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Changes in COVID-19 IgM and IgG antibodies in emergency medical technicians (EMTs)

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Abstract

**Introduction:** Serologic testing can provide a safe and fast approach for assessing SARS-CoV-2 antibodies. These tests can be utilized as a complementary method in diagnosis and patients’ follow-up, and can also be helpful in epidemiological studies. This study aimed to describe temporal changes in the incidence of COVID-19 IgM and IgG antibodies in emergency medical technicians (EMTs) within a specified time period.

**Methods:** All EMTs working for Tehran Emergency Medical Service (EMS) center during May to September 2020 were eligible for this study. Those EMTs who were suspected/probable/confirmed cases of COVID-19, based on WHO defined criteria and were willing to participate, entered the study. The EMTs underwent serology testing four weeks after the occurrence of exposure (in suspected cases) or onset of their symptoms (in probable/confirmed cases). Cases were further confirmed by RT-PCR and/or lung CT, and antibody testing was performed for the second and third time with 12-week intervals. Finger-stick blood sampling was utilized for the specimen collection in three different phases. Samples were then analyzed by a commercial immunochromatography-based kit for qualitative measurement of serum IgM and IgG antibodies against the COVID-19 S-protein antigen.

**Results:** Two hundred eighty-four participants met the inclusion criteria; their mean age was 35.9 (SD = 7.6) years and consisted of 244 (85.9%) males. COVID-19 was confirmed in 169 out of 284 participants. Subsequently, 142 and 122 participants were included in phases 2 and 3 of the study, respectively. The number of seronegative patients exceeded seropositive ones in all three phases. At baseline, 162 (57%) patients were seronegative, 27 (9.5%) were only positive for IgG, 3 (1.1%) were only positive for IgM, and 92 (32.4%) were positive for both antibodies; Seventy-eight (54.9%) were seronegative, and 31 (21.8%) were positive for both antibodies in the second phase; These values were 85 (69.6%) and 8 (6.6%) for the third phase, respectively. Among the people who were positive IgG in the first phase (80 people), 56.3% were still positive in the second phase and 27.5% in both subsequent phases.

**Conclusion:** The results of our study show that there is a significant reduction in COVID-19 antibody seropositivity over time.

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Keywords: COVID-19 IgM antibodies IgG antibodies Emergency medical technicians

1. **Introduction**

Since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in November 2019, it has affected many countries [1,2]. Health care workers (HCWs) are more vulnerable to the disease than the general population [3]; and emergency medical technicians (EMTs) are among HCWs frequently in contact with probable, confirmed and even undiagnosed COVID-19 patients [4]. Considering that EMTs are at constant risk of exposure, they are a population of interest, and their immunity over time has a special importance [5,6].

Seroconversion and serum antibody trends over time in COVID-19 patients have received special attention recently [7-9]. SARS-CoV-2 and its related genus, beta-coronavirus, generally consist of four major proteins: spike glycoprotein (S), nucleocapsid protein (N), small envelope glycoprotein (E), and membrane glycoprotein (M). S protein...
plays a major role in viral attachment [10]. The receptor-binding domain (RBD) in the S1 subunit of S protein is responsible for interacting with ACE2 receptors and facilitating the virus entry into human cells. Thus antibodies against S protein make a vital area for research [10,11]. Serologic testing can provide a safe and fast means for assessing SARS-CoV-2 antibodies. These tests can be utilized as a complementary method in diagnosis and patient follow-ups and can be helpful in epidemiological studies [12,13]. The possibility of seroreversion and a decline in the serum antibody level over time is an important potential problem regarding COVID-19, which might lead to decreased immunity. Although several papers have assessed the persistence of post-recovery COVID-19 antibodies, the exact temporal course of the antibodies in more extended periods is not clear yet [14-16]. Specially, misguided beliefs about the length of time a SARS-CoV-2 infection confers immunity lead some to believe they do not need the vaccine [17,18]. This study aimed to describe temporal changes in the incidence of COVID-19 IgM and IgG antibodies in EMTs within a specified time period.

2. Methods

2.1. Study design and setting

Iran was among the most affected countries by the COVID-19 pandemic and its capital city, Tehran, was among the cities with a very high incidence rate of the disease. During a six month period, from 20 March 2020 until 21 September 2020, 1,301,896 calls were made to Tehran Emergency Medical Services (EMS) dispatch center, lead to 333,374 missions by the EMTs; 10,187 (3.06%) out of the performed missions were related to COVID-19 patients. All EMTs working for Tehran EMS center during May to September 2020 were eligible for participation in this study. The study proposal was approved by the ethical committee of Tehran University of Medical Sciences (TRTUMS.MEDICINE.REC.1399.1160). All subjects gave written informed consent prior to participation.

2.2. Study population

The sample collection was done in three different phases. At first, all EMTs who were suspected/probable/confirmed cases of COVID-19, based on WHO defined criteria [19], and were willing to participate, entered the study. All eligible EMTs who fulfilled the inclusion criteria were registered in a list.

Based on the literature, IgG antibodies reach their highest levels almost 4 weeks after the onset of COVID-19 symptoms [20-22]. Meanwhile, it was reported that antibodies were still detectable 3–6 months after onset of the disease symptoms [23-25]. Considering the content of literature and using experts’ opinion we made a scheduled process for serology testing. The EMTs underwent serology testing four weeks after the occurrence of exposure (in suspected cases) or onset of their symptoms (in probable/confirmed cases). Thereafter, we re-checked the antibodies for the second and third times with almost 12 weeks gap between each test. At the second phase (12 weeks after first test) just confirmed cases (positive findings of RT-PCR and/or lung CT scan) were tested again for antibodies.

2.3. Data collection

A checklist was distributed among participants to record their demographic data and several variables that might potentially affect the serum antibody trend. The checklist included age, sex, height and weight, cigarette smoking, and chronic underlying diseases.

2.4. Antibody assessment tool

Finger-stick blood sampling was utilized for the specimen collection. Our samples were then analyzed by a commercial immunochromatography-based rapid test kit made by Karmapharmaco co. Iran. The kit was used for qualitative measurement of serum IgM and IgG antibodies against the COVID-19 S-protein antigen. The tests were performed according to the manufacturer’s instructions and its accuracy was confirmed in a study [6].

2.5. Statistical analysis

The collected data were added to SPSS software (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp) and analyzed using descriptive statistics for the baseline and demographic variables. The quantitative data were reported as mean and standard deviation (SD) and qualitative data were described with frequency and percentage. The distribution and changes of qualitative data, like any potential changes of antibody frequencies in the study phases, were assessed with Chi-square or Fisher’s Exact test, as appropriate based on test assumption. We also used the independent t-test to compare means of qualitative data in two groups. A p-value of <0.05 was considered statistically significant.

3. Results

Almost 1800 EMTs were working for Tehran EMS center, of whom 284 (15.8%) EMTs were considered as probable/confirmed COVID-19 patient during the study period. The mean age of our study population was 35.9 (SD = 7.6) years consisting of 244 (85.9%) male and 40 (14.1%) female patients. The mean BMI of the participants was 23.5 (SD = 3.9). Based on the findings, of 284 EMTs, 4.2% were cigarette smokers and 18% had at least one chronic medical disease. There was no significant difference between the IgM or IgG positive and negative participants regarding age, medical history, or cigarette smoking (p > 0.05).

In the first phase of the study, these 284 EMTs that all met the inclusion criteria were tested for COVID-19 antibodies. Albeit, out of 284 EMTs participating in the first phase, 169 were positive for COVID-19 based on RT-PCR test or lung CT scan. Subsequently, 142 and 122 EMTs were included in phases 2 and 3 of the study, respectively.

The number of seronegative patients exceeded seropositive ones in all three phases. At the baseline, 162 (57%) patients were seronegative, 27 (9.5%) were only positive for IgG, 3 (1.1%) were only positive for IgM, and 92 (32.4%) were positive for both antibodies. Similarly, among the participants who were included in the subsequent phases, 78 (54.9%) were seronegative, and 31 (21.8%) were positive for both antibodies in the second phase; these figures were 85 (69.6%) and 8 (6.6%) for the third phase, respectively (Table 1).

Comparison of first versus second phases (p < 0.001), second versus third phases (p < 0.001) and also, first and third phases (p = 0.025) showed that seropositivity declined significantly over the study period.

An evaluation of the seronegativity trend between phases (Tables 2-4) revealed that most patients who were negative for both antibodies remained negative in the latter phases. Of the total number of IgM and IgG negative patients in the first phase who participated in the second phase, 33 (76.7%) remained seronegative. This value was 54 (85.7%) and 30 (78.9%) for phase 2 to 3 and phase 1 to 3, respectively. On the other hand, the results demonstrated a considerable decline in seropositivity over time. Of the total number of patients who tested positive for both antibodies in the first phase and engaged in the second phase, 22 (28.8%) remained positive for both antibodies, 25 (32.9%) resulted positive only for IgG, and 29 (38.2%) turned negative. In a similar manner, the study revealed that in the period between the second and third phase, 2 (8%) patients remained positive for both antibodies, 9 (36%) turned positive only for IgG, and 14 (56%) were negative. Likewise, between first and third phase 6 (8.8%) remained positive, 18 (26.5%) were only positive for IgG, and 44 (64.7%) turned negative.

During the period between the first and second phase, 10 (23.3%) patients seroconverted; 3 of them only tested positive for IgG, and others were positive for both antibodies. Between phase two and
three, nine (14.3%) patients seroconverted; two of them only tested positive for IgM, five for IgG, and the other two were positive for both.

In the same way, the seroreversion rate was analyzed, indicating a substantial reduction in antibody levels. During the period between the first and second phase, 45 patients seroreverted, including 16 (76.2%) out of 21 patients who were IgG positive and 29 (38.2%) out of 76 patients who were positive for both antibodies. Similarly, between the second and third phase, 27 patients seroreverted, including 1 (50%) of IgM positive participants, 12 (44.4%) of IgG positive ones, and 14 (56%) of those who were positive for both antibodies.

Fig. 1 shows a summary of seroconversion in our study patients. Out of the total, 117 EMTs participated in all 3 phases of the study. Among these individuals, 80 EMTs (68.4%) were positive for IgG in the first phase and this percentage was 44.4% and 28.2% for the second and third phases, respectively. Out of 117 EMTs, 91 were positive for IgG in at least one of the three phases and 22 (18.8%) were positive for IgG in all three phases (Fig. 1). In other words, among the people who were positive IgG in the first phase (80 people), 56.3% were still positive in the second phase and 27.5% in both subsequent phases (second and third).
Table 4
Frequency distribution and changes in first vs. third phase antibody test results. (p 0.025)

|                          | Negative for both Antibodies | Positive for IgM | Positive for IgG | Positive for both antibodies |
|--------------------------|------------------------------|------------------|-----------------|------------------------------|
| First phase              | Count                        | 30               | 0               | 6                            | 2                            | 38                           |
|                          | % within P.1                 | 78.9             | 0.0             | 15.8                         | 5.3                          | 100.0                        |
|                          | % within P.2                 | 35.3             | 0.0             | 23.1                         | 25.0                         | 31.1                         |
| Positive for IgM         | Count                        | 1                | 1               | 0                            | 0                            | 2                            |
|                          | % within P.1                 | 50.0             | 50.0            | 0.0                          | 0.0                          | 100.0                        |
|                          | % within P.2                 | 1.2              | 33.3            | 0.0                          | 0.0                          | 1.6                          |
| Positive for IgG         | Count                        | 10               | 2               | 14.3                         | 0.0                          | 14.4                         |
|                          | % within P.1                 | 71.4             | 2               | 14.3                         | 0.0                          | 100.0                        |
|                          | % within P.2                 | 11.8             | 66.7            | 7.7                          | 0.0                          | 11.5                         |
| Positive for both antibodies | Count                     | 44               | 0               | 18                           | 6                            | 68                           |
|                          | % within P.1                 | 64.7             | 0.0             | 26.5                         | 8.8                          | 100.0                        |
|                          | % within P.2                 | 51.8             | 0.0             | 69.2                         | 75.0                         | 55.7                         |
| Total                    | Count                        | 85               | 3               | 26                           | 8                            | 122                          |
|                          | % within P.1                 | 69.7             | 2.5             | 21.3                         | 6.6                          | 100.0                        |
|                          | % within P.2                 | 100.0            | 100.0           | 100.0                        | 100.0                        | 100.0                        |

P: phase.

Fig. 1. The distribution and prevalence of positive IgG in emergency medical technicians which participated in all 3-phases of study (n = 117).

There was one EMT who interestingly had positive IgG in all three phases that was diagnosed with re-infection during the study period.

4. Discussion

Humoral immune responses are the most important arm of the immune system against re-infection and strongly associated with protection [26]. Neutralizing antibodies neutralize viruses that enter the body and prevent them from reaching receptor-containing cells, or at least reduce the number of viruses that reach cells or receptors [27].

Previous works have stated that SARS-CoV-2 IgM can be applied as a marker for acute-phase infection. However, it has been reported that COVID-19 IgM antibodies can persist for months post-infection [28]. Antibody seroconversion is reported about 12 days after the onset of symptoms, and most people have neutralizing antibodies 14 to 20 days after the onset [29]. The level of neutralizing antibodies is directly related to age, male gender and disease severity [29,30]. However, these antibodies have been reported to decline in the first few months post-infection. This raises concerns about the quality and quantity of cell and humoral immunity against COVID-19 [31].

Although antibody responses have been identified in most patients from COVID-19, studies on diversion and persistence of antibody responses are still under investigation [10,13,14,27,29]. Humoral immunity against SARS-CoV-2 may not be long lasting in persons with mild illness [32]. The results of our study show that there is a significant reduction in seropositivity over time, which is consistent with other studies. It is not truly possible to conclude beyond our study period because it is likely that the decline in antibody titer will continue. We still need to be cautious about antibody-based “immunity passports,” herd immunity, and perhaps vaccine efficacy over time.

Discrepancies between prevalence reported in our study and the others’ data, may be caused by methodological and technical differences between studies [10,13,14,27,29]. One factor may have been the quality of the kits used for serological testing [28]. The sensitivities and specificities of the antibody detection kits used in different studies are variable; sensitivities ranged from 75% to 100% and specificities were 80% or higher [3].

Another issue especially in countries with a high burden of community infection is the correlation of acquiring infection to working in a hospital. Contact with patients (59%), infected coworkers (11%), and community acquired infection (13%) were reported as the main routes of exposure among Health Care Workers (HCWs), however the opposite results have been reported [33]. In a study, seroprevalence in personnel working with non-COVID-19 patients was 8.6% and another study reported 2.9% seropositivity [34,35]; whilst, much lower seroprevalence was reported in HCWs caring for COVID-19 infected patients, with most studies reporting 0–2%. In another study, the seroprevalence rate was 1.2% and 5.4% among HCWs treating COVID-19 and not treating COVID-19 patients, respectively [36].

The pattern of seroconversion in our study highlighted the possibility of re-infection at various times after the primary infection, which could be considered as a key point for establishing vaccination strategies. Indeed, it should be emphasized that more vaccine boosters may be needed for proper protection against the disease and future studies should evaluate the necessity and timing of vaccines and boosters, even in patients who were previously infected.

4.1. Limitations

A linear trend of seropositivity in our study against the expected exponential rate of transmission growth within people with close contact may support the transmission via community-related exposure to COVID-19, not by co-workers. In addition to technical issues, our sample size is too small to conclude a definitive trend.
The severity of the infection in the participants was not considered; and, the interval between the onset of infection and the time of testing was not exactly controlled. Also this is a qualitative study and the extent of antibody titer reduction over the time is unknown. There was a 9.5% and 17.6% non-attendance of subjects in 2nd and 3rd phases, respectively, that may influence the analysis of the results to some extent.

5. Conclusion

Our research results indicate that there is a significant reduction in COVID-19 antibody seropositivity over time. Although seropositivity decreased during the study period, 24 weeks after the first test and in the third phase, at least one-third of EMTs still had positive antibodies; and except for one, none of the EMTs reported re-infection.

Authors’ contribution

The conception and design of the work by PS, AB and PHS; Data acquisition by PHS, MJ and ZA; Analysis and interpretation of data by FS, AB and AA; Drafting the work by SF, AB and AA; Revising it critically for important intellectual content by PS, PHS, MJ and ZA; All the authors approved the final version to be published; and Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work.

Funding

This study was funded with a grant from Tehran EMS Center. The project was commissioned by the organization and the cost was paid to the contractor.

Declaration of Competing Interest

None declared.

Acknowledgments

We would like to express our commitment to the Prehospital and Hospital Emergency Research Center affiliated to Tehran University of Medical Sciences.

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