ANA positivity. Male gender has also been described as an important risk factor for a more severe course and higher mortality. In this study, male gender was correlated with ANA positivity, but there was no correlation between severe course of infection and ANA positivity.

The positive rate of ANA IIF Hep-2 test was different between our previous [11] and current studies (57.3–25.6%). The female to male ratio was also higher (4.6 vs 3.4). Comparing ANA patterns between old and current studies, the rate of mixed (10.0% vs 21.3%) patterns was found to decrease. The mixed patterns in our study referred to the existence of two or more patterns. The mixed patterns could provide a hint for SSc (43.4%) and SLE (27.8%) [13]. Though, in this study, ANA pattern and titer were found irrelevant with the severe course of infection.

There are a few limitations of this study. The study population might not reflect all aspects of target population. We do not have the detailed clinical data needed to capture all possible comorbidities. The retrospective design of our study did not allow for the analysis interaction between anti-covid antibodies and ANA. The follow-up period of the ANA positive patients was limited. In general, the study data reflects characteristics of geographic properties. Further studies with larger populations are compulsory to interpret the study results in detail.

In conclusion, although males were more prone to produce ANA during COVID-19 infection, this was not correlated with severe course. It would be interesting for future studies to explore in greater detail whether severe course of COVID-19 infection in male is correlated with ANA positivity. Future studies are also needed to address the possible role of host and environmental factors in autoantibody formation in COVID-19 infection.

Methods

Study patients

The present study involved 156 patients from Kocaeli, Turkey. All individuals were Caucasian. Patients included in the present study were recruited sequentially and all had COVID-19 infection. The patients under 18 years old and patients with missing records were excluded from study. Study protocols were approved by the Kocaeli Faculty of Medicine Clinical Research Ethical Committee, Turkey (26.01.2021-2021/19). All methods were carried out in accordance with Declaration of Helsinki. In this retrospective study, all patient records and information was anonymized and de-identified prior to analysis, precluding the requirement of written informed consent.

Anti-nuclear antibody assays

ANA positivity and patterns were assessed by indirect immune-fluorescence on Hep-2 cells (Euroimmun, Luebeck, Germany) using standard techniques. Testing and evaluation were carried out according to the manufacturer's instructions. All samples were screened at a dilution of 1:100 for the presence of ANA and semi-quantitated between 1+ to 4+.

SARS CoV-2 qPCR assays

SARS CoV-2 qPCR was performed as previously described [14]. Diagnosis of COVID-19 infection and detection of virus RNA was performed by a quantitative PCR method. Gene amplification reactions and signal detections were performed in the Light Cycler 480 System (Roche Diagnostics GmbH, Mannheim, Germany). The human RNase P gene was selected as internal control.

Statistical analyses

Raw patients data were transferred to Microsoft Excell and analyzed with Microsoft Access softwares. Statistical analysis of data was carried out, using Graphpad Prism. Univariable analysis was performed using simple logistic regression test on the following variables: age, gender, geographic location, weather condition and diabetes mellitus. Multiple logistic regression analyses were used to assess corelates of ANA positivity. To facilitate categorical analyses, continuous variable such as age was dichotomized at median (age median: 44). The temperature of weather was split into those which were warm (April to September) and cold (October to March). For analyses of the effect of geographic location, addresses were dichotomized into urban and rural. Factors with a p-value ≤ 0.25 in univariable analysis were entered into multiple logistic regression model.

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Tables

Table 1. Correlates of ANA positivity among 156 individuals with SARS-CoV-2

Factors	OR	95%	P-value
Univariable correlates			
Age	1.932	0.929 – 4.121	0.0814
Gender	0.384	0.153 – 0.874	0.0294
Geographic location	0.886	0.353 – 2.442	0.8042
Warm weather	1.307	0.611 – 2.921	0.4989
Diabetes mellitus	1.765	0.513 – 5.477	0.3369
Multivariable analyses			
Gender	0.354	0.139 – 0.816	0.0200
Age	2.124	1.005 – 4.625	0.0518

Table 2. Serum ANA prevalence in the hospitalized COVID-19 cohort, and in previous studies for comparison. All studies used indirect immunofluorescence as method for serum ANA detection.

n	Female (%)	Age range	Any titre	% ≥1:80	% ≥1:160	Location	Reference
18	38.9	25-96	4	22.2	16.7	Turkey	Present study
2	50.0	65-73	2	50.0	0	Japan	[7]
34	50.0	22-90	12	35.3	35.3	Italy	[8, 9]
29	27.6	43-85	10	34.5	34.5	Greece	[10]

Figures

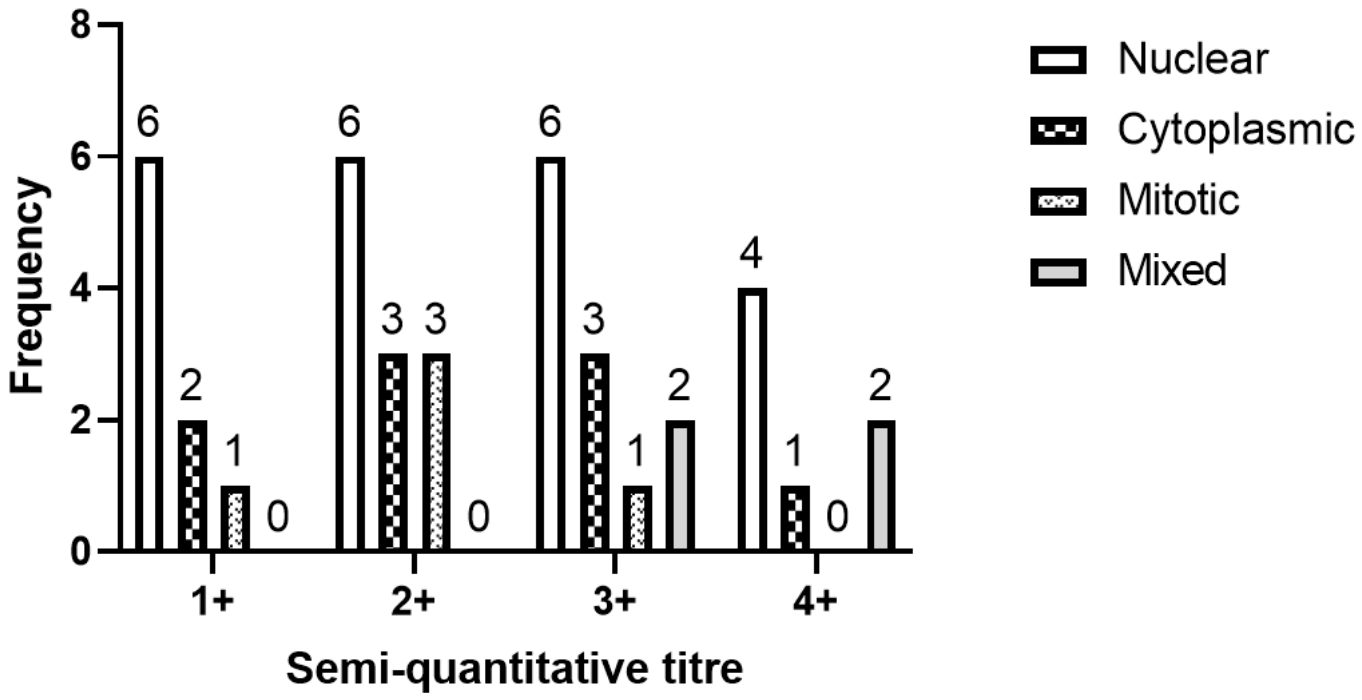

Figure 1

Observed distributions of ANA pattern and semi-quantitative titer. The frequencies of each of the patterns is indicated above each of the bars.