RESEARCH ARTICLE

Screening COVID-19 in Apparently Healthy Individuals

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ABSTRACT

Background: A pneumonia outbreak with an unknown etiology broke out in Wuhan, China, and quickly spread worldwide. Coronavirus 2 is a new coronavirus that causes severe acute respiratory syndrome (SARS-CoV-2). This study will look at coronavirus in people who don't have any symptoms and test the sensitivity, specificity, and accuracy of the tests used to detect COVID-19.

Methods: This study included 300 people who appeared to be in good health. Nasal swabs were taken and placed in a viral transport media (VTM), which was then tested using real-time polymerase chain reaction (RT-PCR) and a rapid test for anti-COVID-19 antibody (IgM, IgG). The reverse method was used to look at blood groups. Finally, a Hemolyzer III was used to count total leukocytes and granulocytes in peripheral blood.

Results: The present study included 176 males (58.7 %) and 124 females (41.3%). The age range was between 10 to 69 years. There is a non-significant difference ($p > 0.05$) between the blood group and carry coronavirus in apparently healthy individuals. There was a highly significant decline ($p \leq 0.01$) in total leukocytes count in positive RT-PCR subgroup was $4.1 \pm 0.1*10^9$. Also total leukocytes count significantly decline ($p \leq 0.05$) in positive of both RT-PCR and the rapid test was $4.5 \pm 0.1*10^9$/L, a non-significant difference ($p > 0.05$) between total leukocytes count and favorable rapid test subgroup comparison with negative test subgroup. This result shows a significant decrease ($p \leq 0.05$) in granulocyte count in the positive RT-PCR test subgroup and in the positive of both RT-PCR and rapid test subgroup was $4.07 \pm 0.11*10^9$/L, $4.1 \pm 0.10*10^9$, respectively. There is a non-significant difference ($p > 0.05$) in the positive rapid test subgroup $4.5 \pm 0.12*10^9$/L and compared with the negative test subgroup.

Keywords: COVID-19, RT-PCR, Rapid test, SARS-COV2, 2019-NCoV.

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INTRODUCTION

A real-time reverse transcription-polymerase chain reaction (RT-PCR) test is used in the coronavirus disease -19 diagnostic panel. Oligonucleotide primers are used in RT-PCR to amplify nucleic acid (RNA or DNA) fragments that a fluorescently labeled probe can then detect. Two regions of the SARS-CoV-2 nucleocapsid (N) gene are amplified, along with an internal control and the RNase P (RP) gene. The presence of all three genes, along with clinical symptoms of COVID-19 infection, is considered presumptive positive for SARS-CoV-2.[1]

They developed an indirect enzyme-linked immunosorbent assay ELISA protocol for detection immunoglobulin G (IgG) and mmunoglobulin M (IgM) against COVID-19 using coating antigens. Antibody responses begin about a week after infection, with multimeric IgM being the first to appear, often at the same time as active infections. As a result, IgM is frequently used as a marker for newly acquired infection. IgM antibody responses are usually transient and fade away once the infection is no longer present. IgG antibodies appear near the end of an active infection and can last for months or even years afterward.[2]

Several biological abnormalities were also described, such as leukopenia and lymphopenia. Therefore, complete blood count (CBC) can potentially contribute to the diagnosis of COVID-19.[3]

Granulocytes are protective during bacterial or fungal infections; however, their role in viral infections is not fully understood.[4]

ABO blood groups have recently been related to COVID-19 infection. previously reported showing that group A is a risk factor while group O seems to be a protective factor against SARS-CoV-2 infection. Importance of the absence of immune antigens defined by group O, as a protective factor for Sars-CoV-2 virus infection presence of anti-A antibodies could be protective against viral entry into lung epithelium.[5] ABO groups are distributed differently based on ethnic origin and, therefore, the results from these studies are highly dependent on the distribution in each region.[6]

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MATERIALS & METHODS

Materials

The study was conducted on 300 healthy individuals after confirming that there were no symptoms of COVID-19 and had not come into contact with a person who had coronavirus and not previously infected with COVID-19. Included 176 males (58.7%) and 124 females (41.3%). The age range was between 10 to 69 years from the employees a request from their department, random healthy campaigns that included popular markets, taxi drivers and schools, and the family and friends from August 2020 to March 2021.

Sample Collection

Nasopharyngeal Swab

Nasopharyngeal (NP) swab is often recommended for diagnosis of early infection. NP samples involve inserting the swabs into the nostril parallel to the palate, maintaining the swab in place for a few seconds to enable secretion absorption and immediate placement of the swab into a sterile tube containing 2–3 mL of viral transport media (VTM).

Blood Sample

Five milliliters of venous blood were withdrawn from each subject by vein-puncture under the aseptic technique by single-use syringe. The blood samples were divided into two parts, one part in two milliliters dispensed in a test tube contains K3 Ethylene Di-amine Tetra-acetic Acid (K3EDTA) for testing complete blood count, and the second part was dispensed in a sterile gel tube and left for about one hour to clot at room temperature and then centrifuged at 1500 round per minute for five minutes to separate the serum and dispensed into sterile plain tubes which tightly closed and stored at -20°C until assayed.

METHODS

All subjects were exposed to the following:

1. Taken complete information
2. Molecular examination of nasopharyngeal swabs:

- RNA Extraction by Viral RNA Mini kit (Qiagen, America) from nasopharyngeal swabs involves denature and disrupt proteins and envelope of SARS-CoV-2 for diagnosis of COVID-19.
- EURO Real-time SARS-CoV-2 PCR assay by (EUROIMMUN, Germany) the most sensitive method of confirming, monitoring, and managing COVID-19 disease during the ongoing pandemic in all affected countries.[7]

3. Leukocytes and granulocytes were counted in peripheral blood using a Hemolyzer III (Analyticon, Germany). It implements the so-called coulter method for counting cells passing through a small aperture.[8]
4. Estimation COVID-19 IgG/IgM Rapid test cassette (Viva Diag, Netherlands) is a lateral flow chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies to COVID-19 in human serum specimen.[9]
5. Evaluation blood group using reverse method when these A, B antigens on red blood cells are allowed to bind with antibodies anti A, anti B in serum forms agglutination.[10]

Statistical Analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0 and Data Analysis via Microsoft Excel 2019. Quantitative data were expressed as mean ± standard error. Qualitative data were expressed frequency and percentage. The following tests were done:

- A two-way analysis of variance (ANOVA) when comparing between over two means
- Examine the specificity, sensitivity and accuracy of each of PCR and rapid test and both.
- Probability (p-value) was considered as below;
  - p-value ≤ 0.05 was considered significant.
  - p-value > 0.05 was considered non-significant.

RESULTS

Demographic characteristics were studied for 300 healthy individuals, including 176 males (58.7%) and 124 females (41.3%). The age range was between 10 to 69 years. They were divided into blood groups 20.7% blood group type A, 8.7% blood group type AB, 28% blood group type B, and 42.6% blood group type O.

Leukocytes

This study explained the relationship between leukocytes count and COVID-19 in asymptomatic individuals, the results showed a highly significant decline in leukocytes count ($p \leq 0.01$) with the positive RT-PCR test subgroup, the mean ± standard error of leukocytes count was 4.11 ± 0.1*10⁹/L, also significant decrease ($p \leq 0.05$) in leukocytes count with the positive of both RT-PCR and rapid test subgroup, where leukocytes count was 4.5 ± 0.1*10⁹/ L, a non-significant decline ($p > 0.05$) in positive rapid test subgroup (6.9 ± 0.1*10⁹/L) in comparison with negative of both RT-PCR and rapid test (7.2 ± 0.1*10⁹/L) as illustrated in Table 1.

Blood Groups

The comparison study between blood groups and positive RT-PCR test subgroup, positive of both RT-PCR and rapid test subgroup, positive rapid test subgroup, shows non-significant differences ($p > 0.05$) between blood group and these subgroups as demonstrated in Table 2.

Table 1: Distribution of study subgroups according to total leukocytes count

Subgroups	*NO (%)*	*Leukocytes *10⁹/L Mean ±SE*	*p-value*
Positive RT-PCR test	79 (26.3)	4.1 ± 0.1**	0.01
Positive of both RT-PCR and Rapid test	41 (13.6)	4.5 ± 0.1*	0.05
Positive Rapid test	49 (16.3)	6.9 ± 0.1	0.07
Negative of both RT- PCR and Rapid test	131 (43.6)	7.2 ± 0.1	

NO: *Number, RT-PCR: Real time Polymerase Chain Reaction, l: liter, SE: standard error, P=probability, **is mean significant p-value ≤0.01, *is mean significant P. Value ≤0.05.*

Table 2: Blood groups in apparently healthy indviduals

	Blood group					
Subgroups	*NO (%)*	*A (%)*	*AB (%)*	*B (%)*	*O (%)*	*p-value*
Positive RT-PCR test	79 (26.3)	16 29.6	6 30%	23 28.7%	34 23.2%	0.9
Positive of both RT-PCR and rapid test	41 (13.6)	8 14.4	2 10%	12 15%	19 13%	0.6
Positive rapid test	49 (16.3)	9 16	2 10%	15 18.8%	23 15.8%	0.7
Negative of both RT- PCR and rapid test	131 (43.6)	21 40	10 50%	30 37.5%	70 48%	$X^2 = 8.44$
Total	300	54	20	80	146	

NO: Number, RT-PCR: Real -time Polymerase Chain Reaction, X^2= Chi square, P=probability.

Table 3: Granulocytes count in positive and negative RT-PCR and rapid test subgroup

Subgroups	*NO (%)*	*Granulocyte *10⁹/L Mean ± SE*	*p-value*
Positive RT- PCR test	79 (26.3)	4.07 ± 0.11*	0.03
Positive of both RT- PCR and rapid test	41 (13.6)	4.1 ± 0.10*	0.04
Positive rapid test	49 (16.3)	4.5 ± 0.12	0.06
Negative of both Rapid test	131 (43.6)	4.7 ± 0.08	

NO: Number, RT-PCR: real time- polymerase chain reaction, SE: standard error, P=probability, l: liter.

Table 4: Sensitivity, specificity and accuracy test for coronavirus tests

	RT-PCR test (%)	*RT-PCR and Rapid test (%)*	*Rapid test (%)*
Sensitivity	94	90.4	90
Specificity	98	98.8	95
Accuracy	96.6%	97.3%	94.6%

RT- PCR: Real time -Polymerase Chain Reaction

Granulocytes

The present study showed significant difference ($p \leq 0.05$) between granulocytes count and carry COVID-19 in apparently healthy individuals, this result explained significant decline in granulocytes count in the positive RT-PCR test subgroup and in the positive of both RT-PCR and rapid test sub group the mean ± standard error of granulocyte was 4.1 ± 0.10*10⁹/L and 4.07 ± 0.11*10⁹/L respectively. A non-significant difference ($p > 0.05$) between positive rapid test subgroup (4.5 ± 0.12*10⁹/L) in comparison with the negative of both RT-PCR and rapid test subgroup (4.7 ± 0.08*10⁹/L) as clarified in Table 3.

Sensitivity, Specificity and Accuracy Test for RT-PCR Test and Rapid Test.

The sensitivity of RT-PCR 94%, specificity 98%, accuracy 96.6%, sensitivity RT-PCR and Rapid test is 90.4%, the specificity is 98.8% and accuracy is 97.3%. Rapid test sensitivity 90%, specificity 95%, accuracy 94.6% as presented in table 4.

DISCUSSION

The severe acute respiratory syndrome coronavirus -2 (SARS-CoV-2) caused by coronavirus Disease 2019 (COVID-19) has emerged at the end of 2019.[11] In this regard, throughout the present study, leukopenia conjugate with COVID-19 agrees with another researcher Li (2020) leukopenia, which is more common in COVID-19 patients than in non- COVID-19 patients.[12] Common clinical laboratory findings include leukopenia, direct invasion by SARS-CoV-2 viral particles damage the cytoplasmic component of the leukocyte and cause its destruction.[3] Leukopenia occurring in COVID-19 patients has been reported by previous studies[13] as observed in our results shown in Table 2 that ABO blood groups are non-significant different with carrying SARS-CoV-2 in apparently healthy individuals. This result agrees with the study that demonstrates that ABO may not play a crucial role in the development of COVID-19, and mortality was not correlated with blood type distribution in COVID-19.[14] These data contrast with those of the previous study conducted by Boudin (2020) that people with blood group A had a significantly higher risk of SARS-CoV-2 infection, whereas people with blood group O had a significantly lower risk.[15] An aspect related to granulocytes in this study explained in Table 3 this result agrees with other researchers such as Tomar (2020) who said these granulocytes play a protective role during bacterial or fungal infections; however, their role in viral infections is not fully understood.[4]

Continuous infiltration of granulocyte at the site of infection and their degranulation and release of nets in response to SARS-COV-2 to raise an immune response produces cytokines and chemokine that might result in the cytokine storm.[16] Wang (2020) also demonstrated that a low ratio of granulocyte coincides with lung injury in COVID-19 patients.[13] Currently, RNA-based molecular tests require upscale laboratories with limited biosafety levels and technical sophistication, and expensive, if some rapid viral diagnostic tests were available, can be easily implemented in the clinical laboratory, the situation would be completely different patients could quickly have blood drawn thus a much wider use than molecular tests.[16]

The real-time PCR method is effective at the detection of COVID-19 despite effective for diagnosing COVID-19, real-time PCR has some limitations of false-positive and false-negative results, a false-positive result is mainly due to contamination that occurs at some point in the entire operating procedure thus, each step must carefully adhere to the relevant operation specifications in the process of PCR detection, a false-negative result is mainly caused by irregularity in the steps that can result in DNA damage.[17]

CONCLUSION

This study explained found 26.3% apparently healthy individuals carried coronavirus antigen, 13.6% have antigens and antibodies, 16.3% have only antibodies these people considered dangerous and tools that spread the disease. Therefore, must be obligated to the protective conditions reported by the World Health Organization (WHO), such as abide by spacing, wearing a mask, and avoiding crowded places. Moreover, the decreased levels of leukocytes and granulocytes in individuals without symptoms these results were statistically significant. Furthermore, blood group anon-significant correlation with coronavirus in asymptomatic individuals. In addition, to clarify the sensitivity of the RT-PCR test, RT-PCR and rapid test, rapid test respectively is (94%, 90.4%, 90%), the specificity is (98%,98.8%,95%) and the accuracy is (96.6%,97.3%,94.6%), this result observed is better to use two tests together for the best diagnosis.

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