Seroprevalence of Coronavirus Disease 2019 (COVID-19) Infection among Blood Donors in a Tertiary Care Hospital in Thailand

Pabhada Limawongpranee, Siriporn Phongjitsiri, Jutarat Mekmullica

Department of pediatrics, Bhumibol Adulyadej Hospital, Medical Services, Royal Thai Air Force, Paholyothin Road, Saimai, Bangkok, 10220 Thailand

Background: In Thailand, five months into COVID-19 from the first report case, low prevalence of 0.0043% was reported. At that time, reverse transcriptase polymerase chain reaction (RT-PCR) for the SARS-CoV-2 virus was performed only in patients who met the national criteria for COVID-19 PCR evaluation.

Objective: To determine the seroprevalence of SARS-CoV-2 virus in asymptomatic blood donors.

Method: An observational descriptive cross-sectional study was conducted at the Department of Blood Transfusion Service, Bhumibol Adulyadej Hospital (BAH), over a two-month period from September 2020 to October 2020. Voluntary blood donors between the ages of 17 and 70 were included. The remaining aliquot of blood from regular serologic collection was utilized to test for total SARS-CoV-2 antibodies. Those who tested positive for total SARS-CoV-2 antibody were subsequently asked to return to BAH within 7 days for a nasal swap RT-PCR for SARS-CoV-2 viral antigen.

Results: A total of 1,550 subjects were enrolled. During the period of the study, COVID-19 vaccine was not available for the participants in the current study. Only two blood donors tested positive for Anti-SARS-CoV2 antibodies (0.13%, 95%CI: 0.04-0.47). The result of an RT-PCR for SARS-CoV-2 antigen using a nasal sample was undetectable. Both individuals had no history of COVID-19 infection, travel to a high-risk country, or confirmed contact with suspected or confirmed COVID-19 patients in the past 3 months.

Conclusion: The seroprevalence rate of healthy blood donor in Thailand during year 2020 was 0.13 percents.

Keywords: Seroprevalence, COVID-19, SARS-CoV-2

Introduction

Coronavirus disease 2019 (COVID-19) is an acute respiratory tract infection caused by severe acute respiratory syndrome virus 2 (SAR-CoV-2). After its first outbreak in China, in late December 2019, it spread out and caused serious public health problems worldwide, later was declared as a global pandemic by the World Health Organization (WHO)[1].

COVID-19's wide range of characteristics of manifestation has been reported including severe pneumonia, mild to moderate symptoms such as fever, cough, shortness of breath, and some with no symptom[2]. Asymptomatic COVID-19 carriers can transmit the SARS-CoV-2 virus to other people and can be the source of disease spreading[3-4].

The identification of viral antigen from bronchoalveolar lavage or nasopharyngeal swab by RT-PCR is the gold standard for the diagnosis of SARS-CoV-2 infection[5]. Other available diagnostic methods include serologic tests detecting immunoglobulin M (IgM), immunoglobulin G (IgG) or total antibodies which are specific to SARS-CoV-2 viral structural protein including spike (S), nucleocapsid (N), envelope (E) and membrane (M)[6]. These serologic tests have been used to detect any past infection or immune response to the virus[7-9].

In Thailand, at five months into COVID-19 from the first report case, low prevalence of 0.0043% was reported[10]. At that time, RT-PCR for the SARS-CoV-2 virus was performed only in patients who met the national criteria for COVID-19 PCR evaluation namely those with history of contact with confirmed COVID-19 cases or travelled from countries with had COVID-19 outbreak.

Many COVID-19 seroprevalence investigation in healthy blood donors had been conducted to evaluate the spread of infection[11-15]. SARS-CoV-2 immunoglobulin level in the population was also studied hoping[11-15]. Variation of seroprevalence in each area was reported[11-15].

The objective of this study was to report the seroprevalence of SARS-CoV-2 virus in asymptomatic blood donors.

Materials and Methods

The present protocol was approved by the Bhumibol Adulyadej Hospital (BAH) Ethics Committee (IRB no. 65/63). All participants received written information about the study and understood it. Written consent was obtained by all.

An observative descriptive cross-sectional study was conducted between September 1st, 2020, to October 31st, 2020 using blood that came to the Department of Blood Transfusion Service, BAH. Blood donation was obtained at a fixed site (BAH) or BAH mobile collection units in Bangkok and Pathumthani province area, Thailand.

Any voluntary blood donors in this study who aged 17-70 years must have been qualified according to the blood donation criteria from the Blood Donation Center (Thai Red Cross)[16]. Furthermore, they must not have been travelling from or transiting in countries which have COVID-19 outbreaks, or have contacted with suspected or confirmed COVID-19 patients within the previous 4 weeks, have no history of COVID-19 virus infection, and did not have a body temperature higher than 37.8 °C on the day of the blood donation. After participants agreed and signed for blood donation and research informed consent, the self-screening questionnaires were given to them.

Eight-millimeter sampling blood of each blood donor was collected in an EDTA plasma tube (Greiner Bio-One, Austria) and was kept for routine serological testing in 4 °C temperature throughout the entire investigation. Lack time from blood collection at the mobile site to BAH blood donation center was < 6 hours. Time from blood collection to blood testing was within 72 hours. Four-millimeter blood were decanted for routine serological tests. The remaining aliquot once confirmed by donor ID to have positive total SARS-CoV-2 antibody testing would be further subjected to our investigation. Those with positive total SARS-CoV-2 antibody testing were then got telephone calls inquiring more specific questions concerning COVID-19 history risks including the history of foreign travel to COVID-19 outbreak countries within the previous three months and history of confirmed contact with COVID-19 cases. In addition, they were inquired to visit BAH for a nasal swap PCR within 7 days for SARS-CoV-2 viral antigen. Participants with positive RT-PCR were then placed under quarantined according to Thailand's public health policy.

The demographic data (age, gender, place of residence, occupation, and associated symptoms) and the SARS-CoV-2 total antibody test results were collected.

Antibody testing

The Elecsys® Anti-SARS-CoV-2 immunoassay (Roche, Switzerland) is a test to detect total antibodies to SARS-CoV-2 viral nucleocapsid

(N) protein by electrochemiluminescence immunoassay performed on cobas e analyzers. The tests have a sensitivity of 99.5 % (97.0 – 100 %) at >= 14 days after PCR confirmation and a high specificity of 99.80 % (99.69 – 99.88%)[7]. The assays were performed according to the manufacturer's instructions. The result which has a Cutoff index (COI) < 1.0 is reported non-reactive. The test which has COI >=1.0 will be repeated one more time for confirmation and if the result is still >=1.0, a reactive result is reported.

Real Time Polymerase Chain Reaction (RT-PCR tests)

Allplex 2019-nCoV Assay from Seegene Inc. (Seoul 05548, Republic of Korea), which is a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in human nasopharyngeal swab was used. When the SARS-CoV-2 envelope protein (E), RNA-dependent RNA polymerase (RdRp), and nucleocapsid protein (N) were found and Ct <= 40, a result reported detected and if Ct>40 or no Ct was detected, a result was reported undetectable.

Sample size

The incidence of COVID-19 in Thailand reported on 20 June, 2020 was 0.0043%[10]. The BAH Blood Service Division received 1536 donations per two months. Estimating a finite population proportion formula was used as follows:

$$n = \frac{Z^2_{1-\alpha/2} P(1-P)N}{D^2(N-1) + Z^2_{1-\alpha/2} P(1-P)}$$

Error (d) was 0.00001 and alpha (α) was 0.05. The sample size of 1,535 was calculated. Therefore, a sample size of 1,550 blood donors was recruited in the study.

Statistical analysis

The data were analyzed by using the Statistics Package for Social Sciences (SPSS) statistic version 27 (IBM Corp, NY, USA). Descriptive statistics were used for the general characteristics of sample groups namely frequency, mean, standard deviation (SD), and percentage.

Results

A total of 1,550 subjects were enrolled in the study between September 1st, 2020, to October 31st, 2020. Demographic characters of subjects were presented in Table 1. Ratio of male and female subjects was 820:730 cases. Mean age was 37.5 years old (standard deviation = 0.59). Eighty-five percent (1,318/1,550) of participants lived in Bangkok and its suburb. Nearly half (747/1,550) of subjects were government officers.

Symptomatology of subjects was shown in Table 2. Most of them (1,461/1,550) was asymptomatic persons. Myalgia, runny nose, and headache were the common symptoms among participants. All of the participants had no risks, history of traveling to high-risk countries and contact confirmed COVID-19 cases.

During the period of the study, COVID-19 vaccine was not available for the participants in the current study. There were only two cases of blood donors who had positive Anti-SARS-CoV2 antibodies (0.13%, 95%CI: 0.04-0.47). After performing quantitative PCR (qPCR) for SARS-CoV-2 antigen through nasal swab, undetectable was reported. Both participants had no history of travel to the high-risk country, and had no confirmed history of contact with suspected or confirmed COVID-19 cases in the past 3 months. All of symptomatic (0/89) participants had negative SARS-CoV-2 antibodies.

Table 1 Demographic data of blood donors

Demographic data	n (%)
Gender	
Male	820 (52.90)
Female	730 (47.10)
Age	
Mean age, years (+-SD)	37.52 (36.93,38.11)
17-30	531 (34.26%)
31-40	372 (24.00%)
41-50	382 (24.64%)
51-60	246 (15.87%)
61-70	19 (1.23%)
Address	
Bangkok	880 (56.77%)
Pathum Thani	389 (25.10%)
Nonthaburi	49 (3.16%)
Others	232 (14.97%)
Occupation	
Medical personnel	71 (4.58%)
Pilots and airhostess	22 (1.42%)
Students	177 (11.42%)
Soldiers and polices	406 (26.19%)
Employee	144 (9.29%)
Merchants	96 (6.19%)
Business owners	77 (4.97%)
Officers	341 (22.00%)
Maids	56 (3.61%)
Teachers	32 (0.21%)
Others	127 (8.20%)

Table 2 Symptoms reported by blood donors

Reported symptoms	n (%)
Asymptomatic	1461 (94.26%)
Symptomatic	89 (5.74%)
Fever	1 (0.06%)
Chill	1 (0.06%)
Fatigue	12 (0.77%)
Myalgia	33 (2.12%)
Cough	21 (1.35%)
Sore throats	22 (1.41%)
Runny nose	25 (1.61%)
Shortness of breath	2 (0.13%)
Chest pain	4 (0.26%)
Headache	31 (2.00%)
Nausea or vomit	2 (0.13%)
Abdominal pain	6 (0.39%)
Diarrhea	7 (0.45%)

Discussion

After the first COVID-19 case was reported in Thailand in late January 2020, the number of infected cases had increased monthly. The monthly country lockdown was announced on 26th March 2020. Stay at home, the use of facial mask, increase personal hygiene and social distancing policies had been enforced by the government official. In June, work from home and online education were enforced among office workers and children of all ages. Air travel and international travel were suspended. After three months, the number of new COVID-19 cases in Thailand declined to near zero[17]. However, until then only a small number of COVID-19 tests were performed and no COVID-19 vaccine was available in Thailand. The new normal policy was continuously promoted while the number of the cases was not increasing significantly.

In October 2020, the outbreak of COVID-19 worldwide was skyrocketed. Report by the United Kingdom government in October 2020 showed 6.46 new COVID-19 infections for every 10,000 people per day (95% credible interval: 5.46 to 8.55) in the community population in England[18]. At the same period Thailand had less than 0.01new COVID-19 per 10,000 cases[17]. The result of this study was collected during the same period as UK data. Our finding revealed the effective government measure in the infection control. This investigation, the seroprevalence of (0.13%, 95%CI: 0.04-0.47) was reported while the subsequent PCR was undetectable.

This number of COVID-19 total antibody test result of this investigation was lower than that in a healthy blood donor from other researches including Italy, Brazil, Netherland, Denmark, and Germany at 2.4-9.0, 4.6-7.1, 4.0, 2.7, 1.7, 0.91%, respectively[11-15]. In April 2020, Italy and Germany had the third and fifth highest COVID-19 infection rate of 32.4 and 18.6 new COVID-19 cases per 10,000 population, respectively [19]. Our finding with low seroprevalence in healthy blood donor was in lieu with Thailand low COVID-19 case report.

Despite the high sensitivity of the Elecsys Anti-SARS-CoV-2 immunoassay diagnostic test, false-positive results should be taken into consideration. However, the current finding showed such a low infection rate and both patients tested negative in RT-PCR. The effectiveness of first year COVID-19 infection control measure of the Thai Government which received a worldwide acclaim should receive a clinical recognition.

From a sensitivity of 99.5% and a specificity of 99.8 of the assays, the positive predictive value (PPV) of the tests in populations of 1, 5, 10, and 20% seroprevalence was 83.4, 96.3, 98.2, and 99.2%, respectively[7].

Moreover, cross-reactivity for SARS-CoV-2 Serological Assays with common cold panel and other diseases such as autoimmune condition was proposed[20-21].

The immunoassay test in the current study could be cross-reactivity with cytomegalovirus (CMV) and Epstein-Barr virus (EBV) which gave the specificity of 99.5% (95% CI 98.6-99.9)[7]. So from this study the low prevalence might be from the true number of infected COVID-19 cases or false-positive results of the immunoassays.

The dynamics of the antibody response to SARS-CoV-2 infection was varied among the different studies[7-9]. In the early phase after PCR-confirmed SARS-CoV-2 infection, the sensitivity of immunoassay increased over time. The sensitivity of the Elecsys Anti-SARS-CoV-2 immunoassay reaches 99.5% (95% CI 97.0–100.0) at ≥14 days after PCR-confirmed COVID-19 infection[7].

However, the study of long-term antibody response to COVID-19 infection was under investigation in different periods after PCR-confirmed COVID-19 infection. So false-negative results should be taken into account.

Limitation of this study was incidence report from a small area that was well controlled of the COVID-19 pandemic.

Strength of this study was the first seroprevalence survey in blood donors conducted in Thailand to reflect the preliminary result of seroprevalence of anti-Sar-CoV-2 antibodies in asymptomatic population before the COVID-19 vaccine has been developed and distributed.

In conclusion, the result implied that most blood donors at ten months pandemic marking remained uninfected and the implementation of the government policy during first nine months of the outbreak. The collaboration of the public and the compliance of the people yielded slow spread of the infection. On the other hand, the low rate of seroprevalence or SAR-CoV-2 antibodies was far from the level of herd immunity of 67% [22] which could prevent the burden of disease.

Even Thailand was the country that successing in first rank of COVID-19 pandemic control in the first wave. The importance of vaccination and social distancing were the best way to prevent a pandemic, morbidity and mortality of a new outbreak shortly.

Conclusion

The seroprevalence rate of healthy blood donor in Thailand during year 2020 was 0.13 precents.

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Conflict of interest

The authors declare no conflict of interest.

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ความชุกของภูมิคุ้มกันต่อเชื้อไวรัสโคโรนา 2019 ของผู้บริจาคโลหิตในโรงพยาบาลตติยภูมิในประเทศไทย

ปภาดา ลิมาวงษ์ปราณี, ศิริพร ผ่องจิตสิริ, จุฑารัตน์ เมฆมัลลิกา

ความเป็นมา: ห้าเดือนหลังจากพบผู้ติดเชื้อไวรัสโคโรนา 2019 รายแรกในประเทศไทย ความชุกของการติดเชื้อไวรัสโคโรนา 2019 ถูกรายงานในระดับต่ำที่ 0.0043% โดยในเวลานั้นการตรวจหาเชื้อด้วยวิธี Reverse transcriptase polymerase chain reaction (RT-PCR) จะทำการตรวจวินิจฉัยเฉพาะในผู้ที่เข้าเกณฑ์การสอบสวนโรคติดเชื้อไวรัสโคโรนา 2019 ของประเทศไทยเท่านั้น

วัตถุประสงค์: เพื่อศึกษาความชุกของภูมิคุ้มกันต่อเชื้อไวรัสโคโรนา 2019 ในผู้บริจาคโลหิตซึ่งมีสุขภาพดี

วิธีการศึกษา: : การศึกษานี้เป็นการศึกษาเชิงพรรณนาแบบตัดขวาง ทำการศึกษา ณ กองบริการโลหิต โรงพยาบาลภูมิพลอดุลยเดช ระหว่างเดือนกันยายน - ตุลาคม พ.ศ. 2563 ผู้เข้าร่วมการศึกษาคือผู้บริจาคโลหิตซึ่งมีสุขภาพดี อายุระหว่าง 17-70 ปี โลหิตที่เหลือจากส่วนที่แบ่งไว้สำหรับตรวจคัดกรองการติดเชื้อในการบริจาคโลหิต ถูกนำมาทดสอบภูมิคุ้มกันต่อเชื้อไวรัสโคโรนา 2019 หากผลการทดสอบเป็นบวกจะมีการแจ้งผู้บริจาคโลหิตกลับอย่างรวดเร็ว และให้มาโรงพยาบาลเพื่อตรวจหาการติดเชื้อไวรัสโคโรนา 2019 ด้วยวิธี RT-PCR ภายใน 7 วัน

ผลการศึกษา: ในช่วงเวลาระหว่างที่ทำการศึกษา ยังไม่มีวัคซีนโควิด 19 ถูกใช้ในประเทศไทย จากผลการศึกษา ผู้บริจาคโลหิตทั้งหมด 1,550 คน พบผู้บริจาคโลหิตเพียง 2 คน มีผลภูมิคุ้มกันต่อเชื้อไวรัสโคโรนา 2019 เป็นบวก (0.13%, 95%CI: 0.04-0.47) และทั้งสองคนได้รับการตรวจหาเชื้อไวรัสโคโรนา 2019 ด้วยวิธี RT-PCR ได้ผลเป็นลบ ทั้งสองคนปฏิเสธประวัติการติดโรคโควิด 19 มาก่อน ปฏิเสธประวัติการเดินทางจากประเทศซึ่งมีการระบาดของโรคโควิด 19 หรือสัมผัสผู้ติดเชื้อหรือสงสัยติดเชื้อไวรัสโคโรนา 2019 ในสามเดือนก่อนหน้านี้

สรุป: ความชุกของภูมิคุ้มกันต่อเชื้อไวรัสโคโรนา 2019 ในผู้บริจาคโลหิตซึ่งมีสุขภาพดีในช่วงปี พ.ศ. 2563 เท่ากับ 0.13% (95%CI: 0.04-0.47)

คำสำคัญ: ความชุกของภูมิคุ้มกัน, ภูมิคุ้มกันต่อเชื้อไวรัสโคโรนา 2019, โรคโควิด 19

**กองกุมารเวชกรรม โรงพยาบาลภูมิพลอดุลยเดช กรมแพทย์ทหารอากาศ*