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COVID-19 Mutation, A Critical Variable between Infectivity and Toxicity

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Abstract**—** *Many research papers have been published on the correlation between COVID-19 mutation and infectivity. Infectious power refers to the ability of COVID-19 to penetrate host cells. Even if the penetrating power is adequate, toxicity does not necessarily increase. Toxicity refers to the rate of virus proliferation after infiltration. If the rate of proliferation is constant, the more contagious and the more toxic. It is the variation of the Spike gene that determines the infectivity. It is the group of genes involved in viral replication that determines virulence. A mutation is too coincidental, whether it occurs in the spike or replication group. Currently, COVID-19 is mutating both groups simultaneously. However, the spike protein's structural alteration, which weakens the infectivity, has not yet been observed. Instead, recent studies are finding more spike mutations that enhance infectivity.*

Keywords**—** *COVID-19, Spike Protein, Receptor Binding Domain, ACE2 Receptor*.

I. THE PURPOSE OF THIS STUDY

COVID-19 is still pandemic across the globe. Currently, what virologists are paying the most attention to is the COVID-19 mutation. It mainly focuses on the mutation of the Spike gene that binds to the host cell receptor. Scientific analyzes are being conducted to see if mutations in COVID-19 make the transmission stronger or weaker. The final purpose of this study is to find answers to those questions by analyzing some previously published papers on whether COVID-19 mutation is explicitly affecting the infectious power by what mechanism, and if the infectious ability is enhanced, toxicity is therefore improved.

II. MUTATION IN COVID-19

A mutation means a difference; a letter change in the genome. This is a tiny fraction of the total 30,000 characters in the Virus's genetic code and means that all COVID-19 in circulation can be considered part of a single clonal lineage [1]. Mutations are a perfectly natural part of any organism, including viruses. The vast majority have no impact on a virus's ability to transmit or cause disease. Most commonly, mutations will render a virus non-functional or do not affect it whatsoever. Whether it is a plant or an animal, mutation is a process of evolution. Evolution is the way an individual survives in response to its environment. However, mutations are not always beneficial to an individual's survival. Viruses have a higher frequency of mutations compared to eukaryotic cells. Eukaryotic cells have a proof-reading function that changes to the original base when a mutation occurs during DNA replication [1]. However, viruses do not have such a correction function, and thus relatively more mutations occur [1]. Mutations lead to structural alterations of the protein according to the central dogma. Mutation primitively means loss of gene function.

III. NO DIRECTION IN MUTATION

Mutations are harmful to an individual's survival. Randomly occurring mutations interfere with the metabolic function of the individual. However, some mutations may help in individual growth. The potential for mutations to affect the transmissibility of COVID-19 in its new human hosts exists [2]. In the case of COVID-19, mutations that make it easier to penetrate host cells are also possible. Eukaryotic cells mutate in response to changes in the external environment, but viruses mutate even without stimulation of external environments such as antiviral drugs. This is because the replication process by reverse transcriptase in the host cell is not performed very precisely. Viruses have zero risks without host cells. This is because viruses can only multiply within host cells without exception. COVID-19 binds and penetrates ACE 2 receptors in epithelial cells in the upper airways, lungs, and blood vessels [2]. The protrusion planted in the cell wall of COVID-19 binds to the receptor. Its protrusion is composed of Spike protein. Mutation of the Spike gene means the alteration of the three-dimensional structure of the overhang [2]. The change of the protrusion develops in three directions. One is that it becomes easier to bind to the receptor, the other is that it becomes difficult to attach to the receptor, and the other is that there is no effect on binding to the receptor [2]. All of the vaccines being developed recently target Spike protein. For vaccine companies, the change in the Spike portion of COVID-19 is a fundamental issue. This is because it is directly related to the efficacy of the vaccine. Recently, virologists have published many research papers on the mutation of the Spike gene. The Spike part is directly associated with host cell penetration among the components of COVID-19. Even if a point mutation occurs in the gene sequence that makes the Spike part, it is improbable that the Spike part will be difficult to bind to the receptor [2]. From the standpoint of COVID-19, the Spike part is directly related to their survival, and any mutations are evolving so that Spike's avidity is minimally affected [2].

IV. MUTATION IN SPIKE STRUCTURE

Viruses are like a robot. Various substances are assembled into a whole virus. When dismantled into each part, there is no

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risk. The components of the Virus are like parts of a robot. Just as a robot cannot operate with only one element, a virus cannot be replicated with one component. Viruses consist of a genome, a small envelope surrounding the genome, a large and thick envelope, and a protruding part located above the envelope.

The protruding part is called Spike. This plays a significant role in host cell invasion. The COVID-19 Virus is about 30 kb in size or consists of 30,000 bases [3]. Among these nucleotide sequences, it has a code that synthesizes a specific protein at regular intervals. When analyzing the viral genome, it is primarily classified into two categories. One is a group of genes involved in viral propagation, and the other is a group of genes involved in penetration into host cells. Viral survival is only related to the latter group of genes. Viruses inevitably mutate during replication. This is because there is no gene repair route.

Nevertheless, the Virus remains alive because the Spike structure that binds to the host cell's receptor remains consistent [3]. Even with a point mutation, the Spike protein structure does not change to a level where it cannot bind to the receptor. Some virologists predict that as the number of mutations in the Spike gene increases, the binding power will decrease [1]. Still, recent studies have reported that mutations lead to increased critical power [1,2,3].

V. The Transmissibility vs The Toxicity over COVID-19

The toxicity and infectivity are different. Toxicity refers to a virus's ability to replicate, and infectivity refers to its ability to penetrate host cells. Toxicity is associated with necrosis or apoptosis of host cells. The infectivity of a virus cannot be defined in one sense. Some scholars view the penetration of host cells as an indicator of infectivity [4].

In contrast, others view the growth rate after penetration into host cells to indicate infectivity [4]. Some scholars see both penetration and growth rate as indicators of infectivity [4]. An accurate understanding of infectivity is necessary for the development of therapeutic agents. The vaccine under development is aimed at stopping penetration. Ramdesivir, used as a substitute for COVID-19 treatment, inhibits the growth of the Virus [4]. Based on one infected patient, as the infectivity increases, the number of viruses entering the host cell increases. The number of viruses that proliferate inside the human body increases proportionally. Increasing the number of viruses means many host cell necrosis or apoptosis [5]. Therefore, infectivity has a significant effect on toxicity. HIV is a representative virus whose replication capacity has declined over time, and its toxicity is weakened. There is a possibility that the replication ability will decrease if COVID-19 is also mutated. Viral toxicity does not mean mere cell destruction [5]. When a virus infects host cells, the cytoskeleton collapses, and the expression of membrane proteins decreases, causing the host cells to escape from the matrix [5]. Host cells that have deviated from the matrix fuse with each other and do not function properly [5]. Host cells must metabolize non-stop for survival. However, the production of proteins necessary for viral proliferation interferes with the normal metabolism of host cells. Eventually, the host cell dies of lack of energy as long as the Virus continues to multiply.

VI. The Double Edge of Mutation, Infectivity, and Toxicity

Recently, attention has been paid to mutations in the spike protein of COVID-19 that binds to the host cell receptor (ACE 2) [1]. Spike gene mutation means a change in the conformational structure of a protein. There are three directions of mutation of the Spike gene [6]. A neutral mutation does not affect the host cell and avidity, a friendly mutation that increases the host cell's affinity. A non-affinity mutation decreases the host cell's affinity [6]. However, no one knows the direction of the mutation. The presence of a point mutation in the Spike gene does not directly affect the binding force. Under the condition that the host cell receptor structure is constant, the spike gene mutation changes the Spike protein's conformational structure. As a result, infectivity may increase or decrease. Recently, research on how mutations in the Spike gene affect human infectiousness is ongoing. The infectivity of COVID-19 depends on the Spike protein's binding capacity, but toxicity is related to the rate of replication. Although the penetration power is good, the toxicity is weak when the growth rate is low. Even if the penetrating power is terrible, the toxicity is vital if the growth rate is fast. Assuming that the growth rate is constant, toxicity, and penetration are proportional. Assuming that the penetrating power is the same, toxicity is proportional to the status of growth. The virus replication inside the host cell is very similar to the automobile factory's assembly process [6]. The engine corresponds to the dielectric, and the body corresponds to the shell. However, the assembly sequence is also the same. The car builds the body and puts the engine in it. Viruses make an envelope that wraps around the RNA mass and replicates the RNA.

In other words, some genes are involved in the process of viral replication. First, they make a long protein fact called OFR [7]. Then, cutting this protein into smaller proteins occurs and creates a membrane structure [7]. Then, RNA replication is performed [7]. Many genes involved in viral replication are also inevitable for mutation. Some mutations do not affect the rate of viral growth, and mutations slow the growth rate. When mutations in some of the gene groups participating in the proliferation process occur, the proliferation process is disturbed due to the alteration of the protein's conformational structure [8]. No mutation accelerates the growth rate. However, there is a difference in the growth rate depending on the infectivity. Viruses with strong infectivity quickly infiltrate many host cells and receive sufficient nutrients for proliferation [7].

A small number of viruses enter a host cell, the growth rate increases. However, when many viruses infiltrate one host cell, it is challenging to supply nutrients necessary for proliferation on time, thus slowing the proliferation rate. In the end, based on one infected patient, the rapid infectivity induces a rapid increase in the Virus. Among Spike genes, RBD mutation has a significant influence on the Virus's

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penetration into host cells [1,4]. The 614th base of the Spike gene is originally Aspartic Acid(D) [9]. However, when a point mutation occurs, Glycine(G) is replaced at position 614. "The original spike protein had a 'D' at this position, and a 'G replaced it,' this mutation makes the protein more functional and more efficient at getting into cells [4,9]. The recent epidemic of COVID-19 is the 614G virus [9]. Some scholars have reported that the 614G mutant virus is more infectious [1,4,9]. They found that the mutated Virus not only replicates about ten times faster -- it's also much more contagious [9].

The D614G strain replicates faster and is more transmissible than the Virus, originating in China, spreading at the beginning of the pandemic [10]. An amino acid change in the COVID-19 spike protein gives coronaviruses their characteristic crown-like projections and allows it to attach to host cells [10]. This single character change in the viral genome – termed D614G – has been shown to increase virus infectivity in cells grown in the lab, though with no measurable impact on disease severity [10]. The D614G virus outcompetes and outgrows the ancestral strain by about 10-fold and replicates extremely efficiently in primary nasal epithelial cells, which are a potentially important site for person-to-person transmission [10].

Glycine is more exposed to the solvent than Aspartic acid, though, between them, Aspartic acid is more water-soluble than Glycine. Glycine is hydrophobic and non-polar, but in fact, has a positive charge. Aspartic acid is hydrophilic and has a negative charge. When the host cell's ACE2 receptor's polarity is negatively charged, the Glycine Spike improves its binding ability [4,10]. However, further studies are needed on the infectivity of the 614G mutant virus. Researchers believe the D614G strain of coronavirus dominates because it increases the spike protein's ability to open cells for the Virus to enter [10]. These crown-like spikes give the coronavirus its name. The D614G mutation causes a flap on the tip of one Spike to pop open, allowing the Virus to infect cells more efficiently and creating a pathway to the Virus's vulnerable core [11]. With one flap open, it's easier for antibodies -- like the ones in the vaccines currently being tested -- to infiltrate and disable the Virus [11]. Several genes are involved in the replication process of viruses in host cells. They are responsible for efficient replication and protein synthesis to increase virulence. Currently, viral treatments aim to block some genes involved in the replication process. For example, even if the action of a restriction enzyme that cuts a long protein is blocked, the Virus's replication process is stopped. The COVID-19 mutation could be interpreted in two respects. One is a spike gene mutation that affects penetration, and the other is a mutation of a large number of gene groups involved in viral growth after penetration. The latter group's genetic variation is directly related to the rate of viral growth. When mutations in Spike Protein and genetic mutations involved in proliferation occur in the direction of lowering infectivity, vaccines are no longer needed [12]. However, this is a low probability.

VII. Spike Mutation, Weak Glycosylation

The Spike protein is composed of two functional subunits, S1, and S2 [1]. S1 contains a receptor-binding domain (RBD) and is responsible for the initial attachment of the Virus to the surface of host cells, and S2 is responsible for membrane fusion that triggers entry of the Virus into the host cells [9]. Each Spike protein is extensively glycosylated with host-derived glycans [13]. The Virus acquires glycosylation by hijacking the host cell's glycosylation machinery during the replication process [13]. Therefore, the Spike protein has its unique glycosylation sites and carries the host cells' signature glycosylation patterns [13]. Glycosylation on the Spike protein helps to increase the stability and solubility of the protein [13, Table 1]. More importantly, glycosylation serves to camouflage the immunogenic epitopes on the protein, enhancing the Virus's ability to evade the host immune response [13]. The Spike protein is also a principal target for vaccine development. Variation in COVID-19 spike proteins has been described as stable nonsynonymous substitutions. Some researchers compared the D614G mutant to the wild type, predicts for increased probability of protein glycosylation at residue 616 of the viral spike protein [14]. But, stable nonsynonymous spike protein mutations of COVID-19, including a stable nonsynonymous R408I variant in the receptor-binding domain, predict decreased viral binding and reduced virulence [14]. The binding of glucose to the Spike protein is called glycosylation. Glycosylation causes structural changes in the Spike protein [13]. Several of these mutations are predicted to increase or decrease protein glycosylation [13]. Spike gene mutations develop in two directions. Some mutations lead to an increase in glycosylation, and some mutations result in a decrease in glycosylation [13]. When the Spike gene mutation reduces glycosylation, host cell penetration becomes difficult. Decreased glycosylation of the protein spike of COVID-19 should reduce viral binding to ACE2 and reduce viral uptake by host cells. This should result in a decrease in viral virulence and possibly attenuation.

TABLE 1.

Spike Protein	Wild type	D614G Mutant
Glycosylation	Decreased	Increased
Stability	Decreased	Increased

VIII. Mutation Frequency and Proliferation

Around March 3, 2020. At the time of the first COVID-19 outbreak, some scholars interpreted the COVID-19 mutation to be very rapid [5]. As of Sept. 2020, many experts interpret the COVID-19 mutation as very slow compared to other prevalent viruses [15]. COVID-19 mutates reasonably slowly for a virus, with any lineage acquiring a couple of changes every month, two to six-fold lower than the number of mutations acquired by influenza viruses over the same period [16]. The higher the frequency of viral mutations, the weaker the infectivity of the Virus in many cases. HIV is representative. However, not all viruses have this pattern. Currently, on a global basis, a vaccine against COVID-19 is being developed. They are making a vaccine on the premise

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that the mutation of COVID-19 is slow. The interest of companies developing vaccines is focused on the Spike protein of COVID-19. In particular, it is a Receptor Binding domain that binds to the ACE 2 receptor. Because RNA-type COVID-19 does not have a gene repair system, there is no means for post-correction of base alterations after the replication process [17]. As the number of copies increases, the number of mutations increases.

The slow COVID-19 mutation means that it maintains its ability to bind to host cells [18]. The Spike protein of COVID-19 is a protruding mass planted in the viral cell wall. We should pay attention to the number of Spike proteins produced in a single COVID-19 virus. The number of Spike proteins in a virus and its infectivity are proportional. If the COVID-19 mutation causes an increase in the number of Spike proteins, the mutation is fatal to a human [19]. The more Spike in one Virus, the easier it is to bind to the ACE2 receptor. Based on a single COVID-19, the number of Spike proteins is more lethal to humans than Spike protein structure changes. Although point mutations in the Spike gene change one amino acid (e.g., from Aspartic acid to Glycine). Frame-shift mutations develop in the direction of increasing or decreasing the number of spike proteins [20]. For COVID-19, if the number of Spikes decreases, it is fatal.

On the other hand, if the number of Spikes increases, it is fatal to humans. The number of mutations in COVID-19 depends on the health status of the infected patient. From a virus standpoint, compared to the healthy group, it is more prone to attack for the underlying disease group with reduced immunity. COVID-19 can replicate faster in patients with an immunocompromised state eventually, as many mutations occur as well [21]. For patients with underlying diseases or taking immunosuppressive drugs, the evolution of the Virus will accelerate [21]. Even if antiviral medications are administered, the number of COVID-19 may be temporarily reduced, but the number is maintained by repeating growth again. As much as that, the chances of cloning increase, and the number of mutations increases. In the end, the attack from COVID-19 is fatal for groups with weakened immunity [21]. According to a case report from physicians who recently treated COVID-19 patients, Spike genes account for 13% of all virus genes and 2% of receptor binding sites [22]. They observed a 57% change in the spike gene and 38% in the receptor-binding site [22]. The COVID-19 Virus mutates rapidly in the body of the infected person and resurrects again and again. This case shows that repetitive viral increases and rapid viral mutations associated with decreased immunity can occur.

IX. Conclusion

Around the time of the COVID-19 mutation, the Spike mutation influences infectivity, and the replication gene mutation affects the rate of proliferation. Replication gene mutations and Spike mutations occur frequently. But there are no mutations that significantly affect infectivity and proliferative power. It means that the contagious and toxic COVID-19 is still strong. However, the safety of Spike's structure is of great help to vaccine efficiency. Like HIV in the past, it is challenging to expect attenuation of the toxicity caused by mutations in COVID-19 [23].

Disclosure

The authors declare that there are no conflicts of interest concerning this study.

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