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Research Article

Differential Reactivity of Anti-SARS-CoV-2 IgM/IgG in Response to Disease Onset in an Egyptian Cohort

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Abstract

Background: During an epidemic, it is neither economic nor time-saving to just rely on molecular diagnostics to identify the emerged pathogen and/or monitor response to treatment. **Aim and Methodology:** In this study we prove if we can distinguish between infection-confirmed (IC; V-qRT-PCR-positive) and infection-unconfirmed (IU; V-qRT-PCR-unchecked) Egyptian cohorts in response to disease onset using IgM/IgG AMP rapid test. **Results:** The IC individuals were 45 of them 22 were females and 23 were males whereas the IU individuals were 90 of them 47 were females and 43 were males. Of the 22 IC females, 31.8% were IgM positive (MP), 59% were IgG positive (GP) and 31.8% were positive for both antibody classes (MGP). Of the 23 the IC males, 91.3% were MGP and 14.2% were only GP. Of the 47 IU females, 21.2% were MP, 61.7% were GP, and 21.2% were MGP. Of the 43 IU males, 16.2% were MP, 51.1% were GP, and 16.2% were MGP. Studying the association between disease severity grade (DSG) and the IgM/IgG prevalence rates revealed 1) a nonsignificant positive correlation (NS-PC) in IC females, 2) a PC in IU females that was NS for IgM and S for IgG, 3) a nonsignificant negative correlation (NS-NC) in IC males, and 4) a NS-PC for both IgM/IgG in IU males. **Conclusion:** Detectability of virus-specific IgM/IgG varied among symptomatic/asymptomatic cases and in the individuals of different DSG.

Keywords: Anti-SARS-CoV-2 IgM/IgG reactivities; Infection-confirmed; Infection-unconfirmed; Symptoms onset; Disease severity.

Abbreviations: V-qRT-PCR-P: Virus quantitative reverse transcription polymerase chain reaction-positive; V-qRT-PCR-U: Virus quantitative reverse transcription polymerase chain reaction-unchecked; IC: Infection confirmed; IU: Infection-unconfirmed; MP: IgM positive; GP: IgG positive; MGP: IgM and IgG positive; DS: Disease severity; PC: Positive correlation; NC: Negative correlation; S: Significant; NS: not significant; RR: Relative reactivity

Introduction

By the end of 2019, a series of pneumonia cases with clinical features similar to that of viral pneumonia was emerged in Wuhan, Hubei Province, China [1]. The causative pathogen was recognized as novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the disease was defined as COVID-19 [2]. Since then, the virus developed unlimited spread all over the world [3]. The main target of SARS-CoV-2 is the human respiratory system [4,5], and broad range of clinical manifestations ranging from asymptomatic infection to fever, cough, shortness of breath, sore throat and other respiratory tract symptoms which might end with death were documented. Some patients rapidly develop acute respiratory distress syndrome, acute respiratory failure and other serious complications such as sepsis [6].

Although the majority of the patients respond positively to some of the recommended anti-viral therapies, considerable morbidity and mortality rates were continued to be reported [7,8].

In an Egyptian study, ~30% seroprevalence rate among PCR negative cases was reported and ~33% of infected patients were negative for the virus-specific antibodies [9]. As most of the available data on SARS-CoV-2 infection has been recruited from symptomatic cases, the missing data from asymptomatic patients remains an issue [6,9]. Of note, asymptomatic patients carry the virus and are able to transmit the infection [10], accordingly, this issue is highly important to be considered especially in the regions with limited resources for routine surveillance services.

Indeed, applying large scale seroprevalence studies irrespective of the symptoms onset is important to know the actual prevalence rate and to estimate the risk of infection spread. Of note, upon emergence of the pandemic, credible diagnostic tools were developed and transported worldwide to monitor the infection spread [11,12]. The commercially available SARS-CoV-2 diagnostic assays include 1) molecular tests which recognizes specific parts of the virus RNA in the respiratory secretions 2) serological tests which detects the virus-specific antibodies within the sera [12]. The value of applying the molecular diagnostic assays is their higher sensitivity with the ability to detect the virus-specific RNA in both symptomatic and asymptomatic humans which eliminates any false-negative results. Additionally, the molecular diagnostics are able to identify infected individuals a week before the onset of the symptoms [13]. In the mild infection, the virus-specific RNA starts to decline by week 3 and subsequently becomes undetectable, whereas, in severe illness the RNA may persist longer than 3 weeks [14].

A previous study demonstrated that presence of the virus RNA does not necessarily mean an active infection [10], which represents one of the molecular diagnostics drawbacks. Additional drawbacks are the complexity, higher cost and the long required time to deliver test results. Of note, a single RT-PCR test kit may cost over 100 USD, while setting up diagnostic/processing lab requires more than 15,000 USD, whereas the analysis time requires 4–6 h, and sample-to-result turnaround time takes longer than 24 h [11,12,15]. Moreover, some studies reported high false-negative results of the RT-PCR assay [16,17]. Therefore, diagnostic alternatives, in particular, a point-of-care rapid diagnostic assays (POCT) are urgently needed [18-20].

The previously validated POCT for detecting influenza antigens showed poor sensitivities, whereas in case of SARS-CoV-2 both the used nucleocapsid-based POCT and the virus RNA detection kits were promising, allowed screening of infection on a large-scale and were supportive in the therapeutic intervention decision [21-23].

Among the POCT of great interest are those used to detect antibody responses upon disease onset because they allow rapid diagnosis of acute infection, complement the molecular diagnostics' results, recognize the old infections and contribute to better understanding of disease burden [24,25]. Indeed, the positive values of using the serological assays in diagnosing human viral diseases including COVID-19 has been widely documented [26,27].

The health authorities of China have indeed utilized qualitative serological assays to detect anti-SARS-CoV-2 IgG/IgM [28]. In a previous work of our team, we studied seroprevalence of anti-SARS-CoV-2-IgM/IgG among individuals of low and high socioeconomic standards within a virus-qRT-PCR-unchecked Egyptian cohort using a rapid test [24].

Certainly, during an epidemic, it is neither economic nor time-saving to rely only on molecular diagnostics especially in developing countries like Egypt. In Egypt and many other developing countries, a very limited population can afford diagnosis/monitoring response to the treatment by the qRT-PCR. These population include individuals who need to be certified

for being virus-free before travel, health-insured employees in multi-national/private sector companies, individuals of high socioeconomic standards and admitted symptomatic subjects to hospitals. Here, we studied differential reactivity of virus-specific IgM/IgG among infection-confirmed (IC; Virus-qRT-PCR-positive) and infection-unconfirmed (IU; Virus-qRT-PCR-unchecked) Egyptian cohorts using anti-SARS-CoV-2 IgM/IgG AMP rapid test and correlated this to symptoms severity.

Materials and Methods

The full descriptions of the studied humans are as the following: 1) The IC (V-qRT-PCR-P) humans included 22 females and 23 males. Of the 22 IC females, 17 were symptomatic (S) and 3 were asymptomatic (AS). Of the 23 IC M, 20 were S and 3 were AS. 2) The IU (V-qRT-PCR-Unchecked) humans included 47 females and 43 males. Of the 47 IU females, 27 were S and 20 were AS. Of the 43 IU males, 26 were S and 17 were AS.

Virus-specific IgM/IgG were analyzed in the collected sera from the two groups using anti-SARS-CoV-2-IgG/IgM AMP (AMEDA Labordiagnostik GmbH; Graz, Austria) rapid kit. This kit contains two recombinant proteins from the virus, the spike 1 (S1) subunit and the nucleocapsid (N). Sera samples of the confirmed humans (n = 45) were kindly provided from Prof. Dr. Wael A Hassan; (Egypt Center for Research and Regenerative Medicine, Cairo, Egypt). The virus-specific RNA was quantified for this group using the TaqPath COVID-19 CE-IVD RT-PCR Kit (A51738; Thermo Fisher Scientific).

Blood samples of the IU individuals (n = 90) were collected from co-workers at the National Research Centre of Egypt. This has been done in compliance with relevant laws and institutional guidelines and according to the ethical standards of the Declaration of Helsinki.

Blood was collected, sera were separated, and freshly used as has been explained in the provided manufacturer instructions of the used kit.

Briefly, 5µl volume from each individual serum samples was applied into specimen well followed by adding two drops from the provided sample buffer to the buffer well. The cassette shows a blue control band that turns red upon the correct performance of the test. IgM and IgG positive reactions are visualized as two separate bands. The reaction was left to develop for 10 min and results were recorded independently by two different members of our team and unclear (confusing) results were judged by a third colleague.

Of note, we transformed the qualitative IgM/IgG band intensities into numerical values, so that the negative serum samples were given zero whereas the samples of very weak reactivity, weak reactivity, moderate reactivity, strong reactivity were given 5, 10, 50, and 100, respectively. Additionally, we transformed the symptom numbers into disease severity grades (DSG), where no symptoms refer to zero DSG, while symptom numbers of 1-2, 3-5, 6-7, and >7 reflect 1, 2, 3 and 4 DSG, respectively.

Statistical analysis

Statistical analysis and plots were done using the GraphPad PRISM version 5 software. Results were expressed as means ± standard deviations (SD). Statistical significance was calculated by comparing the differences between means of different studied groups using the student's t-test. Differences were considered significant when the p value was < .05. Correlation analysis was carried out by calculating the square value of the correlation coefficient (r^2) for nonparametric and non-normally distributed data.

Results

The overall number of females was 69 of whom, 22 were IC and 47 were IU. Of the 22 IC females, 7 (31.8%) were IgM positive (MP), 13 (59%) were IgG positive (GP), and 7 (31.8%) were positive for both IgM/IgG (MGP). Of the 47 IU females, 10 (21.2%) were MP, 29 (61.7%) were GP, and 10 (21.2%) were MGP. Of the 66 studied males, 23 were IC and 43 were IU. Of the 23 IC males, 21 (91.3%) were MGP. Of the 43 IU males, 7 (16.2%) were MP, 22 (51.1%) were GP and 7 (16.2%) were MGP.

Of the 69 studied females, 44 were S and 25 were AS. Of the 44 S females, 17 were IC and 27 were IU. Of the 17 ICS females, 6 (35.2%) were MP, 10 (58.8%) were GP and 6 (35.2%) were MGP. Of the 27 IUS females, 11 were (40.7%) MP, 21 (77.7%) were GP and 11 (40.7%) were MGP. Of the 25 AS females, 5 were IC and 20 were IU. Of the 5 ICAS females, 1 was (20%) MP, 3 (60%) were GP and 1 (20%) was MGP. Of the 20 IUAS females, 6 (30%) were MP, 8 (40%) were GP and 6 (30%) were MGP.

Of the 66 studied males, 46 were S and 20 were AS. Of the 46 S males, 20 were IC and 26 were IU. Of the 20 ICS males, 17 were (85%) MGP. Of the 26 IUAS males, 6 (23%) were MP, 16 were (61.5%) GP and 6 were (23%) MGP. Of the 20 AS males, 3 were IC and 17 were IU. All the 3 ICAS males were (100%) MGP. Of the 17 IUAS males, 1 (5.8%) was MP, 6 (35%) were GP and 1 (5.8%) was MGP.

Differential anti-SARS-CoV-2 IgM/IgG reactivities among the IC (V-qRT-PCR-P) and IU(V-qRT-PCR-Unchecked) humans are presented in (Figure1A & B).

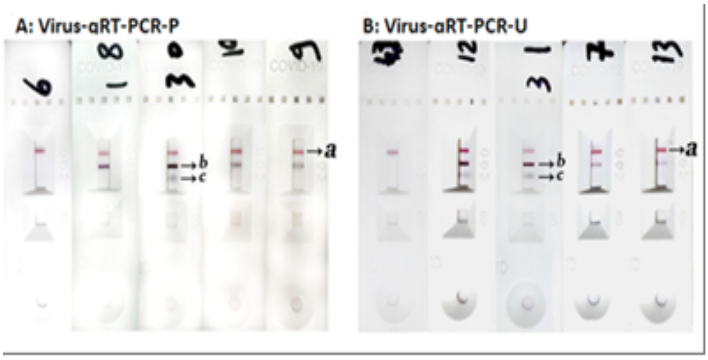

Figure 1. A representative figure showing differential anti-SARS-CoV-2 IgM/IgG reactivities among infection-confirmed (IC; V-qRT-PCR-P (A)) and infection-unconfirmed (IU; V-qRT-PCR-Unchecked (B)). IgM/IgG band intensities ranged from weak to moderate and strong. The upper band (a) represents the positive control, the middle band (b) represents IgG reactivity, and the lower band (c) represents IgM reactivity.

IC: infection-confirmed; IU: infection-unconfirmed; V-qRT-PCR-P: Virus-quantitative reverse transcription polymerase chain reaction positive; V-qRT-PCR-U: Virus-quantitative reverse transcription polymerase chain reaction unchecked

Of note, herein, we transformed the qualitative IgM/IgG band intensities into numerical values, so that the negative serum samples were given zero whereas the samples of very weak reactivity, weak reactivity, moderate reactivity, strong reactivity were given 5, 10, 50, and 100, respectively. The overall IgM/IgG reactivities among males were non-significantly higher (NSH; $p > 0.05$) than females (Figure 2A). The overall IgM/IgG reactivities among the IC group were significantly higher (SH; $p < 0.05$) than the IU one (Figure 2B). In the IC group, IgM/IgG reactivities among males were SH than females (Figure 3A). In the IU group, prevalence rate of IgM was NSH among females than males, while, that of IgG was SH among females compared to males (Figure 3B).

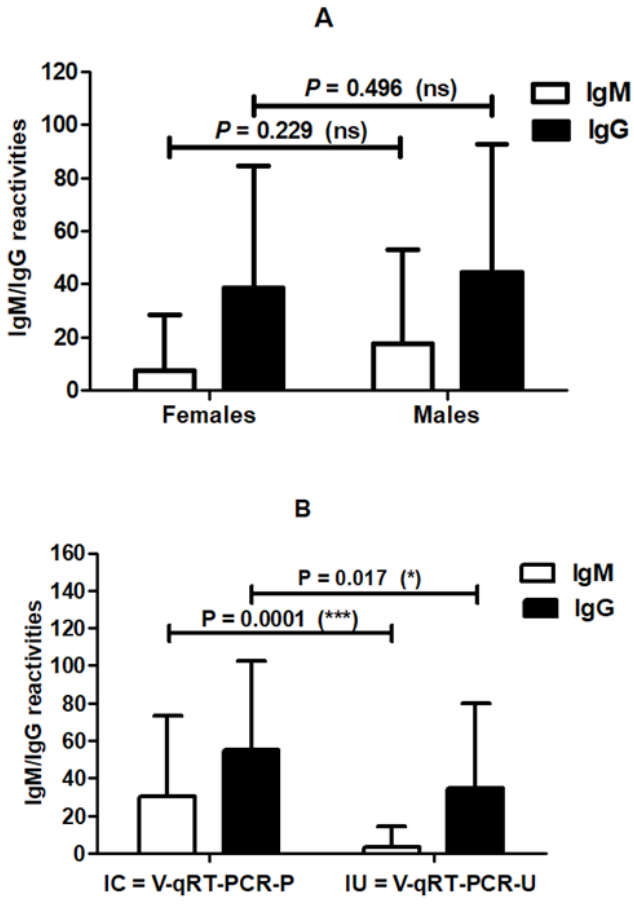

Figure 2. Anti-SARS-CoV-2 IgM/IgG overall prevalence rates among (A) females and males; (B) infection-confirmed (IC) and infection-unconfirmed (IU) humans. IgM/IgG band intensities were transformed into numerical values so that, the negative serum samples were given zero, whereas, samples of very weak reactivity, weak reactivity, moderate reactivity, strong reactivity were given 5, 10, 50 and 100; respectively. Statistical analysis and plots were done using the GraphPad PRISM version 5 software. Results were expressed as means ± standard deviations (SD). Statistical significance was calculated by comparing the differences between means of different studied groups using the student's t-test. Differences were considered significant when the p value was < .05.

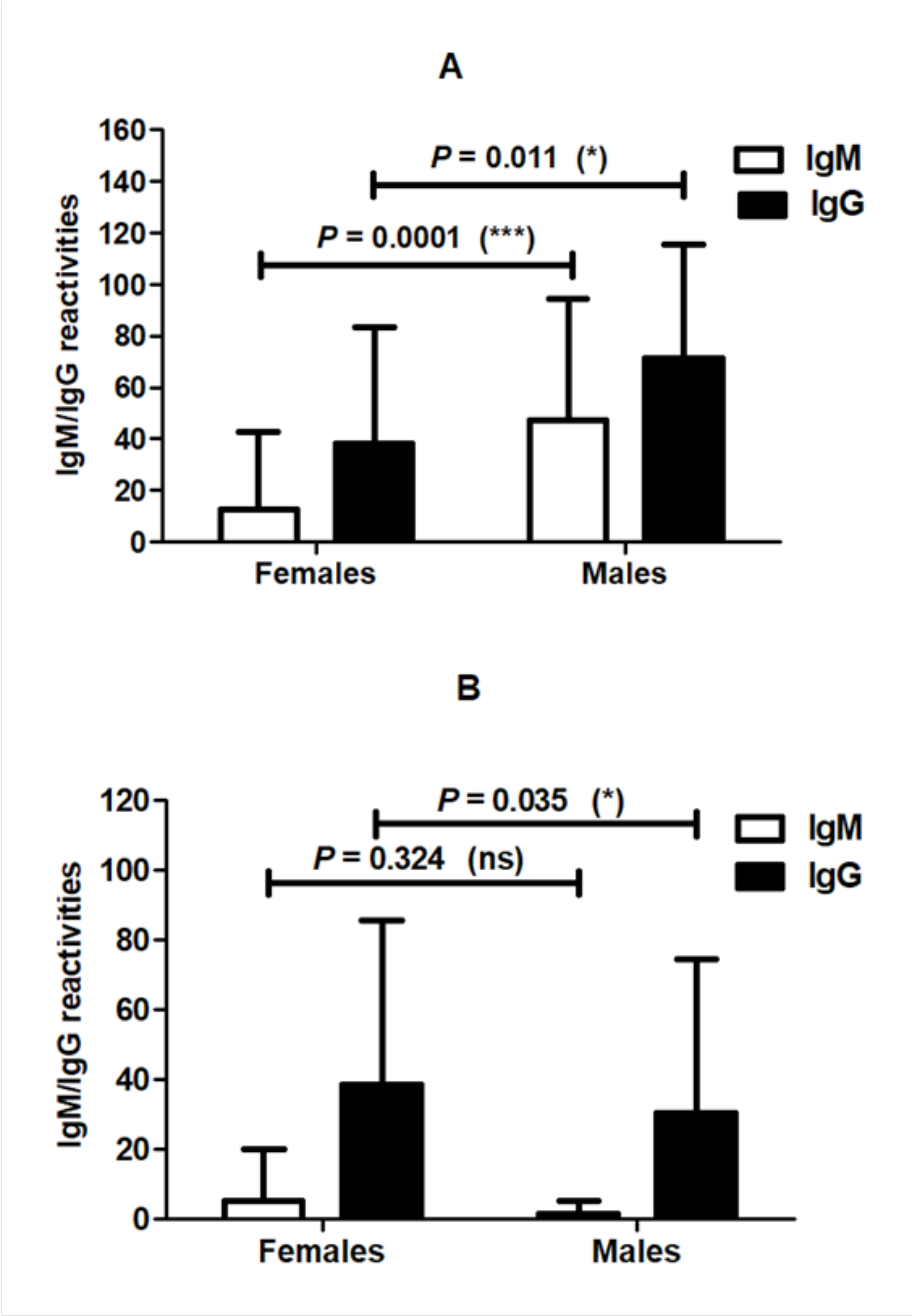

Figure 3. Anti-SARS-CoV-2- IgM/IgG reactivities among both genders of the (A) infection-confirmed (B) infection-unconfirmed humans. IgM/IgG band intensities were transformed into numerical values so that, the negative serum samples were given zero, whereas, samples of very weak reactivity, weak reactivity, moderate reactivity, strong reactivity were given 5, 10, 50 and 100; respectively. Statistical analysis and plots were done using the GraphPad PRISM version 5 software. Results were expressed as means ± standard deviations (SD). Statistical significance was calculated by comparing the differences between means of different studied groups using the Student's t-test. Differences were considered significant when the *p* value was < .05.

In the ICS humans, IgM/IgG prevalence rates were SH among males compared to females (Figure 4A). In the IUS humans, IgM/IgG prevalence rates were NSH among females than males (Figure 4B). In the ICAS humans, prevalence rate of IgM was SH among males compared to females, whereas the IgG prevalence rate was NSH among males than females (Figure 4C). In the IUAS individuals, prevalence rate of IgM was SH among females compared to males, whereas the IgG prevalence rate was NSH among female than males (Figure 4D).

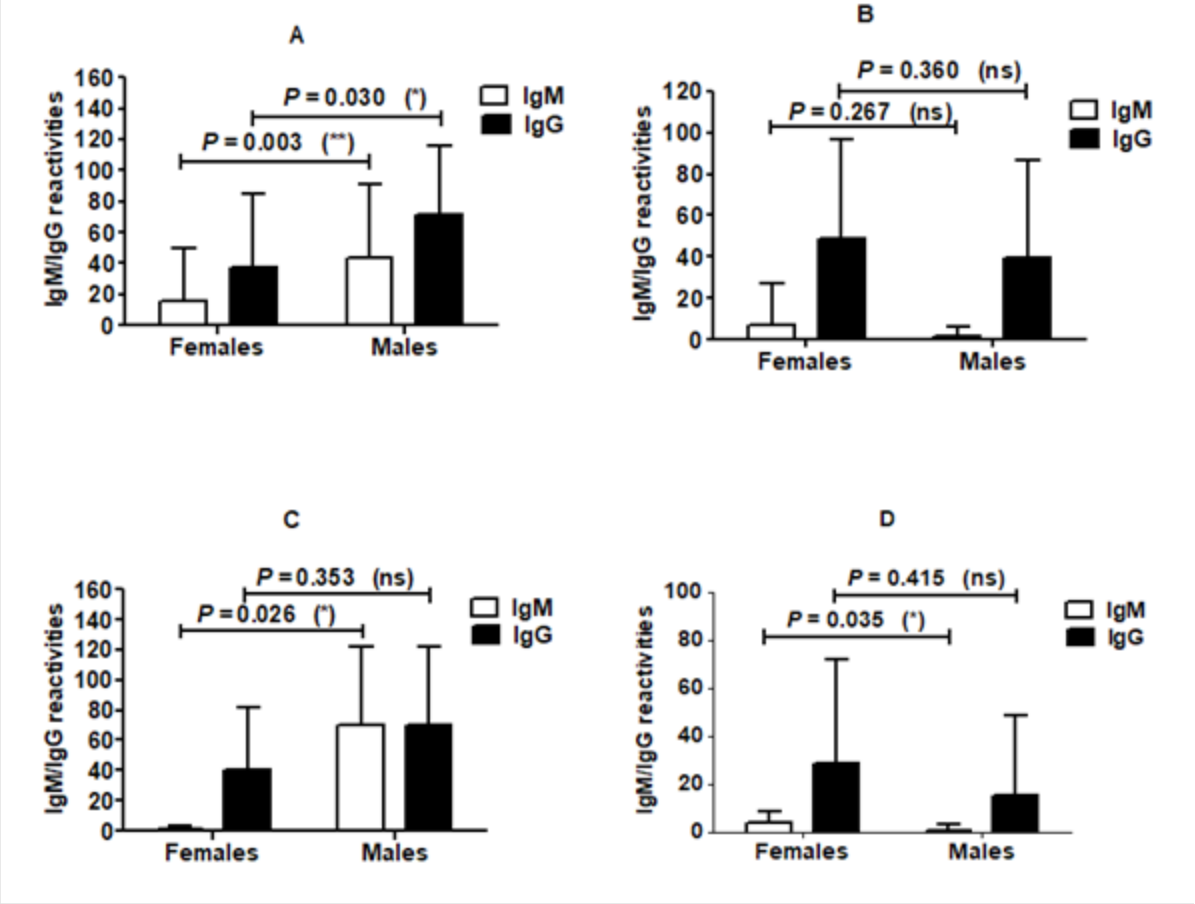

Figure 4. Anti-SARS-CoV-2 IgM/IgG prevalence rates among (A) Infection-confirmed symptomatic (ICS) humans, (B) Infection-unconfirmed symptomatic subjects, (C) Infection-confirmed asymptomatic (ICAS) subjects, and (D) Infection-unconfirmed asymptomatic (IUAS) humans. IgM/IgG band intensities were transformed into numerical values so that the negative serum samples were given zero, whereas, samples of very weak reactivity, weak reactivity, moderate reactivity, strong reactivity were given 5, 10, 50 and 100; respectively. Statistical analysis and plots were done using the GraphPad PRISM version 5 software. Results were expressed as means ± standard deviations (SD). Statistical significance was calculated by comparing the differences between means of different studied groups using the Student's t-test. Differences were considered significant when the p value was < .05.

In addition, we transformed the symptom numbers into disease severity grades (DSG), where no symptoms refer to zero DSG, while symptom numbers of 1-2, 3-5, 6-7, and >7 reflect 1, 2, 3 and 4 DSG, respectively. In the ICS females, the mean reactivities of IgM were 1, 22, 30, 1.25, and 5, whereas of IgG were 40, 40, 44, 27, and 50 for DSG 0, 1, 2, 3, and 4, respectively (Figure 5A). In the ICS males, the mean reactivities of IgM were 70, 47.8, 35, 37.7, and 100 whereas of IgG were 51.9, 48.8, 56.3, 46.7, and 100 for DSG 0, 1, 2, 3, and 4, respectively (Figure 5B).

In the IUS females, the mean reactivities of IgM were 3, 3.5, 13, 1.4, and 6.2, while those of IgG were 17.5, 44.2, 67.2, 34.2, and 77.5 for DSG 0, 1, 2, 3, and 4, respectively (Figure 5C). In the IUS males, the mean reactivities of IgM were 0.5, 2.3, 3.5 for 0, 1, 2 DSG and no individuals showed DSG 3 or 4. The IgG mean reactivities were 14.1, 40.7, 47.1, and 33.3 for DSG 0, 1, 2 and 3, respectively (Figure 5D).

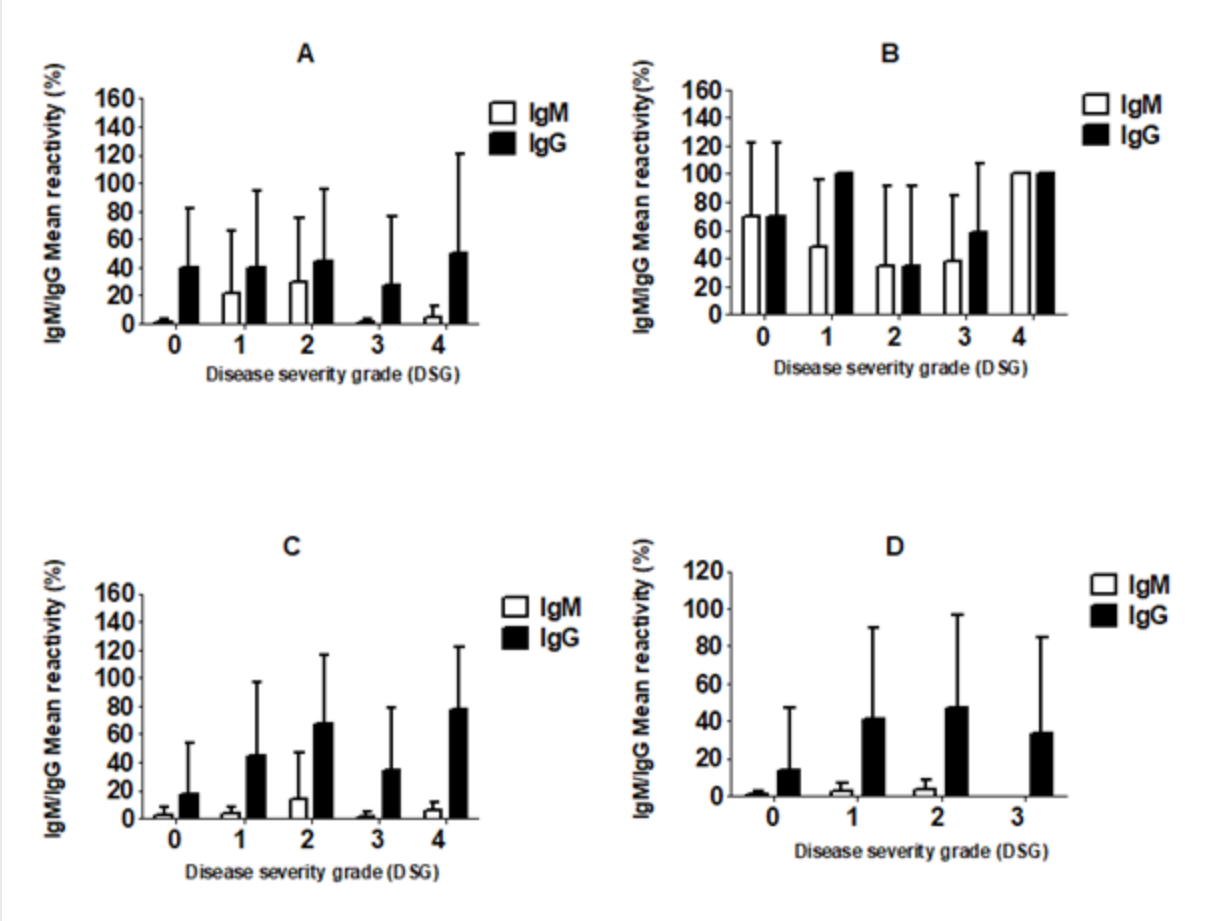

Figure 5. Anti-SARS-CoV-2 IgM/IgG reactivities according to disease severity grades (DSG) among symptomatic (S) females and males of the infection-confirmed (IC) group (A and B, respectively), S females and males of the infection-unconfirmed (IU) one (C and D, respectively). Disease Severity levels were transformed into grades (DSG) according to numbers of recorded symptoms so that, absence of symptoms was given grade zero, whereas, presence of 1-2, 3-5, 6-7, and >7 signs were given DSGs of 1, 2, 3 and 4, respectively. Statistical analysis and plots were done using the GraphPad PRISM version 5 software. Results were expressed as means ± standard deviations (SD). Statistical significance was calculated by comparing the differences between means of different studied groups using the Student's t-test. Differences were considered significant when the *p* value was < .05.

Additionally, the IgM/IgG band intensities were presented as relative reactivities (RRs). In the ICS females, the RRs of IgM were 20, 40, 40, 25, and 50 whereas of IgG RRs were 60, 40, 80, 50, and 50 for DSG 0, 1, 2, 3, and 4, respectively (Figure 6A). In the ICS males, the RRs of both IgM/IgG were 100, 100, 66.6, 88.8, and 100 for DSG 0, 1, 2, 3, and 4, respectively (Figure 6B). In the IUS females, the RRs of IgM were 30, 42.8, 44.4, 14.2, and 75 whereas of IgG were 40, 57.1, 77.7, 85, and 100 for DSG 0, 1, 2, 3, and 4, respectively (Figure 6C). In the IUS males, the RRs of IgM were 5.8, 23, 42.8, and 0 whereas of IgG were 35.2, 61.5, 85.7, and 33.3 for DSG 0, 1, 2, and 3, respectively (Figure 6D).

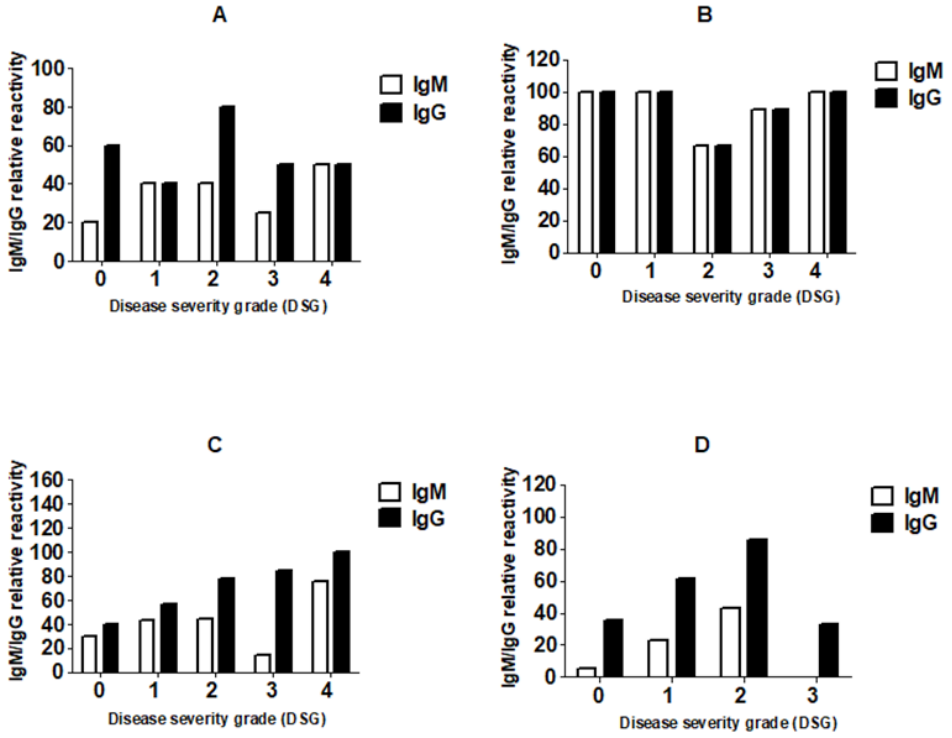

Figure 6. Anti-SARS-CoV-2 IgM/IgG relative reactivities (RRs) according to Disease Severity Grades (DSG) among symptomatic (S) females and males of the infection-confirmed group (A and B, respectively), S females and males of the infection-unconfirmed one (C and D, respectively). Disease Severity levels were transformed into grades (DSG) according to numbers of recorded symptoms so that, absence of symptoms was given grade zero, whereas, presence of 1-2, 3-5, 6-7, and >7 signs were given grades of 1, 2, 3 and 4, respectively. Statistical analysis and plots were done using the GraphPad PRISM version 5 software. Results were expressed as means ± standard deviations (SD). Statistical significance was calculated by comparing the differences between means of different studied groups using the Student's t-test. Differences were considered significant when the *p* value was < .05.

Studying the connection between the prevalence rates of virus-specific IgM/IgG and the age revealed 1) a positive correlation (PC) among IC females that was S for IgM and NS for IgG, 2) a NS negative correlation (NC) for IgM among IU females, 3) a NS-PC for both IgM/IgG among IC males and, 4) a NS-NC for IgG among IU males (Table 1).

Gender	Females (n = 69)				Males (n = 66)			
Infection	IC (n = 22)		IU (n = 47)		IC (n = 23)		IU (n = 43)	
Correlated parameters	IgM/Age	IgG/Age	IgM/Age	IgG/Age	IgM/Age	IgG/Age	IgM/Age	IgG/Age
Correlation coefficient (r^2)	0.496	0.432	-0.195	0.083	0.336	0.332	0.070	-0.209
P-value	0.042 (*)	0.083 (ns)	0.192 (ns)	0.597 (ns)	0.135 (ns)	0.141 (ns)	0.652 (ns)	0.178 (ns)
IC: Infection-confirmed; IU: Infection-unconfirmed								

Table 1: Correlations between the anti-SARS-CoV-2 IgM/IgG band intensities and the age of both genders among the infection-confirmed and infection-unconfirmed subjects.

Studying the correlation between the age and DSG revealed 1) S-PC among IC females, 2) a NS-NC among IC males, 3) a NS-NC among IU females and, 4) a NS-PC among IU males (Table 2).

Infection	IC (n = 45)		IU (n = 90)	
Gender	Females (n = 22)	Males (n = 23)	Females (n = 47)	Males (n = 43)
Correlated parameters	DSG/Age	DSG/Age	DSG/Age	DSG/Age
Correlation coefficient (r^2)	0.525	-0.063	-0.187	0.019
P-value	0.030 (*)	0.787 (ns)	0.178 (ns)	0.893 (ns)
IC: Infection-confirmed; IU: Infection-unconfirmed; DSG: Disease severity grades				

Table 2: Correlations between the disease severity grades and the age of both genders among the infection-confirmed and infection-unconfirmed subjects.

Studying the connection between IgM/IgG prevalence rates and DSG revealed 1) a NS-PC for both IgM/IgG among IC females, 2) a PC among IU females that was NS for IgM and S for IgG, 3) a NS-NC for both IgM/IgG among IC males and, 4) a NS-PC for both IgM/IgG among IU males (Table 3).

Gender	Females (n = 69)				Males (n = 66)			
Infection	IC (n = 22)		IU (n = 47)		IC (n = 23)		IU (n = 43)	
Correlated parameters	DSG/IgM	DSG/IgG	DSG/IgM	DSG/IgG	DSG/IgM	DSG/IgG	DSG/IgM	DSG/IgG
Correlation coefficient (r^2)	0.073	0.009	0.084	0.441	-0.542	-0.223	0.136	0.207
P-value	0.744 (ns)	0.967 (ns)	0.52 (ns)	0.001 (**)	0.480 (ns)	0.306 (ns)	0.382 (ns)	0.181 (ns)
IC: Infection-confirmed; IU: Infection-unconfirmed; DSG: Disease severity grades								

Table 3: Correlations between disease severity grades and anti-SARS-CoV-2 IgM/IgG band intensities of both genders in the infection-confirmed and unconfirmed subjects.

Discussion

In the current study, we compared virus-specific IgM/IgG reactivities of infection-confirmed (IC; V-qRT-PCR-positive) and infection-unconfirmed (IU; V-qRT-PCR-unchecked) Egyptian cohorts using anti-SARS-CoV-2 IgM/IgG AMP rapid test in response to disease onset. In addition, the virus-specific IgM/IgG prevalence rates and/or seroconversions among the both studied groups in response to disease onset have been elucidated.

The observed NSH overall IgM/IgG prevalence rates among males compared to females might be due to the noticed SH IgM/IgG prevalence rates among IC males.

Several factors like nutrition, gender, age, general health, chronic diseases, treatments, immune fitness, severity of the infectious pathogen and the ability to evade induced immunity greatly determine the degree of disease progression or clearance [29].

Previous studies revealed that accuracy of the serological assays was enhanced upon mounting an active immunity to SARS-CoV-2 antigens [30]. Therefore, the blood collection and assay performance time points are of great importance to be considered and could explain the observed SH overall IgM/IgG prevalence rates among males. In line with this speculation, a previous study declared that antibodies require ≥ 8 days after symptoms onset to be released [13].

In contrast to previous reports, our results showed SH IgM/IgG prevalence rates among IC males compared to IC females [30,31]. The noticed both NSH IgM and SH IgG prevalence rates among IU females compared to IU males could be attributed to the higher

probability of either recent or repeated infections of female subjects due to the more tasks and duties performed by females compared to males in Egypt.

In a previous study we found that females belongs either to low or high socioeconomic standard groups showed higher IgM/IgG prevalence rates than males [24].

The both noticed SH IgM/IgG prevalence rates among ICS males and NSH IgM/IgG prevalence rates among IUS females indicate connection between IgM/IgG prevalence rates and the symptoms onset. This also suggests the more likely ability of females to resolve low/moderate infections compared to males. On the other hand, the noticed NSH IgG prevalence rate among ICAS individuals of both genders refers to the importance of including sensitive rapid immunoassays to enhance (complement) the diagnostic accuracy of the virus-qRT-PCR [2,26].

Of note, both ICS individuals from both genders and IUS females with DSG 1 and 2 showed higher IgM mean intensities, while, IUS males along all DSG showed low IgM mean intensities. Both ICS females and IUS males with DSG 4 showed higher IgG mean intensities. The required time for viral-specific IgM/IgG sero-conversion in relation to symptoms onset has been previously reported [29-32].

Additionally, it has been documented that the differences in sero-conversion dynamics were connected to assay design, selected antigen targets, severity of infection, and/or the presence of comorbidities [32-34].

The observed S-PC and NS-PC between the IgM/IgG prevalence rates and the age among both IC females and males suggest that the true infection induces higher IgM/IgG prevalence rates among persons of older age in both genders [35].

On the other hand, the observed NS-NC between the IgM/IgG prevalence rates and the age among both genders in the IU group could be attributed to 1) the higher the age, the less the human mobility and the less they get exposed to the infection and, 2) the higher the age the poorer the capacity of the immune system to respond to infection which makes older age under high risk of disease progression. Accordingly, the older people who are known to have impaired immunity are highly encouraged to perform sports daily to support their immune system to function properly [36].

The NS-PC between the IgM/IgG band intensities and the DSG among the IC females, the NS-PC between the IgM band intensities, the S-PC between the IgG band intensities and the DSG among the IU females, and the NS-PC between the IgM/IgG band intensities and the DSG among the IU males all agree with the speculation that the DSG modulate the IgM/IgG levels [37].

The NS-NC between the IgM/IgG band intensities and both the age and the DSG among the IC males might be due to a case of compromised immunity. Both the S-PC between the age and the DSG among the IC females and the NS-PC among both IU genders could be a matter of an age-related risk factor. This agrees with the fact that the older age infected individuals develop higher disease severities than the younger ones [34]. This might also suggest infection with a different pathogen or presence of co-morbid diseases [38].

The noticed NS-NC between age and the DSG among both IC males and IU females can be a result of unbiased immune functions [37-39].

Conclusion

Irrespective of using or not the SARS-CoV-2-specific qRT-PCR for RNA detection, detection of the virus-specific IgM/IgG using the anti-SARS-CoV-2 IgM/IgG AMP rapid test varied between symptomatic and asymptomatic infections and among individuals of different disease severity grades.

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Ethical considerations: The research project has been approved by the Institutional Ethical Committee of the National Research Centre (Meeting date: 5.11.2020, Decision number: 20166) and the participants have granted informed consents prior to inclusion.

Conflict of Interests: None to declare.

Author contributions: M.M.B. wrote the grant that funded the work, designed the study, discussed the experimental design and the results of the work with all coauthors and put the structure of the manuscript. M.H.N., R.N., and D.N.A. supervised filling the questionnaires and interviewed the virus qRT-PCR unchecked individuals. K.A., W.A.H., Y.K.F., and T.M.H. provided the sera from, and the virus RT-qPCR data of, the hospitalized SARS-CoV-2 patients. M.H.N., R.N., and D.N.A. performed the rapid IgM/IgG detections, made the photos, transformed the raw data into numerical values which they stored into excel files. M.H.N did the statistical analysis, prepared the figures of the manuscript under direct supervision of M.M.B. M.H.N wrote the original draft of the manuscript that was extensively discussed and edited by M.M.B. All authors have read and discussed the final version of the manuscript and agreed on the submission.

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