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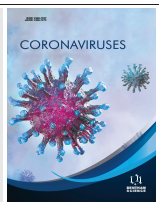
RESEARCH ARTICLE



High Mutation Rate Leads to Fitness Loss for Coronavirus Quasispecies

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Abstract: ***Background*:** RNA viruses evolve very fast, with a mutation rate of 10^3 to 10^5 base substitution per nucleotides per copy. The mutation is a survival strategy for the viruses, which leads them to survive in the new host. Fitness is defined as the replication capacity of the virus in an experimental setup. Generally, the large population passage of the virus leads to fitness gain, but the world data of the coronavirus infection and death shows the flattened curve with time. It is contradictory to the principle of fitness gain due to large population passage. The coronavirus is losing its potency but remains infectious as it is passaging into millions that leads to a decline in the death of COVID patients and high recovery rates. Fitness loss of coronaviruses attributed to a high level of mutation in the RNA genome as well as host immune response. The current outbreak of SARS CoV-2 is surfaced in December 2019 in Hubei province of China and considered as bats/pangolin origin, spreading 235 countries of the world, infecting nearly 31,664,104 people, and claimed nearly 972,221 lives as of September 24, 2020 (Death rate approximately 3%). This coronavirus has passaged into 31,664,104 people from the beginning of this pandemic until September 24, 2020. Now the virus is losing potency rather than being monotonous and continuous in producing virus-related complications. The population is still getting infected at the same rate, but the severity of the disease is reduced due to the potency of the virus diminished due to the passage effect as well as fitness loss of the virus due to high mutation rates. The death rate is reduced to 3% as compared to 6% in June 2020, when this paper was first submitted.

***Objective*:** The purpose of the study is to prove the fact that the coronavirus loses its potency with time but, they remain infective. It becomes more infectious due to mutation of the gene but loses the capacity to kill the host.

***Methods*:** Since the WHO announces the COVID-19 outbreak is an emergency of international concern, every country in the world is taking many measures to mitigate the viral load to their population. Simultaneously, the WHO, CDC USA, CDC Europe, and much other organization is updating the COVID cases and death online daily as reported by the respective country. With the help of the COVID-19 outbreak data published by the European CDC and ourworldindata.org, we correlate the total cases of coronavirus and total death in the top ten affected countries in the world. We also link the trends of total cases *vs.* total death and total new cases *vs.* total new death related to COVID-19 in Germany, Spain, the United Kingdom, Italy, and New Zealand from January 30, 2020, until September 24, 2020. The reason to select these countries for the study is that these countries updating the COVID cases and deaths regularly and said to achieve the peak of COVID related infections and recovering from the pandemic.

***Results*:** We have tried to correlate the high mutation rate of the virus that leads to losing its potency to severe infection and death in the human. Viral extinction through high mutation could be considered as the new anti-viral strategies.

***Conclusion*:** Coronavirus is losing its potency to causing death to the human. The new infection is still being reported from every corner of the world, but the death rate is significantly decreasing.

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1. INTRODUCTION

Since the beginning of the 21st century, three major outbreaks of coronavirus have trembled the world. All of these three outbreaksare zoonotic in nature in which the virus crossed the species barrier and infected humans (un-natural host), causing novel pneumonia (Acute to severe Respiratory Distress). The world noticed the first outbreak in 2003 with severe acute respiratory syndrome coronavirus (SARS-CoV), which was bat origin, emerged in Guangdong province, China, and spread across five continents, infecting approximately 9000 people and claimed 774 lives (death rate 9.6%). The second outbreak was detected in 2012 with Middle East Respiratory Syndrome coronavirus (MERS-CoV), which was of camel origin. This pandemic first surfaced in the Arabian Peninsula spread around 27 countries in the world and infected approximately 2500 person and claimed almost 858 lives (death rate 34%) [1, 2]. The third and current outbreak is surfaced in December 2019 with SARS CoV-2 and considered as bats/pangolin origin, emerged in Hubei province of China, spreading almost all the countries of the word infecting almost 6,272,098 people and claimed almost 379,044 lives as of June 3, 2020 (Death rate approximately 6%), whereas on September 24, 2020, 31,664,104 people are infected and 972,221 people are died (Death rate approximately 3%).

The novel coronavirus COVID-19, which induces novel pneumonia in the human is now renamed as SARS CoV-2, which is also known as HCoV-19 [3, 4]. The Coronavirus is an enveloped, pleomorphic virus ranging from 80-120 nm in diameter. It possesses a 5' capped single-strand positive-sense RNA genome of 26.2 to 31.7 kb in length. The virus belongs to the Coronaviridae family, which is divided into three genera (I, II, and III *i.e.* alpha, beta, and gamma coronavirus, respectively). Group I and III include viruses, whichare mostly animal pathogen. Group II also includes viruses thatare veterinary relevant, but OC43, HKU1, SARS-CoV, and COVID-19 are human pathogen and can get entry to humans through the cellular receptor called Angiotensin-Converting Enzyme 2 (ACE2) (Table **1**). The binding of the viruses to the host cell receptor and fusion with the cell membrane is considered as the first step of viral infection. The lungs epithelial cells are the primary target of the virus. Research on SARS-CoV suggests that the transmission of the virus occurs by binding virus spike proteins to a cellular receptor, which is identified as Angiotensin-Converting Enzyme 2 (ACE2) receptor. ACE2 is a type 1 integral membrane protein, profusely found in the lung alveoli. It is a mono carboxypeptidase, which hydrolyses angiotensin II [5]. The genomic sequence of receptor-binding domain of COVID-19 spike (type I glycoprotein) resembles that of SARS-CoV [6].

Hence, this finding strongly indicates that the entry of the COVID-19 to the lungs epithelial cells host occurs through the ACE2 receptor. On the other hand, the genomic sequence of COVID19 is 50% similar to MERS-CoV and more than 80% similar to SARS-CoV [7, 8], both viruses originated from bats. Likewise, the open reading frame on gene 8 also signifies the COVID-19 virus of bats origin [9]. The Phylogenetic analysis indicates that the COVID-19 belongs to the group II (beta coronavirus) genus, which includes SARS-CoV, and can infect humans, bats, and wild animals [10, 11]. The viral entry to the host is commenced by the interaction of spike protein and the host's lung epithelial cell. The mechanism of pathogenesis needs to be established for COVID-19/ SARS CoV-2. It can be divided into three phases (i) Replication of virus (ii) Hyperactivity of immune system (iii) Destruction of pulmonary alveoli [12]. The spike proteins facilitate the receptor (ACE2) binding as well as fusion with the cell membrane. The spike protein of coronavirus is a class I fusion protein that is assembled in trimers on the virion surface, which give them a "crown" like appearance [13], hence named as "corona". The SARS CoV-2 target is Peripheral Blood Mononuclear Cells (PBMCs), where they replicate themselves inside [14]. The Peripheral Blood Mononuclear Cells (PBMCs) are a group of immune cells that play a key role in providing immunity to the host. The PBMCs originate from Hematopoietic Stem Cells (HSCs) in the bone marrow. The average cell fraction present in the PBMCs is listed in Table **2**. SARS CoV-2 infection leads to lymphopenia, specifically a decrease in T cells and B cells. Evasions of the immune system and lack of IFN α, IFN β, IFN γ responses leads to increase viral load in the patient during the initial ten days. Exaggerated immune response, no significant upregulation of MHC class I, and apoptosis of uninfected lymphocytes are the contributors of acute lymphopenia, severe respiratory distress, and death. Although B cell and T cell do not bear ACE2 receptor they still get infected and die due to direct virus contact [15].

Mutation is a survival strategy for the viruses.The coronavirus has mutated over time. The virus does mutate to survive in the community, and they are interested only in their survival.Becoming more infective is one of the ways to survive and achieve high viral fitness but not necessary to achieve potency to kill the host. According to Dr. Bassetti, San Martino hospital, Italy, the virus was an aggressive tiger in March and April and now become a wild cat, who can infect but not kill. Now the patient with 80 years and above age also sitting on the bed and breathing without a ventilator support system, whereas they were dying in 3 days upon getting infected in March and April [16]. The data shows that the fitness of the coronavirus to infect increased with time, as more number of infection is being reported, whereas the fitness to kill is decreased with time as the data shows, the recovery rate of the patients is more than 90%. The world has 152 recorded 32,730,945 coronavirus cases and 991,224 deaths since the pandemic 153 until September 28, 2020.

The purpose of the study is to prove the fact that the coronavirus loses its potency with time 155 but, they remain infective or become more infectious due to mutation of the gene but loses 156 the capacity to kill the host.

Table 1. Coronavirus family, hosts, diseases, and receptors.

Group	Virus	Host	Receptor	Disease	References
I – Alpha coronavirus	NL-63	Human	ACE2	Respiratory Infection	[17]
	229E	Human	Human APN	Respiratory Infection	[18]
	TGEV, PRCoV	Pig	Porcine APN	Respiratory and enteric infection	[19]
	FeCoV, FIPV	Cat	Feline APN	Respiratory, enteric, and neurologic infection, and hepatitis	[19]
II – Beta coronavirus	OC43	Human	Neu5,9Ac2containing moiety	Respiratory infection and possibly enteric infection	[20]
	HKU1	Human	Neu5,9Ac2containing moiety	Respiratory infection	[19]
	SARSCoV	Human	ACE2	Severe acute respiratory syndrome	[5, 6]
COVID – 19 / SARS CoV-2	Human	ACE 2	**Respiratory;** Acute Respiratory Distress Syndrome, Pneumonia, Rhinorrhoea, Sore throat **Systemic** High WBC count Increase ESR Increase Inflammatory cytokines – IL2, IL7, IL10,TNFa Fever, Cough, Fatigue, Hemoptysis, Cardiac injury, Hypoxemia, Dyspnoea Diarrhoea	[5, 6]	
	BCoV	Cow	Neu5,9Ac2containing moiety	Enteric infection	[21]
III – Gamma coronavirus	IBV	Chicken	Not Determined	Respiratory infection, hepatitis	[22]
	Turkey corona virus	Turkey	Not Determined	Respiratory and enteric infection	[22]

Table 2. Correlation Analysis of COVID cases in Germany; A. Correlation analysis of initial 28 days starting when the 1st death reported B. Correlation analysis of overall data since WHO declared the outbreak as an emergency condition for international concerns.

A					B				
-	**Total Cases**	**Total Death**	**New Cases**	**New Death**	**-**	**Total Cases**	**Total Death**	**New Cases**	**New Death**
Total cases	1.000	-	-	-	**Total cases**	1.000	-	-	-
Total death	0.954	1.000	-	-	**Total death**	0.083	1.000	-	-
New cases	0.752	0.579	1.000	-	**New cases**	0.975	-0.084	1.000	-
New death	0.944	0.915	0.742	1.000	**New death**	0.066	0.597	-0.051	1.000

1.1. Host Receptor Adaptation and Evolution of SARS CoV

Coronavirus, Ebola, Nipah are transmitted by the bats, lead to the outbreak several times in the recent past [23]. The bats and snakes are considered as the natural reservoirs of these viruses [24]. The virus outbreaks are attributed to the viral species jump from reservoir animals to humans. The Influenza type A virus has jumped many times in the past from their reservoir host; birds and pigs, to humans [25]. The human Immunodeficiency virus is also acknowledged being jumping from primates to humans [26]. Similarly, the coronavirus SARS CoV-1 and SARS CoV-2 transmission were also attributed to be an inter-species jump from bat to human in the year 2003 and 2019 [27-30]. The SARS CoV-2 belongs to the beta coronavirus family, which is considered to be transmitted to a human directly from the bats or *via* an intermediate host such as; minks and pangolins [31, 32]. Earlier studies suggest that ancestor SARS CoV was not capable of binding ACE2 [33], but later it acquires the capability of binding to ACE2 due to the plasticity of the spike glycoproteins. The surface spike protein undergoes rapid evolution during epidemic or pandemic situations [34]. Surface glycoprotein recombination and adaptation are considered as the reason for interspecies transmission from the reservoir to the human. The spike protein of coronavirus and host's receptor (ACE2) interaction is considered as the important elements for regulating interspecies transmission and

tissue tropism [35]. Studies also suggest that the SARS spike proteins can recognize the ACE2 receptors of bat, civet, mouse, and raccoon dog for docking and entry leads to interspecies transmission [36-38]. Hence, these animals can also be the carrier of the virus. The spike protein of the coronavirus is incredibly plastic; it can accommodate the addition or deletion of 681 nucleotides without losing its docking and entry capabilities to the ACE2 receptor of lung epithelial cells [39-41].

1.2. Adaptation in Serial Passages and Fitness of Viruses

The viruses are completely depending on the host cell machinery such as surface proteins, translation factors, ribosomes, and Golgy apparatus for their replication and distribution of their progeny. Together with the immune resistance of the host, the high rate of mutation also affects the fitness of the virus to replicate and remain infectious. The coronavirus is an RNA virus and studies suggest that the virus-containing RNA genome evolves very fast, because of the high mutation rate of 10^3 to 10^5 base substitution per nucleotides per copy [42]. Consequently, the virus population becomes the collection of closely related virus species with a non-identical genome called quasispecies [43]. The term quasispecies was first introduced by Eigen, Schuster and their team in 1979 [44]. The quasispecies refers to an RNA virus that has the mutation rate of 10^5 in their genome, hence, the clones and their progeny of the virus population consist of different but related genomes. Therefore, quasispecies is the RNA virus, which is the mixture of mutated clones and their progeny. The mutation makes them adaptable to survive with increased fitness [45]. The diversity in the RNA genome of RNA viruses allows rapid evolution and resistance to host immune system as well as antiviral drugs [46]. Holland and Martinez suggested that the fitness of the RNA viruses change and adapt very rapidly to the new host [47, 48]. The quasispecies of virus evolves due to continuous mutation whose fitness depends on the environment they are replicating [49]. The decreased potency of the virus is attributed to the changing virus population as well as the immunity of the host. Upon 50 rounds of plaque to-plaque passage of an RNA virus FMDV (Foot and Mouth Disease Virus) in a repeated genetic bottleneck leads to fitness decrease [50]. The authors observed FMDV loses its capacity to produce infectious viral progeny as well as a decrease in fitness of the virus through repeated the bottleneck, which leads to FMDV clones' extinction. Viral extinction through high mutation could be considered as the new anti-viral strategies.

2. STUDY DESIGN

We refer to the COVID data provided by the CDC Europe published by ourworldindata.org [51] online until September 24, 2020. The graph is plotted for the total no of cases *vs.* total death and new cases *vs.* new death in the top ten affected countries in the world (Fig. **1**). Furthermore, the individual graph is also plotted for the individual country such as; Germany, Spain, United Kingdom, Italy, and Turkey for the same parameters. These countries are said to achieve the peak of the COVID-19 infection. Fig. (**2**) and Table **2** represent the COVID-19 data analysis for Germany. Fig. (**3**) and Table **3** explain the trends in total no of cases *vs.* total death and new cases *vs.* new death for Spain. Similarly, Fig. (**4**) and Table **4** represent the United Kingdom, Fig. (**5**) and Table **5** for Italy, and Fig. (**6**) and Table **6** represent Turkey for COVID-19 related infection and deaths. Furthermore, we calculated the correlation analysis of the available data using Microsoft Excel by Spearman's Rank Correlation method. This method is a non-parametric method of correlation estimation. It is used to determine the correlation between the ranks of different variables. The degree of correlation is presented as +1, -1, and 0 for Perfect Positive, Perfect Negative, and Uncorrelated, respectively. When the value lies between 0.50 and 0.75, the degree of correlation is high, and when it lies between 0.25 and 0.50, the degree of 231 correlation is low.

3. RESULTS AND DISCUSSION

The trend of total no of cases *vs.* total death and new cases *vs.* new death in the top ten affected countries shows the flattened curve (Fig. **1**). The COVID cases and related death is increasing exponentially until April 2020, later it shows the steady growth in infection and deaths. The infection is not equal to death. The rate of death is decreasing compared to COVID infection since April 2020. The COVID data of Germany start showing the decreasing trends in new coronavirus infection and deaths since May 3, 2020, when for the first time, only 793 new cases are reported in one day, with 74 deaths (Fig. **2**). Later these trends are decreasing continuously. The correlation analysis table also supports this data, where we can observe that the correlation value of total new cases and total new death in the initial 28 days are 0.75 and 0.57, whereas, the overall correlation value until September 24, 2020, shows the total new cases 0.97 and total new death value of -0.08 (Table **2**). Observing the data from Spain, (Fig. **3**), the new cases and new death start decreasing since May 9, 2020, when 743 new cases and 227 deaths are reported in one day for the first time pandemic begins. Later this trend is continuously decreasing until today and the Govt. planning to lift the lockdown sanction in stepwise manners. The correlation analysis table also supports this data, where we can observe that the correlation value of total new cases and total new death in the initial 28 days are 0.86 and 0.75, whereas, the overall correlation values are 0.88 and 0.22 Table **3**. The COVID data of the United Kingdom start showing the decreasing trends in new coronavirus infections and deaths since June 2, 2020, when for the first time, only 1570 new cases are reported in one day with 556 deaths (Fig. **4**). Now, these trends are constantly decreasing, and lockdown is being relaxed. The correlation analysis table also supports this data, where we can observe that the correlation value of total new cases and total new death in the initial 28 days are 0.98 and 0.95, whereas, the overall correlation values are 0.98 and 0.19 (Table **4**). Observing the data from Italy, (Fig. **5**), the new cases and new death start decreasing since May 14, 2020, when 888 new cases and 195 deaths are reported in one day for the first time pandemic begins. Later this

trend is continuously decreasing until today and the Govt. planning to lift the lockdown sanction in stepwise manners. The correlation analysis table also supports this data, where we can observe that the correlation value of total new cases and total new death in the initial 28 days are 0.98 and 0.96, whereas, the overall correlation values are 0.99 and -0.09 (Table **5**). Last but not least, the COVID data of Turkey start showing the decreasing trends in new coronavirus infection and deaths since May 21, 2020, when for the first time, only 972 new cases are reported in one day with 23 deaths (Fig. **6**). Now, these trends are constantly decreasing and lockdown is being relaxed.

Table 3. Correlation Analysis of COVID cases in Spain; A. Correlation analysis of initial 28 days starting when the 1st death reported B. Correlation analysis of overall data since WHO declared the outbreak as an emergency condition for international concerns.

A					B				
-	**Total Cases**	**Total Death**	**New Cases**	**New Death**	**-**	**Total Cases**	**Total Death**	**New Cases**	**New Death**
Total cases	1.000	-	-	-	**Total cases**	1.000	-	-	-
Total death	0.981	1.000	-	-	**Total death**	0.469	1.000	-	-
New cases	0.862	0.756	1.000	-	**New cases**	0.880	0.227	1.000	-
New death	0.983	0.943	0.890	1.000	**New death**	-0.048	0.282	-0.060	1.000

Table 4. Correlation Analysis of COVID cases in the United Kingdom; A. Correlation analysis of initial 28 days starting when the 1st death reported B. Correlation analysis of overall data since WHO declared the outbreak as an emergency condition for international concerns.

A					B				
-	**Total Cases**	**Total Death**	**New Cases**	**New Death**	**-**	**Total Cases**	**Total Death**	**New Cases**	**New Death**
Total cases	1.000	-	-	-	**Total cases**	1.000	-	-	-
Total death	0.989	1.000	-	-	**Total death**	0.208	1.000	-	-
New cases	0.980	0.955	1.000	-	**New cases**	0.989	0.197	1.000	-
New death	0.980	0.983	0.974	1.000	**New death**	-0.088	0.832	-0.048	1.000

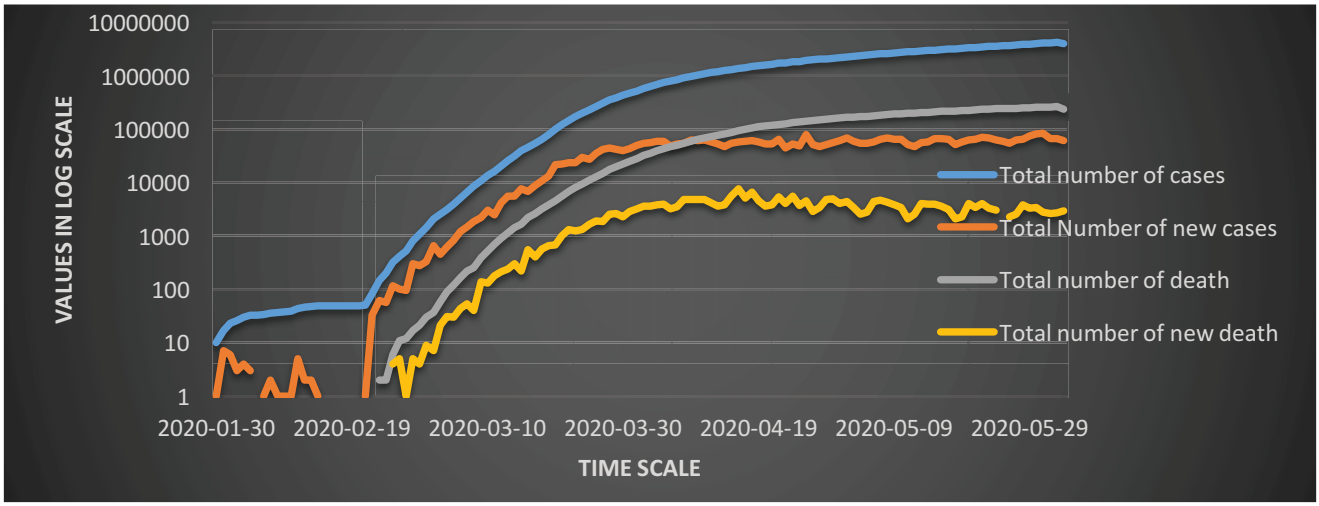

Fig. (1). COVID cases and death for top ten affected countries. The graph is plotted for the total no of cases, total no of death, total no of new cases, and total no of new death with respect to time. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

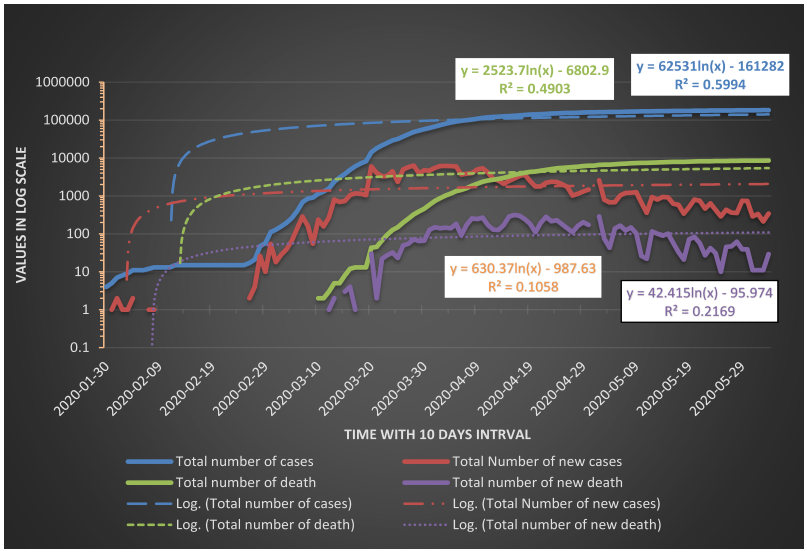

Fig. (2). COVID-19 cases *vs.* death trend analysis and correlation analysis for Germany (Data from 30-01-2020 to 24-09-2020). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

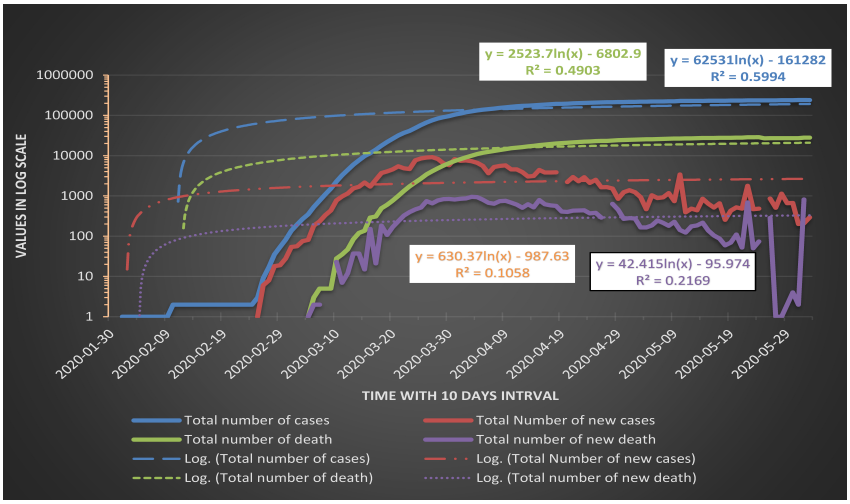

Fig. (3). COVID-19 cases *vs.* death trend analysis and correlation analysis for Spain (Data from 30-01-2020 to 24-09-2020). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 5. Correlation Analysis of COVID cases in Italy; A. Correlation analysis of initial 28 days starting when the 1st death reported B. Correlation analysis of overall data since WHO declared the outbreak as an emergency condition for international concerns.

A					B				
-	**Total Cases**	**Total Death**	**New Cases**	**New Death**	**-**	**Total Cases**	**Total Death**	**New Cases**	**New Death**
Total cases	1.000	-	-	-	**Total cases**	1.000	-	-	-
Total death	0.996	1.000	-	-	**Total death**	-0.032	1.000	-	-
New cases	0.983	0.969	1.000	-	**New cases**	0.995	-0.095	1.000	-
New death	0.980	0.978	0.955	1.000	**New death**	-0.083	0.914	-0.124	1.000

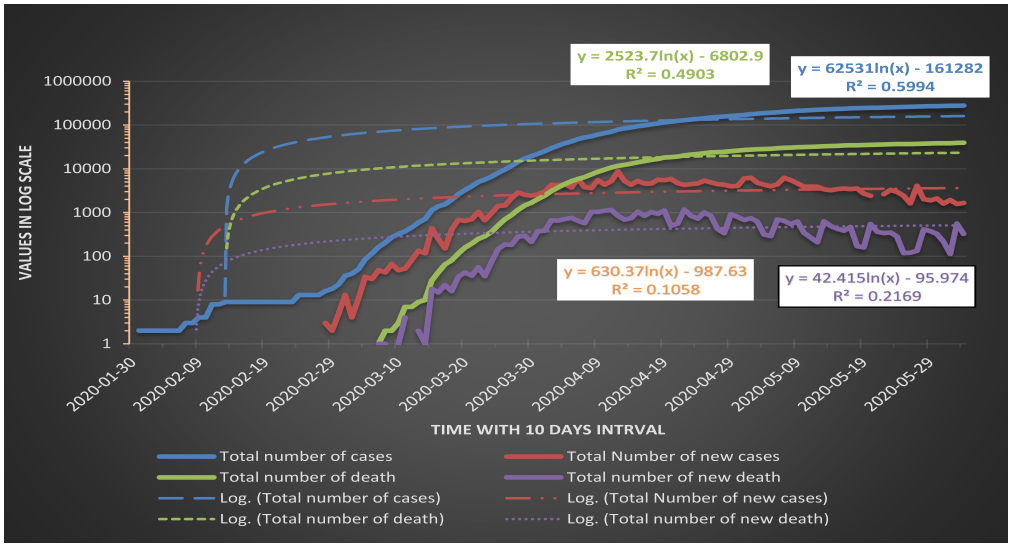

Fig. (4). COVID-19 cases *vs.* death trend analysis and correlation analysis for United Kingdom (Data from 30-01-2020 to 24-09-2020). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

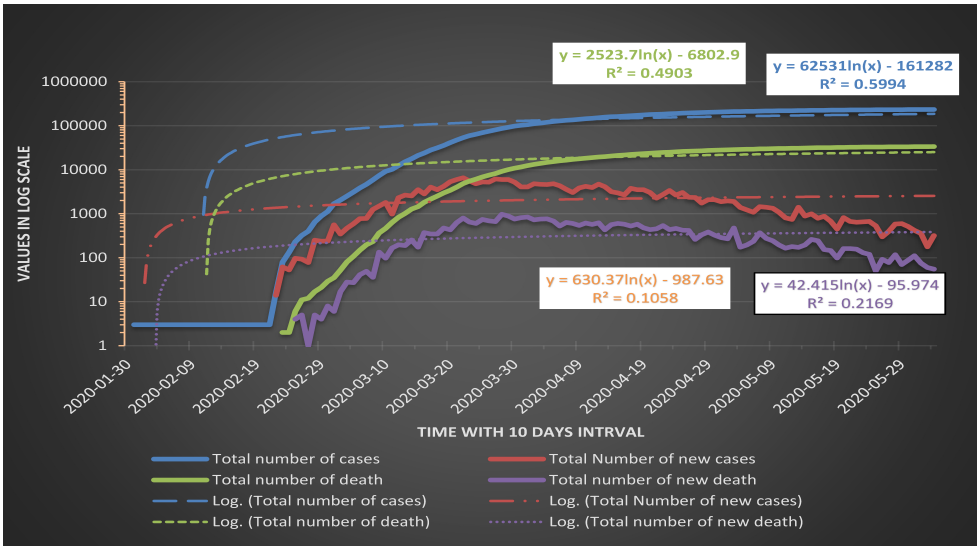

Fig. (5). COVID-19 cases *vs.* death trend analysis and correlation analysis for Italy (Data from 30-01-2020 to 24-09-2020). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 6. Correlation Analysis of COVID cases in Turkey; A. Correlation analysis of initial 28 days starting when the 1st death reported B. Correlation analysis of overall data since WHO declared the outbreak as an emergency condition for international concerns.

A					B				
-	**Total Cases**	**Total Death**	**New Cases**	**New Death**	**-**	**Total Cases**	**Total Death**	**New Cases**	**New Death**
Total cases	1.000	-	-	-	**Total cases**	1.000	-	-	-
Total death	0.999	1.000	-	-	**Total death**	-0.285	1.000	-	-
New cases	0.917	0.904	1.000	-	**New cases**	0.993	-0.323	1.000	-
New death	0.938	0.931	0.947	1.000	**New death**	-0.237	0.891	-0.266	1.000

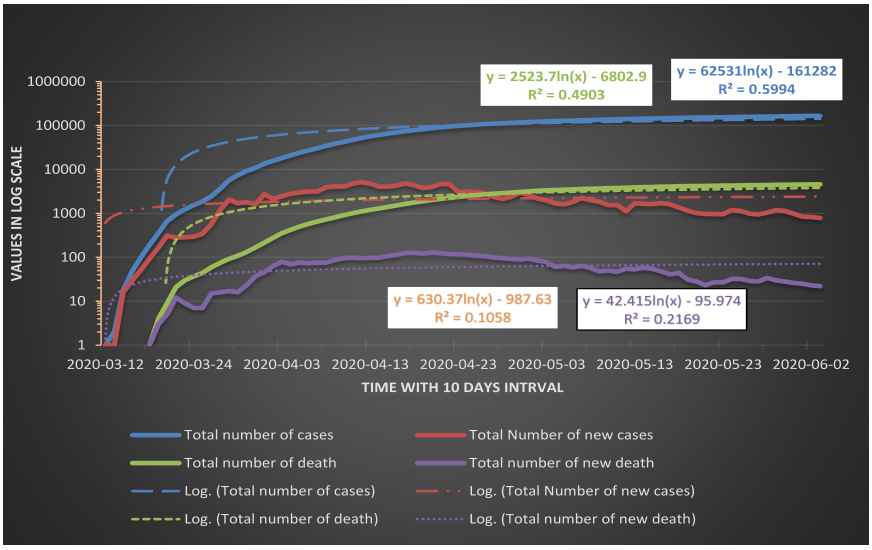

Fig. (6). COVID-19 cases *vs.* death trend analysis and correlation analysis for Turkey (Data from 30-01-2020 to 24-09-2020). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The correlation analysis table also supports this data, where we can observe that the correlation value of total new cases and total new death in the initial 28 days are 0.91 and 0.90, whereas, the overall correlation values are 0.99 and -0.32 (Table **6**).

The degree of correlation of new cases and deaths is high in the initial 28 days whereas, the overall correlation is low or negative for all the countries. It is pertinent to mention that the 28 days is counted from the day, the first death is reported in that particular country. Those data are considered as the baseline for comparing the overall data of the coronavirus infection and deaths. Hence, we can conclude that the coronavirus is losing its potency to causing death to the human. The new infection is still being reported from every corner of the world, butthe death rate is significantly decreasing. The rate of a new infection is also reduced in many countries of the world including China. As we know, to have no specific treatment or vaccine for the SARS CoV-2, but the infected patients are getting recovered with littler death compared to the beginning of the pandemic. We have tried to correlate the high mutation rate of the virus that leads to losing its potency to severe infection and death in the human. Viral extinction through high mutation could be considered as the new anti-viral strategies.

CONCLUSION

It can be predicted that the mutation and evolution of the RNA viruses will continue and some “new” human RNA viruses like SARS CoV-3 or MERS CoV-1 will emerge and the older disease like COVID-19 will disappear or become less significant. The new RNA virus disease will continue to emerge at the uncertain interval and the virus will not be really “new” but rather mutated and evolved from their ancestor one, which will have a high capacity to infect and survive in the new host. Because the large fraction of the genome can be substituted, expanded, contracted, recombined, and rearranged, the direction of the RNA genome evolution of RNA viruses is unpredictable. This usually favors the evolution of RNA viruses, which leads to species jump and an unpredictable “NEW” outbreak.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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