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ESTIMATING EXPOSURE TO SARS-COV-2 AT THE ARAB INTERNATIONAL UNIVERSITY, SYRIA: A SNAPSHOT AFTER A YEAR OF PANDEMIC

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Background: After a year of pandemic in Syria, how prevalent the seropositivity is, and how far we are from herd immunity is a raised question. Aim: Our study aimed to estimate the overall exposure to SARS-COV-2 depending on the COVID-19-specific symptoms as well as the seroprevalence of anti-SARS-CoV-2 IgG antibodies among students and staff at the Arab International University (AIU) in Syria. Methods: All AIU students and staff were invited to voluntarily participate in the study by filling out and submitting an anonymous web-based survey. Responses were evaluated then asymptomatic and pauci-symptomatic respondents were notified of a phlebotomy appointment. Anti-SARS-CoV-2 IgG antibodies were analyzed by ELISA. Results: One third (122 of 381; 32%) of respondents were asymptomatic. The remaining respondents reported COVID-19-specific symptoms (177 of 381; 46.5%), mainly anosmia and/or ageusia (132 of 177; 74.5%), or nonspecific symptoms (82 of 381; 21.5%). Frequencies of symptoms onset showed a strong, positive correlation with the incidence of confirmed SARS-CoV-2 RNA-positive cases reported by the Ministry of Health in Syria. Anti-SARS-CoV-2 IgG antibodies were similarly detected in both symptomatic (21 of 33; 63.6%) and asymptomatic (39 of 61; 63.9%) individuals (P=.97). Conclusions: After a year of pandemic, a 69% exposure to SARS-CoV-2 is roughly estimated amongst asymptomatic and pauci-symptomatic individuals. Further post-vaccination assessment of seroprevalence against SARS-CoV-2 in future largescale studies might provide a more complete picture of the SARS-CoV-2 epidemic in Syria.

Keywords: COVID-19, anti-SARS-CoV-2 IgG, asymptomatic, seroprevalence, exposure, Syria

INTRODUCTION

Since its discovery in Wuhan, China in December 2019,¹ coronavirus disease 2019 (COVID-19) has never been reported in Svria until March 22, 2020. Subsequently, COVID-19 spread in the Syrian community and peak incidence was reached in August 2020, December March/April 2020, 2021, September/October 2021, and February 2022; the curve has got flattened ever since.² COVID-19 reporting system in Syria relies on confirmed SARS-CoV-2 RNA-positive cases and RT-qPCR is generally performed to test

nasopharyngeal swabs collected from hospitalized patients, healthcare workers and travelers. Thus, the predominant portion of COVID-19 cases including asymptomatic and pauci-symptomatic non-hospitalized individuals might remain untested and unidentified; hence, actual incidence is perhaps undervalued.³⁻⁵

On the contrary of nucleic acid amplification test, IgG antibody testing is utilized to diagnose past rather than current infection.⁶ More reliable results are obtained when blood is drawn at least twenty days after the onset of symptoms, if any.⁷ Estimating the

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seroprevalence of anti-SARS-CoV-2 IgG antibodies has then proved to better reflect the burden of infection.^{8&9} Serologic assays usually detect anti-nucleocapsid protein antibodies and/or anti-spike neutralizing antibodies.¹⁰ It has already been demonstrated that anti-nucleocapsid protein antibodies might appear earlier than anti-spike antibodies.^{1,10} Therefore, testing both kinds of antibodies in a single assay might increase the diagnostic sensitivity.

Prior to our study, seroprevalence studies have been conducted in several populations showing various rates of seropositivity among healthy individuals ranging between 1.2% and 24.4%.^{4&5} After a year of pandemic in Syria, how prevalent the seropositivity is, and how far we are from herd immunity is a raised question. Our study aimed to estimate the overall exposure to SARS-COV-2 depending on the COVID-19-specific symptoms as well as the seroprevalence of anti-SARS-CoV-2 IgG antibodies amongst asymptomatic and paucisymptomatic individuals. Study population encompassed students as well as academic and administrative staff at the Arab International University located in Ghabagheb, Daraa Governorate and mostly representing the young adults from various Syrian governorates.

METHODS

Web-based survey

This prospective cross-sectional study was conducted during February and March 2021 where a web-based survey had been developed, validated and announced on the official website of the Arab International University (AIU), Syria. All AIU students and staff were invited to voluntarily participate in the study by filling out and submitting the self-administered anonymous survey including the informed consent. The survey comprised open- and closed-ended questions on demographic information; diagnosis, treatment and course of COVID-19 infection; vaccination against SARS-CoV-2; and potential contact with suspected/confirmed COVID-19 patients. Responses were carefully evaluated and checked for data validity and potential Concerning disease duplication. severity. respondents were grouped into 'severe cases' when oxygen therapy was required, 'moderate

cases' when pulmonary infiltrates were reported but oxygen therapy was not required, 'mild cases' when presenting symptoms but neither oxygen therapy was required nor pulmonary infiltrates were reported, and 'asymptomatic cases' when no symptoms were reported. Respondents, who reported contact with suspected/confirmed COVID-19 patients, were assessed for the risk of acquiring SARS-CoV-2 infection and were assigned exposure risk scores according to number of encounters (once, 1; several, 3; daily, 4), social distancing (more than one meter, 1; less than one meter, 3), and wearing the mask (surely, 1; not necessarily, 2; never, 3) by the participant and the patient each; the higher the sum score the higher the risk. Whereas, respondents who reported no contact with suspected/confirmed COVID-19 patients were assigned a 0 score.

Inclusion and exclusion criteria

Respondents who reported COVID-19specific symptoms including anosmia, ageusia, thoracalgia; dyspnea, and/or pulmonary infiltrates: oxygen therapy: and/or positive SARS-CoV-2-specific antibodies and/or RTqPCR tests as well as those who were already vaccinated against SARS-CoV-2 were excluded from antibody testing. Eligible who provided their contact respondents information along with their approval for participation were notified of a phlebotomy appointment. Electronic informed consent was obtained from all participants and the study was approved by the Research Ethics Committee of the Arab International University (REF; Project No. 3-9-31-1-2021). Time elapsed since the onset of symptoms until the time of phlebotomy was calculated for the participants who reported mild nonspecific symptoms.

ELISA

Blood samples were drawn in plain tubes and collected sera were stored at -30°C until the time of analyses. Anti-SARS-CoV-2 IgG antibodies were analyzed using the COVID-19 ELISA IgG kit (Vircell, S.L., Granada, Spain) according to the manufacturer's instructions.

Statistical analysis

Data analysis was performed using IBM[®] SPSS[®] Statistics, version 25 (IBM corp., New

York, USA). Chi-square test of independence and Kendall's tau correlation coefficient were utilized. Differences between independent groups were compared using Kruskal-Wallis and Mann-Whitney U tests. P-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Results

A total of 381 responses, including 273 (72%) AIU students and 108 (28%) AIU academic and administrative staff, were received between Feb 17th and Mar 19th, 2021. The median age of respondents was 23 years ranging between 18 and 74 years and 70% were female. Two thirds (259 of 381; 68%) of respondents were symptomatic while the remaining 122 of 381 (32%) respondents were asymptomatic. Symptomatic respondents included 177 of 259 (68%) reporting COVID-19-specific symptoms such as anosmia (126 of 177; 71%), ageusia (100 of 177; 56.5%), dyspnea (82 of 177; 46.3%), and/or thoracalgia (60 of 177: 33.9%) and 82 of 259 (32%) reporting nonspecific symptoms such as fever, chill, fatigue, myalgia, sore throat, cough, diarrhea, and/or insomnia. The majority of

symptomatic respondents (243 of 259; 94%) were considered 'mild cases', while 11 of 259 (4%) and 5 of 259 (2%) were deemed cases' 'severe 'moderate and cases'. respectively. The median duration of symptoms was 7 days ranging from 1 to 75 days. Indeed, presenting symptoms, disease severity, and infection duration were independent of age and gender (P>0.05). The peak frequencies of symptoms onset were demonstrated during the months of August 2020 and November 2020 showing a strong, positive correlation ($\tau_b=0.53$, P<0.01) with the incidence of confirmed SARS-CoV-2 RNA-positive cases reported by the Ministry of Health in Syria (Fig. 1). Around two thirds (230 of 381; 60.4%) of respondents were contacts of suspected/confirmed COVID-19 patients and the median exposure risk score was 9 ranging between 4 and 13. Apparently, exposure risk scores showed no significant differences neither between cases who reported COVID-19-specific symptoms, nonspecific symptoms, and no symptoms [H(2)=1.41, P=.49] nor between severe/moderate. mild and asymptomatic cases [H(2)=1.52, P=.46].



Fig. 1: Incidence of confirmed SARS-CoV-2 RNA-positive cases reported by the Ministry of Health in Syria (grey area, right scale) and onset of symptoms reported by participants in our study (n=381, black line, left scale). The peak frequencies of symptoms onset were demonstrated during the months of August 2020 and November 2020 showing a strong, positive correlation (τ_b =0.53, P<0.01) with the incidence of confirmed SARS-CoV-2 RNA-positive cases reported by the Ministry of Health in Syria, where peak incidence was reached during the months of August 2020 and December 2020.

| | | Anti-SARS-CoV-2 IgG antibodies | | |
|--|--------------|-----------------------------------|--------------|---------------------|
| | | IgG-Negative | IgG-Positive | |
| | | (n=34) | (n=60) | |
| Age (years) | Median | 28 | 23 | P=.056 ^a |
| | Range | 21-74 | 19-74 | |
| Gender | Female | 22 (64.7%) | 43 (71.7%) | P=.48 ^b |
| | Male | 12 (35.3%) | 17 (28.3%) | |
| Symptoms | Asymptomatic | 22 (64.7%) | 39 (65%) | P=.97 ^b |
| | Symptomatic | 12 (35.3%) | 21 (35%) | |
| Infection duration | Median | 4.5 | 3 | P=.69 ^a |
| (days) | Range | 2-12 | 1-15 | |
| Contact with | No | 13 (38.2%) | 16 (26.7%) | P=.24 ^b |
| suspected/confirmed COVID-19 patients | Yes | 21 (61.8%) | 44 (73.3%) | |
| Exposure risk score | Median | 6 | 6 | P=.47 ^a |
| | Range | 0-12 | 0-13 | |
| Time elapsed since | Median | 211.1 | 201.5 | P=.76 ^a |
| the onset of symptoms (days) | Range | 32-395 | 65-305 | |

Table 1: Characteristics of participants in anti-SARS-CoV-2 IgG antibodies testing by ELISA (n=94).

^a P-value was obtained using Mann-Whitney U test.

^b P-value was obtained using Chi-square test.

Anti-SARS-CoV-2 IgG antibodies were analyzed by ELISA in a total of 94 serum samples, and 60 of 94 (64%) participants were IgG-positive and 34 of 94 (36%) participants were IgG-negative (Table 1). Median antibody S/CO index for IgG-positive samples was 27.32 ranging between 7 and 93. Anti-SARS-CoV-2 IgG antibodies were similarly detected in both symptomatic (21 of 33; 63.6%) and asymptomatic (39 of 61; 63.9%) individuals $[\chi^2(1, N=94) = .001, P=.97]$ and hence no significant difference in antibody S/CO indices established (Mannwas Whitney U=955, P=.68). Likewise, seropositivity and antibody indices were independent of age, gender, and contact with suspected/confirmed COVID-19 patients (P> 0.05). The median time elapsed since the onset of mild nonspecific symptoms until the time of phlebotomy was 201 days, ranging between 32 and 395 days, and showing no significant difference between IgG-positive and IgGnegative cases (P>0.05). Moreover, antibody S/CO index did not show significant correlation with exposure risk score, duration of symptoms, or time elapsed since the onset of symptoms (P> 0.05).

Discussion

Apparently, about half (46.5%) of the population at the Arab International University in Syria were exposed to SARS-CoV-2 and suffered COVID-19-specific symptoms such as anosmia, ageusia, thoracalgia, and/or dyspnea. Specifically, anosmia and ageusia were the most noticeable of all (74.5%) as previously reported.¹¹Most importantly, they were all convinced they were infected. Despite lack of lab testing in the majority of cases, this is evidenced by the strong positive correlation between the frequencies of symptoms onset and the incidence of confirmed SARS-CoV-2 RNA-positive cases reported by the Ministry of Health in Syria. The other half (53.5%) of the university population were either asymptomatic pauci-symptomatic and were mostly or unaware of their exposure to SARS-CoV-2. Strikingly, two thirds (64%) of whom were SARS-CoV-2 seropositive, a proportion far higher than reported in other ever populations.4,5,7&11-14 This evidently is attributed to the poor adherence to social distancing, which is against the recommendations of the national regulatory authorities.

It seems that the community popular perception is primarily linked to social suspected/confirmed distancing from symptomatic COVID-19 patients. which showed no correlation with seropositivity, antibody index, presenting symptoms, or even disease severity according to our exposure risk scoring system. This is ascribed to unaccounted potential contact with subclinical cases, who are responsible for the transmission of infection,^{5&13-15} while social distancing might be probably unattained. Albeit detectable up to ten months post-infection, seropositivity and antibody index were not correlated with time elapsed since the onset of symptoms. Waning over time,^{4,8,16&17} antibody production might be boosted, however, due to subclinical reinfection upon unconscious contact with asymptomatic cases. This postulation can be confirmed/nullified further future in longitudinal studies.

Overall, depending on both COVID-19specific symptoms i.e., anosmia and ageusia, as well as SARS-CoV-2 seropositivity, a 69% exposure to SARS-CoV-2 among students and staff of the university is roughly estimated. In addition to the limitation of our sample-size, however, reaching out to herd immunity is still questioned as new variants are evolving and the seropositivity of anti-SARS-CoV-2 antibodies is evidence of exposure rather than effective immunity.4&18 Levels of anti-SARS-CoV-2 IgG antibodies were mainly low to moderate among asymptomatic/pauci-symptomatic participants. Furthermore, since our ELISA test assayed anti-spike glycoprotein and both antinucleocapsid protein antibodies, such antibody levels never accounted solely for the neutralizing antibodies responsible for immunity.¹⁹ Moreover, suspected re-infection was reported afterward by two participants in spite of seropositivity. This accorded well with previous studies that reported lower antibody levels among asymptomatic/pauci-symptomatic individuals than severe in COVID-19 patients, 1,4,8,9,13,17,20&21 probably owing to exposure to a number of viral particles below the infectious dose.⁹ However, inducing anti-SARS-CoV-2 neutralizing antibody production upon re-infection in one asymptomatic patient has already been reported.²² This suggests that lower IgG titers do not necessarily indicate inefficient immunity against SARS-CoV-2 as

memory lymphocytes should commit humoral immunological response upon subsequent exposure.

As previously observed, ^[4] the majority of symptomatic participants enrolled in our study were mild cases, thus hindering exploring severity-related associations. Age and gender were independent of presenting symptoms, disease severity. infection duration. seropositivity, and antibody index. Undoubtedly, our study group were mostly young adults and age-related associations might not be obvious due to our bias toward youth age group. Nevertheless, age-related COVID-19 morbidity and mortality are still debated.¹³ In addition, blood samples were drawn at least one month after the onset of symptoms; hence no false negative results are anticipated due to inappropriate sampling interval.^{1,7} Still, false negative results due to post-infection decline of antibody levels cannot be excluded; an unexplained reported phenomenon.⁸

Conclusions

Our study roughly estimated a 69% exposure to SARS-COV-2 after a year of pandemic in Syria depending on the COVID-19-specific symptoms as well as the seroprevalence of anti-SARS-CoV-2 IgG antibodies amongst asymptomatic and paucisymptomatic individuals. This estimate has been made prior to the national immunization campaign in Syria as the only two excluded respondents due to vaccination against SARS-CoV-2 were vaccinated abroad. Attentively, further post-vaccination assessment of seroprevalence against SARS-CoV-2 in future large-scale studies might provide a more complete picture of the SARS-CoV-2 epidemic in Syria.

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نشرة العلوم الصيدليـــة جامعة (أسيوط



تقدير التعرّض لفيروس SARS-CoV-2 في الجامعة العربية الدولية، سورية: لقطة بعد عامٍ من الجائحة وفيقة زرزور ' – وفاء الحبّال '، '* – علاء عريج ' – أحمد كنامة ' – رغد سرّي '

لقسم الكيمياء الحيوية والأحياء الدقيقة ، كلية الصيدلة ، الجامعة العربية الدولية ، درعا ، سورية قسم المخبر، مستشفى الأسد الجامعي ، جامعة دمشق ، ص.ب. ١٠٧٦٩ ، دمشق، سورية

الخلفية: بعد مُضيِّ عامٍ من الجائحة في سورية، يتساءل المرء عن مدى انتشار الإيجابية المصلية، وإلى أي مدىً نبعد عن مناعة القطيع.

الهدف: هدفت دراستنا إلى تقدير التعرض لفيروس SARS-COV-2 اعتمادًا على الأعراض النوعية لمرض COVID-19 إضافة إلى الانتشار المصلي لأضداد SARS-CoV-2 IgG ضمن الطلاب والموظفين في الجامعة العربية الدولية (AIU) في سورية.

الطرق: تمّت دعوة جميع الطلاب والموظفين في الجامعة للمشاركة الطوعية في الدراسة عن طريق ملء استبيان إلكتروني مغفل الاسم عبر الويب. إثر تقييم الردود، تم إخطار أصحابها الذين لم يبدوا أية أعراض أو أبدوا أعراضاً طفيفة بموعد سحب الدم ليتمّ تحليل أضداد IgG المضادة لڤيروس SARS-CoV-2

النتائج: لم تظهر أية أعراض لدى ثلث المشاركين (١٢٢ من ٣٨١؛ ٣٣%) بينا ذكر الباقون أعراضاً نوعية لمرض 19-COVID (١٧٧ من ٣٨١؛ ٢٦,٥٤%)، كان أهمها فقدان الشم و/أو الذوق (١٣٢ من ١٧٧؛ ٢٤,٥٤%)، أو أعراضاً غير نوعية (٨٢ من ٣٨١؛ ٢١,٥٢%). أبدى تواتر ظهور الأعراض في مجموعة دراستنا ارتباطاً إيجابياً قوياً مع معتل الإصابات بثيروس 2-SARS-CoV المؤكدة باختبار RT-PCR التي أبلغت عنها وزارة الصحة في سورية. تم كشف أضداد IgG المضادة لفيروس 2-SARS-cov واللاعرضيين (٢١ من ٣٣، ٣٣,٦٣%) واللاعرضيين لفيروس 2-SARS-بالمثل في كل من الأفراد العرضيين (٢١ من ٣٣، ٣٦,٦٣%) واللاعرضيين

الاستنتاجات: بعد عامٍ من الجائحة، تم تقدير التعرض لفيروس 2-SARS-CoV بحوالي ٦٩% ضمن الأفراد اللاعرضيين وأولئك الذين عانوا أعراضاً طفيفة. إنّ إعادة تقييم الانتشار المصلي بعد حملة اللقاح ضد 2-SARS-CoV في دراسات مستقبلية واسعة النطاق سيعطي صورةً أكثر اكتمالاً لجائحة 2-SARS-CoV في سورية.