Evaluation of Diagnostic Significance of Novel Corona Virus

S S Haque

Author's Affiliation: Biochemist, Department of Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna, Bihar 800014, India.

Abstract

In the current scenario viral diseases continue to emerge a serious public health hazards. Viruses are parasite like molecules that cannot reproduce by itself, for the production of number of viruses it requires cell machinery. Coronaviruses (CoVs) (order Nidovirales, family Coronaviridae, and subfamily Coronavirinae) are a positive sense, single-stranded RNA genome of 26 to 32 kilobases (kb) in length, and enveloped viruses. It genomes is largest in RNA viruses. Many diagnostic tests for coronavirus disease 2019 (COVID-19) are available so far. An RT-PCR test is considered very reliable because it detects less number of virus particles in swabs. D-dimer one of the markers of inflammation, its levels were higher in COVID-19 patients. In this review we discuss about the various diagnostic test for corona virus and their reliability

Keywords: Coronaviruses; Diagnosis; RT-PCR; D-Dimer; Prognosis

Introduction

Corona viruses, belongs to the family Coronaviridae are a large family of viruses that cause "the common cold" or up to 30 percent of upper respiratory tract infections in adults, prevalent almost all part of the world. First described in detail in the 1960s, the coronaviruses single-stranded RNA viruses roughly 26,000 to 32,000 bases long that measure approximately 120 nm when visualized under an electron microscope it appear like a crown (coronam is the Latin term for crown) due to the presence of Club-shaped spike glycoproteins on the envelope. Four major types of these viruses exist. They're known by the Greek letters alpha, beta, delta and gamma. Alpha and beta infect mammals, including bats, pigs, cats, and humans. Gammacoronavirus commonly infects birds such as poultry, while Deltacoronavirus infects both birds and mammals.

In structurally complete viral particle, four major structural proteins: the spike (S) protein, nucleocapsid (N) protein, membrane (M) protein, and the envelope (E) protein, encoded by coronaviral genome play an important role.[1-3] The S protein play an important role in the attachment of the virus to the host cell surface receptors and help in the fusion between viral and host cell membranes to finally support the viral entry into the host cell.[4-6] N protein helps in the binding to the CoV RNA genome, making up the nucleocapsid.[7] N protein also involved in other aspects of

Corresponding Author: S S Haque, Biochemist, Department of Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna, Bihar 800014, India.

E-mail: sshaq2002@yahoo.co.in

the CoV replication cycle and the host cellular response to viral infection.[8] The N is localized into the endoplasmic reticulum (ER)-Golgi region has proposed a function for it in assembly and budding.[9,10] The diagnosis of corona virus largely varies from molecular biology to immunology. The real-time reverse transcription-PCR (RT-PCR) most preferred testing method.

Currently, virus nucleic acid Real Time-PCR (RTPCR), CT imaging and some hematology parameters are the primary tools for clinical diagnosis of the infection.[11] The virus nucleic acid RT-PCR test has become as the current standard diagnostic method for diagnosis of COVID-19. A real-time PCR test kit has certain limitations: 1) these tests have turnaround times is high and is complicated in operation; for the generation of results it takes on average over 2 to 3 hours. 2) The PCR equipment is expensive and trained technicians to operate. 3) In RT-PCR sometimes results are false negatives for COVID-19.[12] There are certain limitations that make RT-PCR unsuitable for use in the field for rapid and simple diagnosis and screening of patients. Therefore, there is an urgent need to develop a rapid, simple to use, sensitive, and accurate test to quickly identify of SARS-CoV-2 patients.

Testing of specific antibodies such as IgM and IgG of SARS-CoV-2 in patient blood is a good choice for rapid, simple, highly sensitive and it provides the first line of defense during viral infections, high affinity IgG responses play an important role for long term immunity and immunological memory[13] It was reported that after SARS infection, IgM antibody could be detected in patient blood after 3 - 6 days and IgG could be detected after 8 days.[14,15]

Currently, development of serological tests (i.e., blood tests for specific antibodies) is still going on.[16-18] Zhang et al. detected immunoglobulin G and M (IgG and IgM) from human serum of COVID-19 patients using an enzyme-linked immunosorbent assay (ELISA) which uses SARS-CoV-2 Rp3 nucleocapsid protein in which 90% amino acid sequence homology to other SARS-related viruses has been reported.[16] Xiang et al. also detected SARS-CoV-2 IgG and IgM antibodies in suspected cases.[17] For detection of COVID-19 some other protein or cellular markers are currently used. By Guan et al infected patients had elevated levels of C-reactive protein and D-dimer and in some pateints low levels of lymphocytes, leukocytes, and blood platelets are found.[19] The problem with these biomarkers is that they are also show abnormal in other illnesses. For the improvement of specificity a multiplex assay with both antibody and small molecule markers can be used.

In epidemic areas Chest CT may be considered as a primary tool for the current COVID-19 detection. Chest CT outperformed lab testing in the diagnosis of 2019 novel coronavirus disease (COVID-19). The recent study found that CT should be used as the primary screening tool for COVID-19. CT has limited sensitivity and specificity for COVID-19 than RT-PCR testing. Chest CT should be considered a supplemental diagnostic tool, particularly for patients who show symptoms. Chest CT is a conventional, noninvasive imaging modality with high accuracy and speed. On the basis of available data reported in recent literature, most of the patients with COVID-19 had characteristic CT features of the disease[20-22], such as different degrees of ground-glass opacities with and/or without crazy-paving sign, multifocal organizing pneumonia, and architectural distortion in a peripheral distribution. Initially Chest CT showed patchy ground-glass opacity, and it rapidly progressed to segmental mixed consolidation and ground-glass opacity, and it resolved in left upper lobe, but showed multifocal ground-glass opacities after few days, and they resolved after some time. The RT-PCR test also shows positive results. By CT findings alone it is difficult to distinguish COVID-19 pneumonia from other viral pneumonia; however, the utility of chest CT increases to detect early change of COVID-19 in cases which RT-PCR tests show negative results.

After COVID-19 outbreaks, the risk of thrombosis and bleeding has attracted much attention. D-dimer is a fibrin (relatively small protein) fragment degradation product that is often used to measure and assess clot formation by fibrinolysis. The liver produces several important proteins involved in the coagulation process, one of which includes fibrinogen. A single fibrinogen molecule is a symmetrical dimer that is made up of three pairs of three different polypeptide chains, which include α, β and γ, each of the intertwined polypeptide chains that comprise a single fibrinogen molecule is held together by disulfide bonds. D-dimer elevations were seen in 3.75–68.0% of the COVID-19 patients.[23-25] D-dimer level is higher in severe cases and may be used as a prognostic biomarker for the community-acquired pneumonia (CAP) and chronic obstructive pulmonary disease (COPD) patients[26-28], and D-dimer > 1 µg/ml is one of the risk factors for mortality in adult in patients with COVID-19. However, the role of D-dimer in

COVID-19 patients requires further investigation. A D-dimer concentration is a sensitive test for the diagnosis of thrombotic states, including pulmonary embolism and DIC.[29] Therefore, increase in D-dimer levels in COVID-19 patients' play an important role in disease severity, pulmonary complications, and risk of venous thromboembolism in the setting of a pro-thrombotic state. This would assist with risk stratification and therapeutic intervention that might reduce COVID-19 related morbidity and mortality.

Conclusion

Identification of the rapid and early laboratory diagnosis is critical to diagnose novel corona virus, control the pandemic, and reduce the economic impact of COVID-19 worldwide. RT-PCR test is gold standard for SARS-CoV-2 identification, which uses conserved regions of the viral genome and the reduced rate of false-negative results are due to a large number of genetic variations, mismatches between primers, probes, and target. IgM and IgG antibody detection of SARS-CoV-2 is a supportive molecular diagnostic tool. The immunological and molecular tests are not suitable for point-of-care diagnosis due to time-consuming, kits and equipment are expensive. Typical CT findings can help early screening of suspected cases and diagnosis of COVID-19. D-dimer levels increases in case of inflammation in COVID-19 patients and have limited predictive value for thrombosis, so in the treatment of COVID-19 patients, so the value of D-dimer levels should be observed dynamically.

References

1. Masters PS The molecular biology of coronaviruses. Adv Virus Res. 2006; 66: 193–292.

2. Mortola E, Roy P. Efficient assembly and release of SARS coronavirus-like particles by a heterologous expression system. FEBS Lett. 2004;576(1–2):174–8.

3. Wang C, Zheng X, Gai W, Zhao Y, Wang H, Wang H, et al. MERS-CoV viruslike particles produced in insect cells induce specific humoural and cellular immunity in rhesus macaques. Oncotarget. 2017;8(8):12686–94.

4. Siu Y, Teoh K, Lo J, Chan C, Kien F, Escriou N, et al. The M, E, and N structural proteins of the severe acute respiratory syndrome coronavirus are required for efficient assembly, trafficking, and release of virus-like particles. J Virol. 2008;82(22):11318–30.

5. Kirchdoerfer RN, Cottrell CA, Wang N, Pallesen J, Yassine HM, Turner HL, et al. Prefusion structure of a human coronavirus spike protein. Nature. 2016; 531(7592):118–21.

6. Song HC, Seo M-Y, Stadler K, Yoo BJ, Choo Q-L, Coates SR, et al. Synthesis and characterization of a native, oligomeric form of recombinant severe acute respiratory syndrome coronavirus spike glycoprotein. J Virol. 2004; 78(19):10328–35.

7. De Haan CA, Rottier PJ. Molecular interactions in the assembly of coronaviruses. Adv Virus Res. 2005;64:165–230.

8. McBride R, van Zyl M, Fielding BC. The coronavirus nucleocapsid is a multifunctional protein. Viruses. 2014;6(8):2991–3018.

9. Tooze J, Tooze S, Warren G. Replication of coronavirus MHV-A59 in sac-cells: Determination of the first site of budding of progeny virions. Eur J Cell Biol. 1984;33(2):281–93.

10. Klumperman J, Locker JK, Meijer A, Horzinek MC, Geuze HJ, Rottier P. Coronavirus M proteins accumulate in the Golgi complex beyond the site of virion budding. J Virol. 1994;68(10):6523–34.

11. Jin YH, Cai L, Cheng ZS, et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (COVID-19) infected pneumonia (standard version). Mil Med Res. 2020;7(1):4.

12. Gallagher J. Are Coronavirus Tests Flawed? BBC News wwwbbccom/news/health-51491763. 13 Feb. 2020.

13. Racine R, Winslow GM. IgM in microbial infections: taken for granted? Immunology letters. 2009;125(2):79-85.

14. Lee HK, Lee BH, Seok SH, et al. Production of specific antibodies against SARScoronavirus nucleocapsid protein without cross reactivity with human coronaviruses 229E and OC43. J Vet Sci. 2010;11(2):165-167.

15. Wan ZY ZX, Yan XG IFA in testing specific antibody of SARS coronavirus. South China J Prev Med 2003;29(3):36-37.

16. Zhang W.; Du R.-H.; Li B.; Zheng X.-S.; Yang X.-L.; Hu B.; Wang Y.-Y.; Xiao G.-F.; Yan B.; Shi Z.-L.; et al. Molecular and Serological Investigation of 2019-nCoV Infected Patients: Implication of Multiple Shedding Routes. Emerging Microbes Infect. 2020,9(1), 386–389. 10.1080/22221751.2020.1729071.

17. 17. Xiang J.; Yan M.; et al. Evaluation of Enzyme-Linked Immunoassay and Colloidal Gold- Immunochromatographic Assay Kit for Detection of Novel Coronavirus (SARS-Cov-2) Causing an Outbreak of Pneumonia (COVID-19) medRxiv, March 1, 2020. 10.1101/2020.02.27.20028787.

18. Cai X.; Chen J.; et al.A Peptide-Based Magnetic Chemiluminescence Enzyme Immunoassay for Serological Diagnosis of Corona Virus Disease 2019 (COVID-19). medRxiv, February 25, 2020. 10.1101/2020.02.22.20026617.

19. Guan W.-J.; Ni Z.-Y.; Hu Y.; Liang W.-H.; Ou C.-Q.; He J.-X.; Liu L.; Shan H.; Lei C.-L.; Hui D. S. C.; et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N. Engl. J. Med. 2020, 10.1056/NEJMoa2002032

20. Huang P, Liu T, Huang L, et al. Use of chest CT in combination with negative RTPCR assay for the 2019 novel coronavirus but high clinical suspicion. Radiology 2020 Feb 12:200330 [Epub ahead of print].

21. Lei J, Li J, Li X, Qi X. CT imaging of the 2019 novel coronavirus (2019-nCoV) pneumonia. Radiology 2020 Jan 31:200236 [Epub ahead of print].

22. Shi H, Han X, Zheng C. Evolution of CT manifestations in a patient recovered from 2019 novel coronavirus (2019-nCoV) pneumonia in Wuhan, China. Radiology 2020 Feb 7:200269 [Epub ahead of print].

23. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395(10223):507–13.

24. Wu J, Liu J, Zhao X, Liu C, Wang W, Wang D, Xu W, Zhang C, Yu J, Jiang B,et al. Clinical Characteristics of Imported Cases of COVID-19 in Jiangsu Province: A Multicenter Descriptive Study. Clin Infect Dis. 2020;29: ciaa199.

25. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020; 395(10229):1054-62.

26. Querol-Ribelles JM, Tenias JM, Grau E, Querol-Borras JM, Climent JL, Gomez E, Martinez I. Plasma d-dimer levels correlate with outcomes in patients with community-acquired pneumonia. Chest. 2004;126(4):1087–92.

27. Fruchter O, Yigla M, Kramer MR. d-dimer as a prognostic biomarker for mortality in chronic obstructive pulmonary disease exacerbation. Am J Med Sci. 2015; 349(1):29–35.

28. Snijders D, Schoorl M, Schoorl M, Bartels PC, van der Werf TS, Boersma WG. D-dimer levels in assessing severity and clinical outcome in patients with community-acquired pneumonia. A secondary analysis of a randomised clinical trial. Eur J Intern Med. 2012;23(5):436–41.

29. Olson JD. D-dimer: an overview of hemostasis and fibrinolysis, assays, clinical applications. Adv Clin Chem. (2015) 69:1–46. doi: 10.1016/bs.acc.2014.12.001