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Review Article

Most Concern Strains of Coronavirus-2 and Diagnostic Protocol in Iraqi's Hospitals: A review

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BSTRACT

The new Severe Acute Respiratory Syndrome Coronavirus-2 is responsible for the current global pandemic. The emergence of covid-19 variants on different continents has caused great concern in global human health. These variants affected many places around the world including China, Europe, U.K., and United States. The most variant of concern is Beta (lineage B.1.351), Epsilon (lineage B.1.429), and Kappa (lineage B.1.617). These variants allowed the virus to become higher transmissible in the population, and undetected because a large number of mutations accumulate in the spike (S) protein, especially within the amino-terminal domain (NTD) and receptor-binding domain (RBD). The consequences of these variants are stimulated high virulence, frequent re-infection, and increased resistance for monoclonal antibodies. In Iraq the diagnosing process starts with a PCR test to confirm the infection, however, if the result comes up negative with persistence symptoms, the PCR need to be followed by CT the most supportive procedure for diagnosing the infection, where the infection is classified into three types depends on symptoms of cystic fibrosis as High confidence, intermediate confidence, and low confidence. The diagnosis procedure infection summaries that all series of diagnostic tests need to be done even if the PCR results are negative.

Keywords: Coronavirus-2, Diagnostic, Iraq.

Mutations, Variants, and Spread

Mutations occur naturally from viral replication. Mutation rates tend to be higher in RNA viruses than DNA. Viral reproduction in the cells of the body means the reproduction of the virus hundreds of billions of times, which may cause changes in the genetic makeup, which are errors in the genetic makeup of the virus and its 30,000 letters, which are called a mutation, and this leads to a change in the methods of dealing with viral diseases increase its transmissibility, increase virulence, and reduce the effectiveness of vaccines against it (Grubaugh *et al*., 2020; Sanjuán and Domingo-Calap, 2016).

When coronaviruses share the same distinct mutations, it is known as the variant. Adequate accumulation of mutations in a strain may cause viruses to act differently in their behavior. These strains are known as subspecies. In general, Viruses obtain mutations over time, till result in an emerging variant, when it became grew in a population (Almubaid and Al-Mubaid, 2021).

The potential consequences of emerging variants

Emerging variants could come with consequences listed below; increasing morbidity, transmissibility, and mortality, undetected by diagnostic tests, susceptibility to neutralizing antibodies and antiviral drugs is decreased. Capability to bypass natural immunity leading to reinfections, where vaccinated individuals still have the chance to be infected. Multi-inflammatory symptoms of long COVID infection make it worse. Immunocompromised individuals or children become more vulnerable (Weisblum *et al*., 2020).

Variants of interest are terms called if it meets one or more of these criteria pending validation and verification of these properties. The cause of an increased number of cases or unique outbreak clusters considers as a character for these variants. The spread of this variant should remain within national levels, otherwise, it becomes a "variant of concern" (Griffiths *et al*., 2021). "Variant of high consequence" takes place if the effects of protective measurement decreased significantly for a specific variant (Baloch *et al*., 2020).

Strains of SARS-CoV-2

More than eight virus strains throughout time and variability characteristics have been registered in different countries listed below in (Table 1) (Watanabe *et al*., 2020).

Table 1: Strains of SARS-CoV-2 in different countries

Number	Major outbreak area	First appearance	Features
1	China	12/30/2019	All COVID viruses may have evolve from here, among which the A2 virus is the closest to the virus genes on bats and pangolins.
2	China and other Asian countries	12/24/2019	Virous virus strains evolved from A1 virus in the A family
3	Europe	1/28/2020	S protein variation
4	U.k. the Netherlands, Hong Kong	1/21/2020	NSP2, NSP3 and ORF3 protein variants
5	U.S West Coast, Canada	1/19/2020	Most of ORF8 protein variants have two NSP13 protein variants
6	Spain, Australia, South Korea, China	1/10/2020	All are viruses that have evolved directly from A2
7	Europe, South America	2/16/2020	Variations of three consecutive sites on S protein and N protein
8	France U.S East Coast	2/21/2020	S protein variation and ORF3 protein variation

The Other Strains Of SARS- Cov-2

Alpha variant Lineage B.1.1.7

Alpha variant Lineage B.1.1.7 was investigated in the United Kingdom in October 2020. It has other names like the first Variant Under Investigation and Alpha variant and 20I/501Y.V1. The emergence of B.1.1.7 was around September 2020 and then rapidly become the dominant circulating SARS-CoV-2 variant in England (1). the United States is one of 30 countries that have the B.1.1.7 (Callaway, 2021; Galloway *et al*., 2020). The conformation of the receptor-binding domain is affected by a mutation in the S protein (N501Y). Besides B.1.1.7 lineage-defining mutations, the S protein has 13 other ones. Out of these mutations, there is a deletion at positions 69 and 70 (del69–70) that is hypothesized to increase transmissibility Fig. (1). This deletion will affect at least one Real Time-Polymerase chain reaction (RT-PCR) based diagnostic assay by causing S-gene target failure (SGTF). the variants with the del69–70 and B.1.1.7 variant produce a negative result for the S-gene target and a positive in the other two targets. SGTF was used as a proxy in the U.K for identifying B.1.1.7 cases. B.1.1.7 compared with other SARS-CoV-2 variants, consider more efficient transmission in the United Kingdom as multiple lines of evidence indicated. This explains the faster epidemic growth in areas of B.1.1.7 than in other areas U.K. diagnoses with SGTF increased faster than did non-SGTF diagnoses in the same areas (Corum and Zimmer, 2021).

The spike protein includes Mutations as follow (Corum and Zimmer, 2021):

- N501Y, the mutation may not aid the virus to evade current vaccines. But still, assist viruses to bind firmly to human cells.
- P681H, create new spike proteins in a more efficient way that may help infected cells.
- The H69–V70 and Y144/145, deletions, evade some antibodies by altering the shape of the spike.

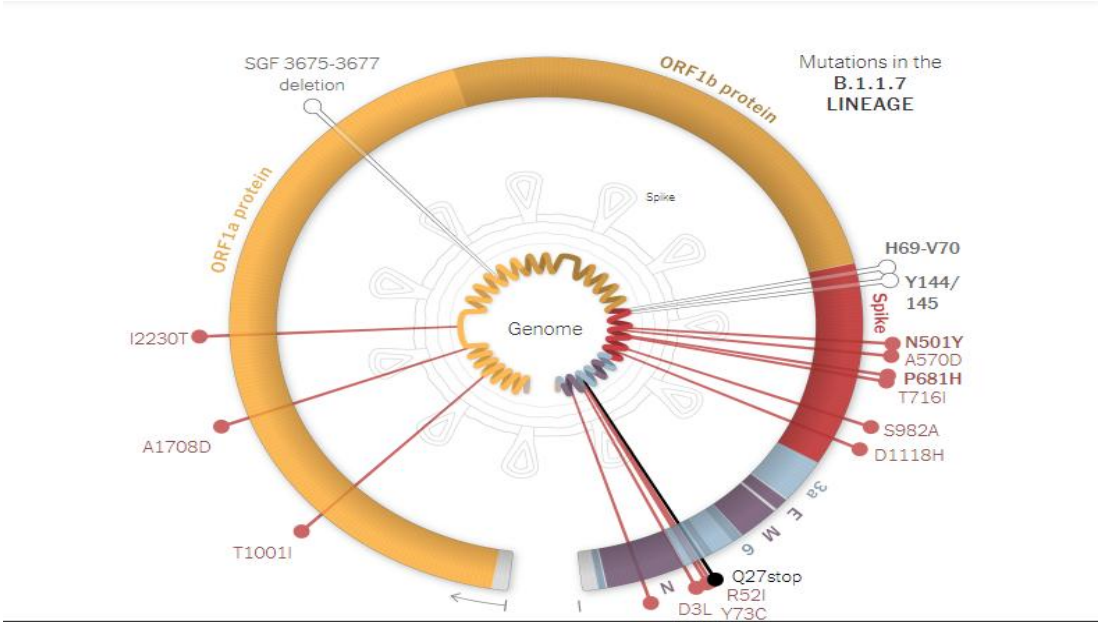

Fig. 1: Mutation in the B.1.1.7 lineage (Corum and Zimmer, 2021)

Lineage B.1.351

The beta variant is called 20H/ 501Y.V2 (formerly 20C/ 501Y.V2). It is among several variants that are particularly important, it was first reported on 18 December 2020 in Nelson Mandela Bay/ South Africa, followed by other cases discovered globally like the United State. By

the end of December 2020, the alternative was also identified in Zambia and seemed to be the dominant alternative in the country (Novazzi *et al*., 2021).

Three mutations (K417N, E484K, N501Y) in the spike region consider the particular interest of the B.1.351 genome (Corum and Zimmer, 2021) the following five mutations are generating less concern as can be seen in Fig. (2).

Previous mutations in the receptor-binding domain (RBD) allow the variant to attach more easily to human cells. Two of these mutations, E484K and N501Y, are within the receptor-binding motif (RBM) of the receptor-binding domain (RBD). The N501Y mutation was detected in the United Kingdom. It is good to note that two mutations found in 501.V2, E484K, and K417N, are not found in Variant of Concern 202012/01. Also, 501.V2 does not have the 69-70del mutation found in the other variant (Tegally *et al*., 2021).

Mutations near the tip of the spike protein include (Tegally *et al*., 2021):

- **N501Y**, assist virus to bind tightly to human cells. This mutation also occurred in the B.1.1.7 and P.1 lineages.
- **K417N**, assist the virus to bind tightly to human cells.
- **E484K**, may assist the virus to avoid a few types of antibodies

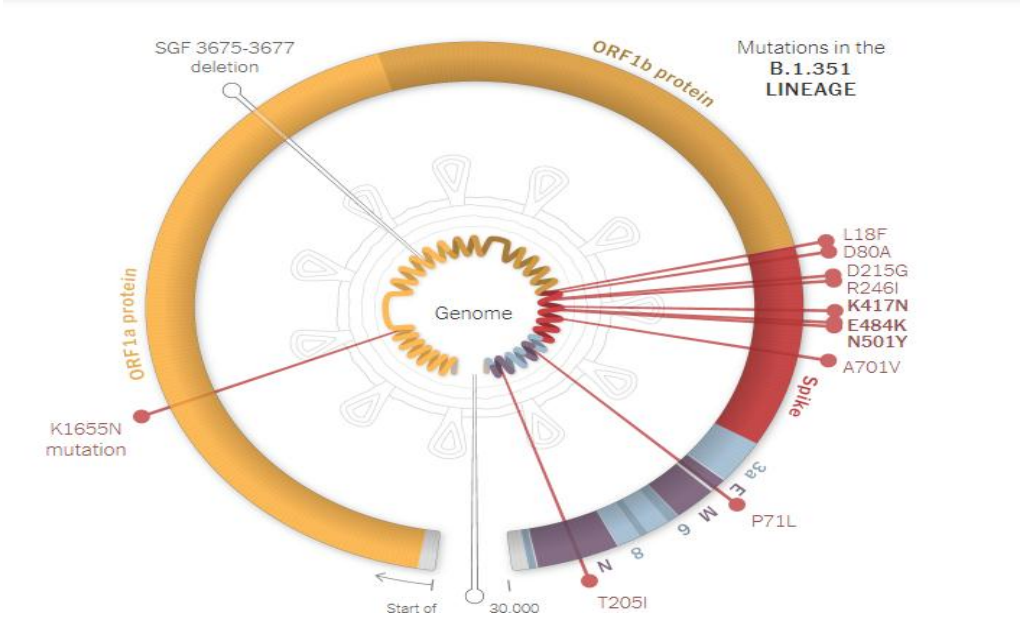

Fig. 2: Mutation in the B.1.351 lineage (Tegally *et al*., 2021).

Lineage P.1

Gamma or Brazil variant is another type of SARS-CoV-2 variant, that is responsible for COVID-19. It has 17 unique changes in amino acid, 10 on spike protein, including three considered a concern: N501Y, E484K, and K417T (figure 3). The variant emerged on 6 January 2021 in Tokyo-Japan (Faria *et al*., 2021; Naveca *et al*., 2021). 28-AM-1 and 28-AM-2 are the two distinct sub-variants included in P.1, these two have the concern mutations N501Y, E484K, K417T, independently of each other in the Brazilian Amazonas region. It must be differentiated between P.1 and P.2 lineage which is called 'B.1.1.28.2' originated from Rio de Janeiro. P.2, unlike P1 it has E484K mutation. P1 and P2 are similar in key mutations, although they arose independently (Toovey *et al*., 2021).

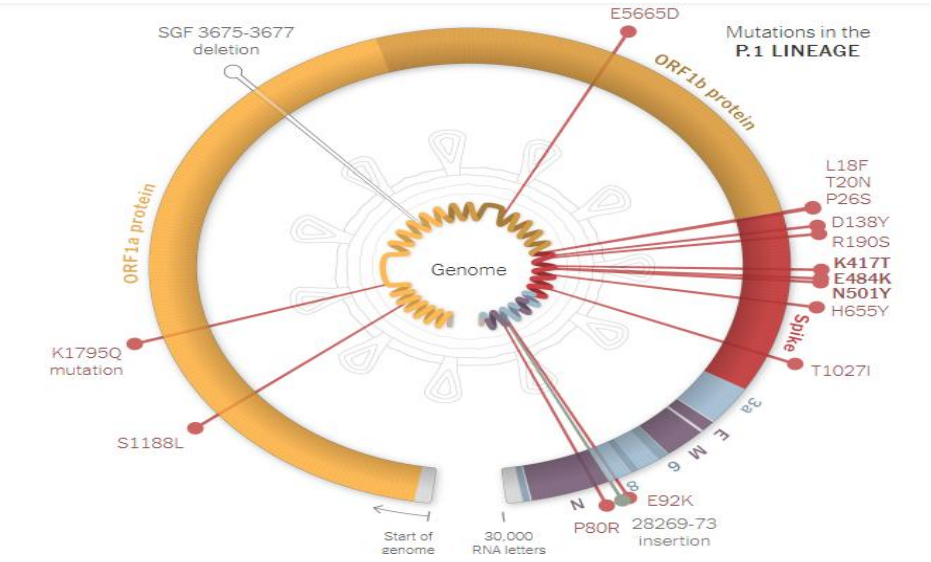

Fig. 3: Mutation in the P.1 lineage (Toovey *et al*., 2021).

Lineage B.1.429

Epsilon Lineage B.1.429, called CA VUI1 or CAL.20C (Alaa *et al*., 2021), the spike proteins S-gene of this variant has five distinct mutations (I4205V and D1183Y in the ORF1ab-gene, and S13I, W152C, L452R. where the L452R appeared earlier in unrelated lineages. The transmission of B.1.429 is likely to be more than others, however, further investigation is needed to manifest this (Zhang *et al*., 2021). This variant is listed as "variants of concern," by CDC. It was first observed by scientists in the united states, showing a 20% increase in transmissibility of variants mean to have a significant impact on neutralization by antibodies (Shen *et al*., 2021).

Lineage B.1.525

Public Health England (PHE) called Eta Lineage B.1.525. It as VUI-202102/03 it carries E484K- mutation that found in P.1, P.2, and 501.V2, the san AH69/AV70 deletion in positions 69 and 70 of amino acids histidine and valine. This variant is different in terms of carrying F888L mutation. The S2 domain of the spike protein has the replacement of phenylalanine (F) with leucine (L). As well as the E484K-mutation. B.1.525, detected in 23 countries, by March 5, including Australia, Spain, and the US (Cullen, 2021).

Lineage B.1.617

This Kappa variant is also a “variant of concern” after B.1.1.7, B.1.351, and P.1. It appeared in October 2020 from the state of Maharashtra in India (Cherian *et al*., 2021). Its receptor-binding domain (RBD) of the spike protein comes with two important mutations, the E484Q and L452R. these mutations may lead to increase angiotensin-converting enzyme-2 (ACE2) receptor binding. As result, the transmission capability of this variant is enhanced and the disease is greatly spread. Further, the effect of mutations leads to reduced binding to the selected monoclonal antibodies which reflect in aiding immune escape and conferring increased virulence (Cherian *et al*., 2021).

B.1.617.1, B.1.617.2, and B.1.617.3 are the three sub-lineages of the variant. The first two lineages charged the second wave of the pandemic in India, and together account for around 70% of the SARS- CoV-2 genomes sampled in the country, according to the Data from the Global Initiative on Sharing All Influenza Data (GISAID) database. The B.1.617.2 variant with its mutations like E484Q, L452R, and P681R tend to be more important as its prevalence jumped from 1% to 70% in two months (Shu *et al*., 2017). By late February 2021, these variants are widely been in circulation among various population groups worldwide (Cherian *et al*., 2021).

Mutations of Concern

It is known as the ones that could make the coronavirus more virulent.

The D614G Spike Mutation

At position 614, G (glycine) replaced D (aspartic acid). Since July 2020, D614G could be considered a pandemic variant in Europe through China and the rest of East Asia. (Zhukova *et al*., 2020). In terms of transmissibility, D614G had a moderate effect as PHE confirmed internationally. D614G prevalence globally associated with (anosmia) loss of smell as one of the COVID-19 symptoms. This mutation had a stable protein that has an affinity of binding of the RBD to the ACE2 receptor of epithelial cells (Butowt *et al*., 2020).

The N501Y Spike Mutation

N501Y indicates a change in amino-acid position 501 from asparagine (N) to tyrosine (Y). B.1.1.7, B.1.351, and P.1 lineages and several variants of concern, have the N501Y mutation. The position of this mutation near the tip of the coronavirus spike, likely to change the shape of the protein to be a tighter fit with human cells (Liu *et al*., 2021).

The E484K Spike Mutation

"Eek" is nicknamed for the mutation. At position 484, the glutamic acid (E) is replaced by lysine (K). E484K enhances the capacity of the virus to avoid the immune system by making altering the shape of the coronavirus spike protein and is called an escape mutation as at least one trial has shown. B.1.351 and P.1. had these mutations including B.1.1.7 lineage origin Oregon and Britain (Musafer *et al*., 2022).

The L452R Spike Mutation

At position 452, the leucine (L) is that replaced by arginine (R). This mutation arose independently in multiple lineages, including B.1.617 and B.1.429. this mutation is improved ACE2 receptor binding ability and causes a reduction in attached antibodies on this mutant, so it will fold differently spike protein. L452R, capable of making the coronavirus resistant to T cells (Tchesnokova *et al*., 2021).

The P681H Spike Mutation

In January 2021, novel SARS-CoV-2 variants have a significant characteristic feature that appeared in Nigeria (B.1.1.207) and U.K. (B.1.1.7), causing enormous frequency worldwide, as what occurred to 'D614G' (Maison *et al*., 2021). For effective serology tests, the following series of questions in need of further examination? (Siddell *et al*., 2019). Sensitivity, specificity, reproducibility, specimen collection timeline, state of disease, and when rapid diagnostic tests (RDTS) are appropriate.

Protocol of Diagnosis and Tests in an Iraqi hospital

Virus Corona's tests and its antibodies, there are four types of tests: two types of tests: antibodies (Abs) including Chromatography, Enzyme-linked immunosorbent assay (ELISA), Fluorescent and PCR-Ag. The first test; is Chromatography and also called rapid test that depends on antibodies against the virus in the blood. IgM, can be found in the infected body after three to seven days of infection and persist till two weeks and be symptomatic, the positive of IgM appear in the acute phase then disappear. IgG; start after one week from infection and peaked after three to four weeks, then starts to decline gradually from two to three months. The false-positive result means IgM and IgG may be related to another infection. It is similar to IgM in immune response however, it delays apparency in blood as can be seen in table 1 (Rashid *et al*., 2020).

These antibodies cannot be used as infection diagnosis of the virus but it is useful to be rapid and supportive test before PCR and CT scan and operation to detect the virus. The negative results do not mean that there is no infection, antibodies (Abs) levels might be very low in the blood.

Whereas, in positive results, it means that there is infection from 80% to 88%. This could justify why it is required to do the test after 14 days from the infection (Rosanna *et al*., 2020).

Table 2: Explain the test result of Abs with consideration of infection symptoms

IgM	IgG	Cases Results
-ve	-ve	Negative
+ve	-ve	False +ve, Acute infection
-ve	+ve	False +ve, previous infection, Acute or recent infection

Note: positive +ve, Negative -ve

Enzyme-linked immunosorbent assay (ELISA) test

ELISA test sensitivity is about 98.3%, and it depends on the medical history of the patient, and also may cause errors in the people suffering from Lipemic and hemolyzed it use only in particular cases. This test is not recommended for a patient who donate blood recently (Craig *et al*., 2020). This test does not show cross-rection to IgM antibodies. A negative result may be caused by the incubation time of infection, 6% of people do not have Abs in their recovery period as well as the immunocompromised people. Some cases give false-positive results when it is infected with other types of viruses, this method requires 75 minutes to be completed, also, positive samples may need to dilute (Liu and James, 2021).

Fluorescence immunoassay (FIA) test

This test is used in the following cases; early mild asymptotic acute patients. Test restrictions, the sample should be fresh, the blood collecting time should not be exceeding 24hr. people suffering from Lipemic and hemolyzed should not use their serum. Sometimes this test gives false-positive results (Liu and James, 2021).

PCR-Ag diagnosis

The samples are serum, plasma, blood and throat swab, and nasopharyngeal secretion. It recommends using this test after two to seven days from the symptoms. Use in early diagnosis of clinical infection. The fact of SARS-COV2 infection was confirmed by detection of RNA but this method is an accurate 75% because the test's sensitivity depends on collecting of samples and the technician experience, the RNA quantity is sometimes too low for detection. This test is expensive and cannot be used to detect the previous infection therefore we need to detect antibodies (Abs) and antigens (Ags) at the same time. When the patient is symptomatic with negative PCR, the test should be repeated after two to three days. While is the symptomatic patient after ten days, needs to repeat PCR and Abs (Mustafa, 2020).

RESULTS EXPLAINING PCR

Positive mean infection either symptomatic or asymptomatic. A negative result does not refer to no infection for the following reasons; low viral load, a mistake in sample collection and transport. Therefore, we need to consider three main factors (symptoms, PCR, and CT scan). The Biosafety of the worker an isolation unit and PCR lab, they have to take TB vaccine, their blood group should be O not A and they have to take Anti-Corona supplements. Workers need to be checked for infection before starting working in these labs and should wear PPE (Mustafa, 2020).

CT scan is the most supportive procedure for the diagnosis of the infection, where the infection is classified into three types depending on symptoms of cystic fibrosis in the test and its intensity,

high confidence, intermediate confidence, and low confidence. The negative result of a CT scan does not mean no infection, the virus might exist in the pharynx and not reach the lung. Also, blood analysis evaluates the patient status and infection intensity. Radiology tests consider as support procedures for the other tests. As can see in (Table 2,3) this study (Afshar *et al*., 2021). The early diagnoses of covid-19 are very important as mentioned in (Table 4).

Table 3: IgM and IgG presence refers to the active immune response against the virus

IgM	IgG	PCR	Time
-ve	-ve	+ve	Incubation from 0-5 days
+ve	-ve	+ve	Acute infection 5-10 days
+ve	+ve	+ve	Acute infection
+ve	-ve	-ve	False negative
-ve	+ve	-ve	Swab should be repeated after 72hrs to assure recovery
-ve	+ve	+ve	New infection after recovery from the first infection

Note: positive +ve, Negative -ve

Table 4: Clarifies the speed of detection in covid-19 infection, it aids in lowering the infection's intensity and controlling the disease. It is a comparison between two patients at different times (between early and late diagnosis cases from teaching laboratories- Baghdad).

Patient 1 (Diagnoses after 2 days)	Patient 2 (Diagnoses after 7 days)
PCR (+ve)	PCR (+ve)
CT-SCAN 0 % Infected lung	CT-SCAN 45 % Infected lung
CBC Lymph (High) WBC (Within normal)	CBC Lymph (Low) WBC (Low)
D-DIEMER The normal (50 -500) After few days 50 Then 104	D-DIEMER The normal (50 - 500) After few days 250 Then 610
Serological IgG -ve IgM -ve	Serological IgG +ve IgM +ve
PT PTT INR Within normal	PT PTT INR Within normal

Note: Polymerase chain reaction (PCR), computerized tomography (CT), complete blood count (CBC, white blood cell (WBC), prothrombin time (PT), partial thromboplastin time (PTT), international normalized ratio (INR).

CONCLUSION

It could be true that mutations occur naturally, however, they tend to be higher in RNA than DNA viruses. The manifest effect of mutation is making the virus more resistant against the vaccine. Further, these mutations share several characteristics and look similar, the mutation of concern D614G, N5OIY, and E484K. are the ones that responsible for the devastating effect around the world. The complete blood picture, D-dimer, Prothrombin time Test, PCR, X-Ray, and CT scan. All these tests may assist when an accurate diagnosis is required. These tests are done even if the PCR results are negative.

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سلالات فيروس كورونا 2 الأكثر اثارة للقلق والبروتوكول التشخيصي في المستشفيات العراقية– مقال مراجعة

الملخص

المتلازمة التنفسية الحادة الجديدة لـ Coronavirus-2، هي المسؤولة عن الوباء العالمي الحالي. تسببت بظهور متغيرات covid-19 في قارات مختلفة وخلقت قلق كبير على مستوى صحة الإنسان عالمياً. أثرت هذه المتغيرات على العديد من الأماكن حول العالم بما في ذلك الصين وأوروبا والمملكة المتحدة والولايات المتحدة. أكثر المتغيرات المثيرة للقلق هي بيتا (النسب ب 1.351) و إبسيلون (النسب ب 1.429) وكابا (السلالة ب 1.617). سمحت هذه المتغيرات للفيروس بأن يصبح أكثر قابلية للانتقال بين السكان، ولم يتم اكتشافه بسبب وجود عدد كبير من تراكم الطفرات في بروتين السنبلة (S)، خاصة داخل المجال الطرفي الأميني (NTD) ومجال ربط المستقبلات (RBD). عواقب هذه المتغيرات تتمثل بتحفيز الفوعة العالية، وتكرار إعادة العدوى، وزيادة المقاومة للأجسام المضادة وحيدة النسيلة. تبدأ عملية التشخيص في العراق باختبار تفاعل البوليميراز المتسلسل لتأكيد الإصابة، ومع ذلك، إذا كانت النتيجة سلبية مع استمرار الأعراض، يجب اتباع فحص PCR والتصوير المقطعي المحوسب CT، وهو الإجراء الأكثر دعمًا لتشخيص العدوى، تصنف العدوى إلى ثلاثة أنواع اعتماداً على أعراض التليف الكيسي إذا كانت عالية، متوسطة او منخفضة. إجراءات تشخيص العدوى يمكن تلخيصها بوجوب إجراء جميع الاختبارات التشخيصية حتى لو كانت نتائج تفاعل البوليميراز المتسلسل (PCR) سلبية.

الكلمات الدالة: فيروس كورونا 2، تشخيصي، العراق.