Review Article

Covid 19: Transmission, Case Fatality Rate, Protective Measures, Laboratory Diagnosis, and Possible Laboratory Features

Mathew Folaranmi Olaniyan, Musa Abidemi Muhibi, Tolulope Busayo Ojediran
Department of Medical Laboratory Science, Edo University, Iyamho, Nigeria

Abstract

Covid 19 is a coronavirus disease caused by Covid 19 virus or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) formerly referred to as novel coronavirus or Wuhan coronavirus or 2019-nCoV causes a deadly respiratory infection. It is an infectious viral agent and a positive-sense (+ssRNA), single-stranded RNA virus that causes acute respiratory disease which can be mild, moderate, or severe illness including death. It is a zoonotic infection and can also be transmitted from human to human. The virus enters the cell by binding with cell that has angiotensin I converting enzyme 2 (ACE2) receptor using the spike. ACE2 receptor is found on the cell membranes of cells in the lungs, arteries, heart, kidney, and intestines. The first outbreak was reported in Wuhan, China, on December 31, 2019, hence the initial name Wuhan coronavirus. Globally, it has a fatality rate of 6.7% with a fatality rate of 3.5% in Nigeria as at April 18, 2020. The infection is air borne through droplets from infected person during coughing, spiting or sneezing. It can also be contracted by touching eyes, nose, or mouth with contaminated hands. Covid 19 may elicit both inflammatory and acute phase immune responses while the mechanical innate immune defense can be overcome to cause severe pneumonia The virus enters the lung through ACE2 receptors on the cell membrane of the lung to destroy cilia resulting into the accumulation of dead tissues, cells/dirts/wastes, and fluids thereby displacing the normal air content of the lung which will eventually bring about dry cough, and difficulties in breathing. The infection can be prevented through basic protective measures which include regular washing of hands with soap and water followed by sanitizing hands with alcohol-based sanitizer, social distancing, avoidance of gathering, quarantine measure applicable to especially those from endemic areas, self-isolation for those who are positive or manifesting related signs and symptoms, use of personal protective equipment, early diagnosis, and adequate intervention. The use of soap and alcohol are effective as soap can break through the lipid layer of the virus to become smaller particles which are washed away by water while alcohol is capable of lysing the virus. Covid 19 is diagnosed in the laboratory by real-time reverse transcription polymerase chain reaction Panel. Covid 19 may result into abnormal liver function tests due to abnormal fat retention, elevated plasma creatinine and urea which may be due to kidney damage, elevated $C0_2$, and decreased oxygen, level due to severe pneumonia, decreased and elevated anti and pro-inflammatory cytokines respectively which may be manifested as fever, acute phase response, decreased erythropoietin due to possible kidney damage, prolong prothrombin time/activated partial thromboplastin time and depleted platelet count which may manifest as disseminated intravascular coagulation. This work reviewed the transmission, case fatality rate, basic protective measures, laboratory diagnosis, and possible laboratory features of Covid 19.

Keywords: Case fatality rate, Covid 19, laboratory diagnosis, laboratory features, protective measures, transmission

Introduction

Covid 19 is a coronavirus disease caused by Covid 19 virus or Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) formerly referred to as Novel Coronavirus or Wuhan coronavirus or 2019-nCoV belongs to a group of virus known as coronavirus otherwise referred to as informally known as the Wuhan coronavirus or 2019 novel coronavirus (2019-nCoV) It is an infectious viral agent and a positive-sense (+ssRNA), single-stranded RNA virus that

Access this article online

Quick Response Code:

Website: www.matrixscimed.org

DOI: 10.4103/MTSM.MTSM_6_20

Address for correspondence: Prof. Mathew Folaranmi Olaniyan, Department of Medical Laboratory Science, Edo University, Iyamho, Nigeria.
E-mail: olaniyanmat@yahoo.com

How to cite this article: Olaniyan MF, Muhibi MA, Ojediran TB. Covid 19: Transmission, case fatality rate, protective measures, laboratory diagnosis, and possible laboratory features. Matrix Sci Med 2020;4:64-9.

Received: 19-04-2020, **Revised:** 19-04-2020, **Accepted:** 26-04-2020, **Published:** 14-07-2020

causes acute respiratory disease. Currently, there is an outbreak of 2019–2020 Wuhan coronavirus.[1-5] It shares some similarities with SARS-CoV (79.5%) and bat coronaviruses (96%), which makes an ultimate origin in bats likely. Covid 19 has been associated with bat betacoronaviruses.[6-8] The first outbreak of Covid 19 was reported in Wuhan, China, on December 31, 2019, hence the initial name Wuhan coronavirus.[9-11] Majorly Covid 19 affects lungs which may lead to respiratory failure and death.[1-5] This is because the virus enters the cell using the glycoprotein spike to connect the angiotensin I converting enzyme 2 (ACE2) as entry point which is most abundant in the type II alveolar cells of the lungs. ACE2 is also abundant in the glandular cells of gastric, duodenal and rectal epithelium, endothelial cells, and enterocytes of the small intestine. It is also found in the cell membranes of arteries, heart, and kidney.[1-5]

The virus enters the lung through ACE2 receptors on the cell membrane of the lung to destroy cilia resulting into the accumulation of dead tissues, cells/dirts/wastes and fluids thereby displacing the normal air content of the lung which will eventually bring about dry cough, and difficulties in breathing.[1-5] The normal function of cilia in the lung is to prevent accumulation of dead tissues or cells/dirts/wastes and fluids which are removed through productive cough as a form of innate/mechanical immune response or defense but this function is reversed in on the entry of Covid 19 virus into the lung as it will damage the cilia to negatively affect the physiological function.[1-5]

The basic reproduction number of Covid 19 virus is between 1.4 and 3.9 which implies that if the infection is left without clinical intervention, the virus typically results in 1.4–3.9 new cases per established infection.[12-15] The illness caused by Covid 19 virus ranged from mild to severe resulting in death.[16,17] Covid 19 may elicit both inflammatory and acute phase immune responses while the mechanical innate immune defense can be overcome to cause severe pneumonia.[1-5]

Table 1: 2019-nCoV rRT-PCR

MH step	Cycles	Temp	Time
UNG incubation	1	25°C	2 min[30,31]
RT incubation	1	50°C	15 min[30,31]
Enzyme activation	1	95°C	2 min[30,31]
Amplification	45	95°C	3 s[30,31]
		55°C	30 s[30,31]

RT: Reverse transcription, UNG: Uracil-N-glycosylase

Table 2: Covid 19 real-time reverse transcription polymerase chain reaction diagnostic panel results interpretation

nCoV_N1	Covid 19_N2	Covid 19_N3	RP	Result interpretationa
+	+	+	±	Covid 19 detected[30,31]
If only one, or two, of three targets is positive			±	Inconclusive result[30,31]
-	-	-	+	Covid 19 not detected[30,31]
-	-	-	-	Invalid result[30,31]

RP: human RNase P

Transmission

Covid 19 virus is primarily a zoonotic infection. The infection was linked to a large seafood and animal market. Covid 19 virus can be transmitted from infected human to an uninfected human through close contact, specifically through respiratory droplets from coughs and sneezes within a range of about 6 feet (1.8 m).[18-20] Specifically, the infection is air borne through droplets from infected person during coughing, spiting or sneezing. It can also be contracted by touching eyes, nose, or mouth with contaminated hands when the hands have come in contact with the virus on objects or air. Contaminated money and bottoms of automatic teller machine can also serve as vehicle of transmission. Coronavirus RNA has also been demonstrated in stool samples from infected patients.[21] The virus is capable of infecting human during incubation period of 1–14 days and there is possibility transmission from asymptomatic cases. The virus is able to transmit along a chain of at least four people. Animals sold for food are possible intermediary because many of the first identified infected individuals were workers at the Huanan Seafood Market because of their rate of contact with animals.[16,17]

The receptor binding protein of the nCoV spike (S) protein has adequate affinity to the receptor of Angiotensin converting enzyme 2 (ACE2). The virus uses Angiotensin converting enzyme 2 (ACE2) which is also found on kidney as receptor to enter cell. In a drug docking experiments for the potential protease inhibitors, the viral 3C-like protease M (pro) from the ORF1a polyprotein has been modeled.[18-20]

Case Fatality Rate of Covid 19 infection

Globally, 2,074, 529 confirmed cases and 139,378 deaths with a fatality rate of 6.7% as on April 18, 2020, while the fatality rate as at this time in Nigeria was 3.5% (19 deaths against 542 confirmed cases).[1,21-27]

Risk Assessment

Covid 19 outbreak is a novel public health problem that calls for aggressive interventions. The risk from these outbreaks depends how well the virus spreads from human to human, the severity of illness it causes availability of vaccine, treatment medications and responses to stem the spread.[1-5] Covid 19 virus has posed a high threat to public health globally as the illness caused by the virus may result to death (at the rate of about 6.7%) and can easily be spread between humans as found all over the world.[3,4] The infection has become pandemic. The risk of infection majorly depends on exposure through contacts with the droplets of infected individuals. Currently, health-care workers caring for Covid 19 virus patients, other close contacts through any form of gathering involving Covid 19 virus patients.[1-5]

Basic Protective Measures against Covid 19 Virus

- Building resistance against Covid 19 virus[1,5]
- Individuals presenting with dry cough, difficulty in breathing, fever, fatigue, and other respiratory symptoms should present themselves for proper diagnosis and management[5]
- Health-care provider should follow recommended infection control procedures[1,17]
- People who have had close contact with someone infected with Covid 19 virus should report to appropriate health facilities for laboratory test or self-isolate for 14 days until they are confirmed negative by laboratory test[19]
- Regular washing of hands with soap and water for at least 30 s and thereafter disinfect the hands with an alcohol-based sanitizing solution (using alcohol of 60%–90% grade)[20]
- During coughing and sneezing, cover mouth, and nose with flexed elbow or tissue or sterile handkerchief and discard the tissue/handkerchief immediately into a closed bin and wash hands as earlier recommended[24]
- In public or at socials, maintain a distance of at least 3 feet distance from people[25]
- Avoid touching eyes, nose, and mouth as we use hands to touch surfaces which can be contaminated with the virus, if necessary wash and sanitize the hands as recommended before doing so[26]
- Report fever, cough difficulty breathing or any respiratory symptom as early as possible for medical care[4]
- Regular hand washing with soap, alcohol, and potable water after touching animals and animal products; avoid touching eyes, nose or mouth with hands; and avoid contact with sick animals or spoiled animal products[4]
- Avoid contact with animals especially in the market (e.g., stray cats and dogs, rodents, birds, and bats)[5]
- Avoid contact with potentially contaminated animal waste or fluids on the soil or structures of shops and market facilities[4]
- Avoid consumption of raw or undercooked animal products such as meat, milk, or animal organs[5]
- Early diagnosis[5]
- Quarantine measures of suspected individuals[5]
- Self-isolation if exposed and isolation of positive cases[5]
- The use of personal protective equipment[5]

Laboratory Diagnosis

Two 1-step quantitative real-time reverse-transcription polymerase chain reaction assays

This is a rapid assay that allows for the detection of detection of 2019n-CoV in human samples, that can allow early identification of patients.[27,28]

Daniel *at al.*[28] developed two 1-step quantitative real-time reverse transcription polymerase chain reaction (rRT-PCR) assays to detect two different regions (ORF1b and N) of Covid 19 genome. They designed primer and probe sets that will react with Covid 19 and its closely related viruses such as SARS coronavirus. These assays also involved the use of positive and negative controls [Table 1].

Daniel *at al.*[28] reported that RNA extracted from cells infected by SARS coronavirus was used as a positive control and DNA plasmids was used as positive standards, the detection limits of these assays were found to be below 10 copies per reaction. All in the assays. Samples from two 2019-nCoV-infected patients tested positive against the negative control samples that tested negative as expected.

Detection of Covid 19 by real-time reverse transcription polymerase chain reaction[29]

Corman *et al.,*[29] made use of cell culture supernatants containing typed coronaviruses and other respiratory viruses including samples from all collections comprised sputum as well as nose and throat swabs. RNA was extracted from clinical samples using MagNA Pure 96 system (Roche, Penzberg, Germany) and from cell culture supernatants with the viral RNA mini kit (QIAGEN, Hilden, Germany) [Table 2].

Real-Time reverse transcription polymerase chain reaction panel for detection Covid 19

Centers for Disease Control and Prevention, Respiratory Viruses Branch, Division of Viral Diseases.[29,30] describes the use of rRT-PCR assays for the *in vitro* qualitative detection of Covid 19 in respiratory specimens and sera. The Covid 19 primer and probe sets are designed for the universal detection of SARS-like coronaviruses (N3 assay) and for specific detection of Covid 19 (N1 and N2) assays.[30,31]

Biosafety Precautions

To carry out the assay or handle the samples those involved must wear personal protective equipment such as gowns, gloves, eye protection when working with clinical specimens. The samples must be processed in a certified class II biological safety cabinet following biosafety level 2 or higher guidelines.[29,30]

Acceptable Specimens

1. Nasopharyngeal or oropharyngeal aspirates or washes[30,31]
2. Nasopharyngeal or oropharyngeal swabs[30,31]
3. Bronchoalveolar lavage[30,31]
4. Tracheal aspirates and sputum[30,31]
5. Serum.[30,31]

Note: Swab specimens should be collected only on swabs with a synthetic with aluminum or plastic shafts while swabs with calcium alginate or cotton tips with wooden shafts are not acceptable.[30,31]

Storage of Specimen

1. Store specimens at 4°C for up to 72 h after collection or at −70°C or lower if there will be a delay in the extraction[30,31]

2. The Nucleic acids extracted from the specimens should be stored at −70°C or lower.[30,31]

Criteria for the Rejection of Specimen

- Reject all specimens not kept at 2°C–4°C (≤4 days) or frozen at −70°C or below[30,31]
- Reject any incomplete labeling of specimen or documentation[30,31]
- Reject inappropriate specimen type[30,31]
- Reject insufficient specimen volume.[30,31]

Reagents and Equipment Required

Reagents and supplies

1. rRT-PCR primer/probe sets[30,31]
2. Positive template contro[30,31]
3. TaqPath™ 1-Step RT-qPCR Master Mix, CG[30,31]
4. Molecular grade water, nuclease-free[30,31]
5. Disposable powder-free gloves[30,31]
6. P2/P10, P200, and P1000 aerosol barrier tips[30,31]
7. Sterile, nuclease-free 1.5 mL microcentrifuge tubes[30,31]
8. 0.2 mL PCR reaction tube strips or 96-well real-time PCR reaction plates and optical 8-cap strips[30,31]
9. Laboratory marking pen[30,31]
10. Cooler racks for 1.5 microcentrifuge tubes and 96-well 0.2 mL PCR reaction tubes[30,31]
11. Racks for 1.5 ml microcentrifuge tubes[30,31]
12. Acceptable surface decontaminants[30,31]
13. DNA AwayTM[30,31]
14. RNAse[30,31]
15. 10% bleach (1:10 dilution of commercial 5.25%–6.0% sodium hypochlorite).[30,31]

Equipment

1. PCR work station (ultraviolet lamp; laminar flow)[30,31]
2. Vortex mixer[30,31]
3. Microcentrifuge[30,31]
4. Micropipettes of 2 µl, 10 µl, 200 µl and 1000 µl[30,31]
5. Multichannel micropipettes (5–50 µl)[30,31]
6. 2 × 96-well cold blocks[30,31]
7. −20°C (nonfrost-free) and −70°C freezers; 4°C refrigerator[30,31]
8. Real-time PCR detection system[30,31]
9. Nucleic acid extraction system.[30,31]

Extraction of nucleic acid

1. Amount and quality of sample template RNA determine the performance of rRT-PCR amplification-based assays therefore RNA extraction procedures should be qualified and validated for recovery and purity before testing specimens[30,31]
2. Commercially available extraction procedures that have been shown to generate highly purified RNA extraction include[30,31]
    - bioMérieux NucliSens® systems
    - QIAamp® Viral RNA Mini Kit
    - QIAamp® MinElute Virus Spin Kit
    - RNeasy® Mini Kit (QIAGEN)
    - EZ1 DSP Virus Kit (QIAGEN)
    - Roche MagNA Pure Roche MagNA Pure 96 DNA
    - Viral NA Small Volume Kit and Invitrogen ChargeSwitch® Total RNA Cell Kit.
3. Retain residual specimen and nucleic extract and store immediately at −70°C[30,31]
4. Only thaw the number of specimen extracts that will be tested in a single day[30,31]
5. Do not freeze/thaw extracts more than once before testing.[30,31]

Quality control

Due to the sensitivity of rRT-PCR, these assays should be conducted using strict quality control and quality assurance procedures.[30,31]

1. The laboratory personnel must be conversant with the method/procedure and instruments to be used[30,31]
2. There must be separate areas and dedicated pipettes, microcentrifuge, microcentrifuge tubes, pipette tips, gowns and gloves for assay reagent setup and handling of extracted nucleic acids[30,31]
3. The flow of work must always be from the clean area to the dirty area[30,31]
4. Laboratory personnel involved must wear clean disposable gowns and new, previously unworn, powder-free gloves during assay reagent setup and handling of extracted nucleic acids.[29,30] Whenever contamination is suspected gloves must be changed[30,31]
5. Store primer/probes and enzyme master mix at appropriate temperatures as prescribed in the package.[29,30] Do not use expired reagents[30,31]
6. Capp all reagent tubes and reactions as much as possible[30,31]
7. Clean and decontaminate working surfaces[30,31]
8. Extracted nucleic acid or PCR products must not be brought into the assay setup area[30,31]
9. Use aerosol barrier (filter) pipette tips only[30,31]
10. Use PCR plate strip caps only[30,31]
11. Do not use PCR plate sealing film.[30,31]

Assay controls

1. Assay controls should be run concurrently with all test samples[30,31]
2. Positive template control (PTC) with an expected value range[30,31]
3. Negative template control (NTC) added during rRT-PCR reaction setup[29,30]
4. Human specimen extraction control (HSC) extracted concurrently with the test samples provides a nucleic acid extraction procedural control and a secondary negative control that validates the nucleic extraction procedure and reagent integrity[30,31]
5. All clinical samples should be tested for human RNAse P gene to determine specimen quality.[30,31]

Real-time reverse transcription polymerase chain reaction assays

1. The assays involve preparation of stock reagent setting rRT-PCR Primers/Probe, use of PTC, NTC, HSC and equipment preparation[30,31]
2. Assays should be carried out according to the manufacturer's instructions.[29,30]

Equipment preparation

1. Clean and decontaminate all work surfaces, pipets, centrifuges and other equipment prior to assays using RNase or 10% freshly prepared sodium hypochlorite[30,31]
2. Put on AB 7500 Fast DX and allow block to attain optimal temperature[29,30]
3. Perform plate setup and select cycling protocol on the instrument[30,31]
4. Set Instrument: Detector (FAM); Quencher (None); Passive Reference: (None); Run Mode: (Standard);
5. Sample Volume (20 μL).[30,31]

Fluorescence data should be collected during the 55°C incubation step[31,32]

Possible laboratory features[32-34]

1. Abnormal liver function tests due to liver: microvesicular steatosis steatosis, (abnormal retention of fat (lipids) within a cell or organ)[32-34]
2. Prolong prothrombin time and the activated partial thromboplastin time, low fibrinogen, depleted platelet count, high levels of fibrin degradation products, including D-dimer, schistocytes manifesting as disseminated intravascular coagulation possibly due to impaired liver function[32-34]
3. Low level of oxygen (hypoxemia) and elevated carbon dioxide (hypercapnia) due to severe pneumonia[32-34]
4. Abnormal renal function tests including elevated urea/creatinine, electrolyte/fluid imbalance due to damage to the kidney in advance stage[32-34]
5. Low erythropoietin level which may result from kidney[32-34]
6. Elevated pro-inflammatory and decreased anti-inflammatory cytokines due to inflammatory responses as a result of the infection as manifested by symptoms like fever
7. Acute phase response leading to elevated positive acute phase and decreased negative acute phase proteins[32-34]
8. Decrease in blood minerals as a result of gastrointestinal disorders because the virus can infect enterocytes through ACE2 on the intestinal cell membrane to cause enteropathy thereby decreasing the absorption of nutritive minerals.[32-34]

Conclusion

Covid 19 is a coronavirus disease caused by Covid 19 virus or SARS-CoV-2 that enters host cell through ACE2 receptor using the spike can be detected by Real-Time RT-PCR Panel. It can be contracted through contact with the droplets from the infected person which can be avoided by washing hands, use of alcohol-based sanitizer, personal protective equipment, self-isolation, social distancing, quarantine measures, and social distancing. As on April 6, 2020, the case fatality rate globally was 5.5%; and in Nigeria, it was 2.2%. Possible laboratory features include abnormal levels of hematological, immunological, liver and renal parameters.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Nigeria Centre for Disease Control. Updates on COVID-19 (Coronavirus Disease 2019). Available from: https://covid19.ncdc.gov.ng/. [Last updated on 2020 Apr 06].
2. Dan F. What you need to know about the Wuhan coronavirus. Nature 2020. Available from: https://www.nature.com/articles/d41586-020-00209-y. [Doi: 10.1038/d41586-020-00209-y]. [Last accessed on 2020 May 10].
3. Zhang YZ, Wu F, Chen Y M, Pei YY, Xu L, WangW, *et al*. Wuhan Seafood Market Pneumonia Virus Isolate Wuhan-Hu-1, Complete Genome. GenBank. Bethesda, Maryland, United States: National Institutes of Health, Archived from the Original on; 21 January, 2020. Available from: https://www.ncbi.nlm.nih.gov/nuccore/MN908947.2 [Last retrieved 2020 Jan 13].
4. World Health Organization. Surveillance Case Definitions for Human Infection with Novel Coronavirus (nCoV): Interim Guidance v1, (Report). World Health Organization; 2020. Available from: http://hdl: 10665/330376.W HO/2019-nCoV/Surveillance/v2020.1. [Last accessed on 2020 April 18].
5. United States: Centers for Disease Control and Prevention (CDC). Novel coronavirus (2019-nCoV). Wuhan, China: Archived from the Original on; 10-14 January, 2020. Available from: https://emergency.cdc.gov/han/han00426.asp?deliveryName=USCDC_511-DM17544, [Last retrieved on 2020 Jan 16].
6. Huang P. How Does Wuhan Coronavirus Compare with MERS, SARS and the Common Cold? NPR.org. Archived from the original on; 2 February, 2020. Available from: https://www.npr.org/sections/goatsandsoda/2020/01/22/798277557/how-does-wuhan-coronavirus-compare-to-mers-sars-and-the-common-cold. [Last retrieved on 2020 Feb 03].
7. Perlman S. Another decade, another coronavirus. New England J Med 2020;382:769-2.
8. Wu JT, Leung K, Leung GM. Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: A modelling study. Lancet 2020;395:689-97.
9. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, *et al*. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270-3.
10. Benvenuto D, Giovannetti M, Ciccozzi A, Spoto S, Angeletti S, Ciccozzi M. The 2019 new coronavirus epidemic: Evidence for virus evolution. bioRxiv: 202010. Journal of Medical Virology doi: 10.1002/jmv.25688. [Doi: 10.1101/2020.01.24.915157].
11. Callaway E, Cyranoski D. Why snakes probably aren't spreading the new China virus. Nature 2020. [Doi: 10.1038/d41586-020-00180-8].
12. Randhawa GS, Soltysiak MP, El Roz H, de Souza CP, Hill KA, Kari L. Machine learning-based analysis of genomes suggests associations between Wuhan 2019-nCoV and bat Betacoronaviruses. bioRxiv 2020; PLOS ONE doi: 10.1371/journal.pone.0232391.
13. World Health Organization. Novel Coronavirus – China. World Health Organization; Archived from the original on 23 January, 2020.

Available from: https://www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/ https://www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/. [Last retrieved 2020 Feb 01].
14. Cohen J. Wuhan seafood market may not be source of novel virus spreading globally. Sci 2020. Available from: https://www.sciencemag.org/news/2020/01/wuhan-seafood-market-may-not-be-source-novel-virus-spreading-globally. [Doi: 10.1126/science.abb0611. ISSN 0036-8075].
15. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, *et al*. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: A study of a family cluster. Lancet 2020;395:514-525.
16. Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, *et al*. Transmission of 2019-nCoV infection from an asymptomatic contact in germany. N Engl J Med 2020;382:970-1.
17. BBC News "Coronavirus Declared Global Health Emergency. Online. Archived from the original on; 30 January 2020. Available from: https://www.bbc.com/news/world-51318246. [Last retrieved on 2020 Jan 30].
18. Wee SL, McNeil DG Jr., Hernández JC. W.H.O. Declares Global Emergency as Wuhan Coronavirus Spreads. The New York Times. Archived from the original on; 30 January, 2020. Available from: https://www.nytimes.com/2020/01/30/health/coronavirus-world-health-organization.html. [Last retrieved on 2020 January 30].
19. World Health Organization. Statement on the Second Meeting of the International Health Regulations (2005) Emergency Committee Regarding the Outbreak of Novel Coronavirus (2019-nCoV); Archived from the Original on 30-31 January, 2020. Available from: https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov) [Last retrieved 2020 Jan 30].
20. World Health Organization. Novel Coronavirus (2019-nCoV) Situation Reports no. 8. Archived from the original on; 26 January, 2020. http://www.who.int. [Last retrieved on 2020 Feb 01].
21. Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, *et al*. First case of 2019 novel coronavirus in the United States. New England J Med 2020;382:929-36.
22. Kupferschmidt K. Study claiming new coronavirus can be transmitted by people without symptoms was flawed. Science 2020. Available from: https://www.sciencemag.org/news/2020/02/paper-non-symptomatic-patient-transmitting-coronavirus-wrong
23. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet 2020;395:470-3.
24. World Health Organization. NHK. 24 January 2020. Archived from the Original on. World Health Organization; 26 January, 2020. Available from: https://www.cgtn.com/special/Battling-the-novel-coronavirus-What-we-know-so-far-.html. [Last retrieved on 2020 Feb 03].
25. NBC News. How Does Coronavirus Spread? Archived from the Original on; 28 January, 2020. Available from: https://www.nbcnews.com/news/asian-america/coronavirus-spreads-so-does-concern-over-xenophobia-n1125441. [Last retrieved on 2020 Jan 29].
26. CDC. Transmission of Novel Coronavirus (2019-nCoV). Archived from the Original on 27-28 January, 2020. Available from: http://www.cdc.gov. [Last retrieved 2020 Jan 29].
27. Worldometer Wuhan Coronavirus Death Rate. Archived from the Original on; 31 January, 2020. Available from: http://www.worldometers.info. [Last retrieved 2020 Feb 02].
28. Daniel KW, Yang P, Samuel MS, Kenrie PY, Pavithra K, Yingzhi L, *et al*. Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. Clin Chem 2020;66:549–55 . [Doi: 10.1093/Clinchem/Hvaa029].
29. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, *et al*. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25:2000045. doi: 10.2807/1560-7917.ES.2020.25.3.2000045 .
30. CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients; 2020. Available from: https://www.cdc.gov/corona virus/2019-nCoV/guidelines-clini cal-specimens.html. [Lasr accessed on 2020 May 05].
31. CDC. Biosafety in Microbiological and Biomedical Laboratories 5th ed. EditionPublisher: US Department of Health and Human ServicesEditor: Lewis Casey Chosewood; Deborah E. WilsonISBN: 978-0160850424. Available from: https://www.cdc.gov/labs/BMBL.html. [Last accessed on 2018 Oct 24].
32. Giani M, Seminati D, Lucchini A, Foti G, Pagni F. Exuberant plasmocytosis in bronchoalveolar lavage of the first patient requiring extracorporeal membrane oxygenation for SARS-CoV-2 in Europe. J Thorac Oncol 2020;15:e65-6.
33. Lillicrap D. Disseminated intravascular coagulation in patients with 2019-nCoV pneumonia. J Thromb Haemost 2020;18:786-7.
34. Mitra A, Dwyre DM, Schivo M, Thompson GR 3rd, Cohen SH, Ku N, *et al*. Leukoerythroblastic reaction in a patient with COVID-19 infection. Am J Hematol 2020;10.1002/ajh.25793. doi: 10.1002/ajh.25793. PMID: 32212392 PMCID: PMC7228283 DOI: 10.1002/ajh.25793.