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Research Article

Phantom Behavior of EBOV & MARV: Multiple Integration of EBOV & MARV into All Types Viral Genomes

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ABSTRACT

Ebola hemorrhagic fever (EHF), triggered bythe Ebola virus. EBOV have fatal epidemic sickness with the highly pathogenic rate, exceptionally infection rate, and particularly very high mortality rate. In order to prevent the disorder from spreading further, it is necessary to have a lookup of the most unsafe Ebola virus at the molecular level. This research explores of EBOV/MARV virus has some similarity with the other viruses. They may arise because of error in their replication process. That's why they have no cure and more dangerous from existing viral diseases. So our research want to uncover the mystery how new emerging viruses are closely related to preexisting viruses. The phylogenies strategies are capable to divulge the degree of the evolution whose processing is conservative at present. Phylogenic tree can mirror the relationship of the genomes in diverse virus world. With the help of phylogenetic and genomic integration analysis we explore with whom its closets and how much they are closer to each other. For phylogenetic analysis we focused on MEGA7 and nBLAST used for genomic level analysis. From research, we found that ZAIRE strain of EV showed maximum evolutionary relationship with other viruses. From the genomic level analysis, found that most integrated part is VP40, VP24, NP & GP. The integrated part targeted as to the design of new drug molecules. Still, very little or no data on evolutionary relationships among these viruses (EBOV/MARV) to other viral genomes available. We identified and analyzed all 742 complete genomes out of 5672 of all types of (DNA, RNA, retrotrancribing and unclassified) viruses which infects only humans and vertebrates. The main objective of the current study is to find the nearest lineage of the Ebola virus and MARV among all viruses. The phylogenetic analysis and integrated genome can help us to find a readymade cure against the immediate outbreak. The protocol employed in the present study could be used in the future or side by side for the functional identification of new drugs for a different range of viruses. It's sizable to have a continuous concern of the Zaire virus because it's have more potency to evolute.

Keywords: EBOV, MARV, Phylogenetic analysis, Genomic integration, evolution, Ebola hemorrhagic fever (EHF).

INTRODUCTION

Viral haemorrhagic fevers (VHFs) caused by RNA families of viruses like Rhabdoviridae, Arenaviridae, Flaviviridae, and Filoviridae [1]. These cause severe illness like Lassa fever, yellow fever, dengue fever, rift valley fever etc. [2].Ebola is a deadly lethal virus that causes haemorrhagic

fever (HF) mostly in humans. Basis of infection behavior, it is zoonotic that means infect both humans and nonhuman primates (antelopes, monkeys, gorillas, and chimpanzees) [3]. According to ICTV classification of viruses, Ebola belongs to the order monengivirales & family is Filoviridae [4]. Ebola have five strains; *Zaire, Sudan,Tai Forest,Bundibugyo* and *Reston Ebolavirus*, initial first four cause human to human infection and Reston also cause nonhuman primates infection [5, 6].Ebola spread firstly in West African countries. Ebola virus first case was reported in 1976 in the Democratic Republic of Congo [7]. All EV strains data clearly mentioned in Table 1[8].

Table 1: Ebola virus strains information

S. No	Type of virus strains	Now present abbreviation	First time appearance/ Year	Mortality rate	Most affected area of world
1	Zaire Ebola virus	EBOV	Yambuku/1976	80- 90%	Whole Africa
2	Bundibugyo Ebola virus	BDBV	Uganda/2007- 08	30%	Uganda
3	Reston Ebola virus	RESTV	Reston, Virginia/1989	Death not reported	Philippines, Texas and Italy
4	Sudan Ebola virus	SUDV	Nzara , Sudan/1976	40- 60%	Most part of Sudan
5	Tai Forest Ebola virus/ Ivory coast Ebola virus	TAFV/ CIEBOV	African forest/1994	Non-fatal	DRC

The Viruses world have so many types of viruses and they infect host in so many ways. The NA (Nucleic acid) of viral genomesiseither DNA or RNA clearly represent in Figure 1 [9].

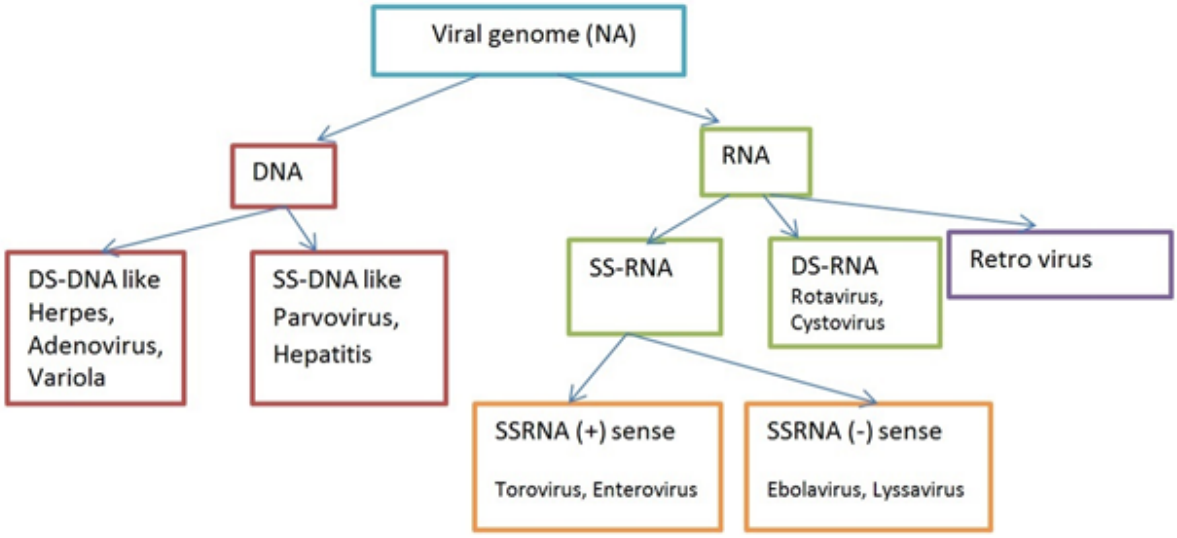

Figure 1: Genomic composition of virus

Two very important actions occur that time when virus infects a new host cell. One is that production of proteins/enzymes (virion proteins) and second one is viral replication.Every group of viruses assembled them in different ways because of differences in their genomes [10].EV genome is made up of ~19kb (18,959- 18,961) nucleotides with RNA genome with sequentially arranged of seven structural proteins [4, 11]. Those proteins which are required for replication and transcription are nucleoprotein (NP), matrix protein VP35, matrix protein VP30 and RNA dependent RNA polymerase (L) [12-14]. Rest three protein likes glycoprotein (GP), matrix protein VP40 & matrix protein VP24 participation in the formation of filamentous virions. These filamentous virions again infect normal host cell, whole genome composition shown in Figure 2 [15, 16].

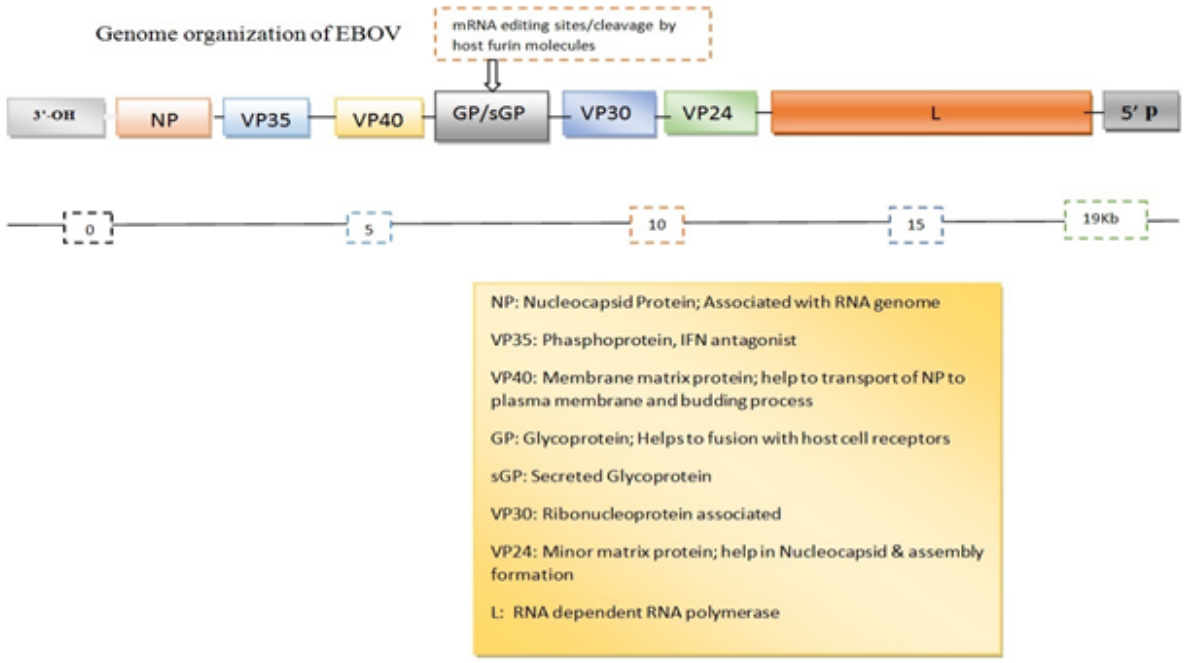

Figure 2: Genomic organization of EBOV at molecular level

Ebola virus infection occurs through direct contact with infected humans & other animals, their mucus and other viral containing fluids [17, 18]. Immunological response in EBOV is that virus firstly attacks on immune system and then on vascular system. In immune system they attack on dendritic cells and macrophages cell. Dendritic cells are very necessary for proper working of T cells these T cells destroy the infected cells. Macrophages cells also eat infected cells [19]. In laststage EBOVvirus attacks on endothelial cells cause leakage of blood from blood vessels [19, 20]. EBOV life cycle is 21 days. The incubation period is 8 to 10 days [21]. In initial symptoms chills, fever and muscles pain etc. In last day's condition is worse condition for patient because of diarrhea, severe abdominal pain, damage of blood vessels and multiple organs failure [22].Many scientist and researchers works on EBOV to develop new drugs or vaccines which are effectively work on this. But till now no therapeutic agents are known. We will prevent Ebola virus infection but not stop [21]. The primary treatment is supportive which includes balance electrolyte in body, anticoagulants in early and procoagulants in last stage of infection. Symptomatic treatment also provide in EBOV [23].

MATERIALS AND METHODS

Phylogenetic analysis and genome integrations of EBOV & MARV into the other viral genomes at molecular level, soavailable genomes of all types of viruses downloaded from National Center for Biotechnology Information whose website is http://www.ncbi.nlm.nih.gov.In which most of the sequences were downloaded on September 26, 2016 that time total number of complete genomes of viruses were available **(5672)**. This is huge amount of genomes so, applied data mining method for filtration of sequences. Research focused only those viruses which infect humans and vertebrates. Separate Filoviridae family (EBOV & MARV) genomes also downloaded in which five strains of EBOV and two strains of MARV. Phylogenetic analysis conducted with the help of MEGA7 (Molecular evolutionary genetics analysis 7.0 for bigger datasets whose website is

http://www.megasoftware.net/.For multi-sequence alignments, ClustalW uses revolutionary alignment methods. In these, the most similar sequences, that is, those with the satisfactory alignment rating are aligned first. Then step by step extra far-off corporations of sequences are aligned till a world alignment is obtained. This heuristic approach is fundamental because finding the international superior solution is prohibitive in both reminiscence and time requirements. The algorithm starts by computing a rough distance matrix between each pair of sequences based totally on pair-wise sequence alignment scores. These rankings are computed the usage of the pair-wise alignment parameters for nucleotide sequences. The length of the tree branch represents the evolutionary distance amongst the sequences. The approach used to assemble a phylogenetic tree and examine the sequences is Neighbor-Joining Method written by Saitou and Nei [24], due to the fact that nucleotide sequences of the Ebola virus and other considered viruses in various year are extensive similar. Therefore, the effects of the phylogenetic tree will not appear the phenomenon such as Long-branch attraction, which will severely have an effect on the shape of the tree. This algorithm permits the complete distance of the machine tree to be minimum via the willpower to make the closest (or adjacent) classification unit pairwise. A node only connects two adjacent gadgets in a bifurcate tree. Through the adjoining nodes being merged into new nodes step with the aid of step, the corresponding topological tree can be set up.

There are many other available methods to construct a phylogenic tree, such as Maximum Parsimony (MP) methods, Unweighted Pair-group Method with Arithmetic Means (UPGMA), Maximum Likelihood (ML). However, these strategies are much less suitable for the evaluation of EBOV&MARV against all virus world. Maximum Likelihood, which was utilized in phylogenetic evaluation on the issue of inspecting gene frequency statistics in the early time, additionally primarily based on the analysis of molecular sequences. During the process of ML analysis, the unique choice mannequin to analyze a given set of sequence facts will make each topology structure with the most probability ratio, and then pick out one of the largest possibility topology as the optimizing tree. In the analysis of the maximum possibility method, what will reflect on consideration on in most cases is no longer topology but every topology structure of branches. It takes a long time to calculate the maximum likelihood ratio and to estimate the size of branches. ML is a kind of mature method with fantastic statistical theory, however it’s tough to locate out a kind of sensible and alternative model when it comes to the building of EBOV & MARV phylogenic tree.

MP, which basically used in earliest morphological characters study, is now delivered into the evaluation of the evolution of molecular sequences. The principle claims that the satisfactory theory to give an explanation for a method is having the minimum assumption. In that case, all feasible topology shape will be calculated, and the topology tree with the least replacement will be regarded as optimizing tree. Felsenstein pointed out that if the quantity of base changes for the duration of the manner of evolution is small, the MP technique is very reasonable, but when a lot of base changes took area in the sequences, MP method would make mistakes [25- 27]. The reconstruction of the phylogenetic tree with the aid of 1000 instances bootstraps method is used to affirm the credibility of the tree branch, as the number displayed on the branch as shown in figures 4, 5, 6, S1, S2, S3, & S4). If the value is larger than 50 that means the tree is plausible to be analyze. Which have larger than 50 value further selected as references sequences for genomic integration and this done with the help of nBLAST program from (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

Changes of the virus nucleotides are complicated and subtle, and there are so many factors may

additionally greatly impact the function or structure of the proteins. One of the crucial factors is that the virus is characterised through the nucleotide sequence. Comparing to the proteins of the virus, the nucleotide sequence is consider to more stable. Apart from the differences of amino acid sequences, spatial changes of the protein may additionally even divers the function, however, solely the order of the base in RibonucleicAcid (RNA) is able to differ itself from other sorts of sequences. The EBOV and MARVnucleotide sequences (five strains of EBOV and two strains of MARV) are retrieved from NCBI website. There are many complete genome of the virus in a number years, so with the help of data mining approach, total collected genomes of different viruses are**742** out of **5672** as clearly shown in Figure 3.

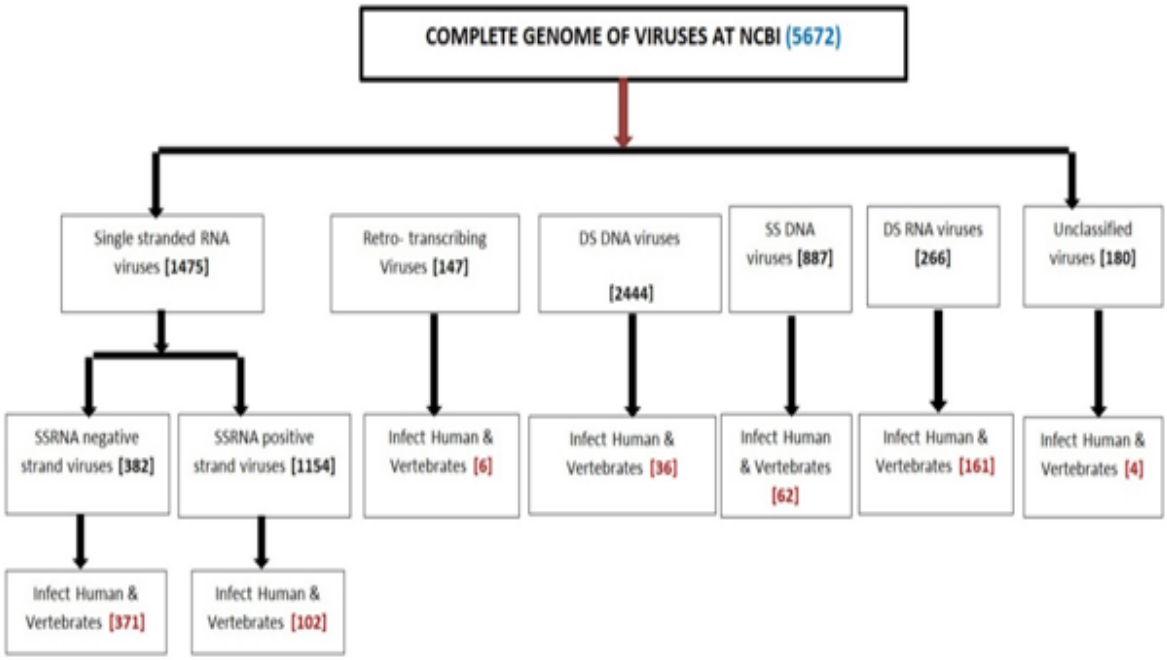

Figure 3: Data mining of complete genomes of viruses

The construction of phylogenetic tree with neighbor-joining (NJ) Method is in a position to reveal the relationship of each EBOV & MARV strains with other viral genomes. It's the most suitable way to assemble the phylogenetic tree. The length of branch stands for the close or far-off relationship. The relationship of every strains of EBOV & MARV in diversed virus worlds is homologizing. There is an fascinating phenomenon in (Figure 4, 5, 6, S1,S2 etc.) that some of strains of EBOV and MARV sequences are in different branches. The genetic glide is obvious through so many years that's one of the reasons why they have the one of a kind contamination charge and mortality rate.

From phylogenetic analysis this research concluded that ZAIRE strain of EV showed maximum evolutionary relationship with all types of selected viruses. In figures 4, 5, S1, S2, S3, S4 highlight the evolutionary history of ZAIRE EV with DSDNA viruses (simian virus, human herpes virus), Retroviruses (HIV-2), SSDNA viruses (Boca virus), DSRNA viruses (Diarrheal rotavirus), SS (-) SENSE RNA viruses (Influenza C virus, Measles virus) and SS (+) SENSE RNA viruses (Dengue virus, Japanese encephalitis, Zika virus, SARS corona virus, Chikungunya). In Figure S5, unclassified sequences have not showed any relationship EBOV & MARV.

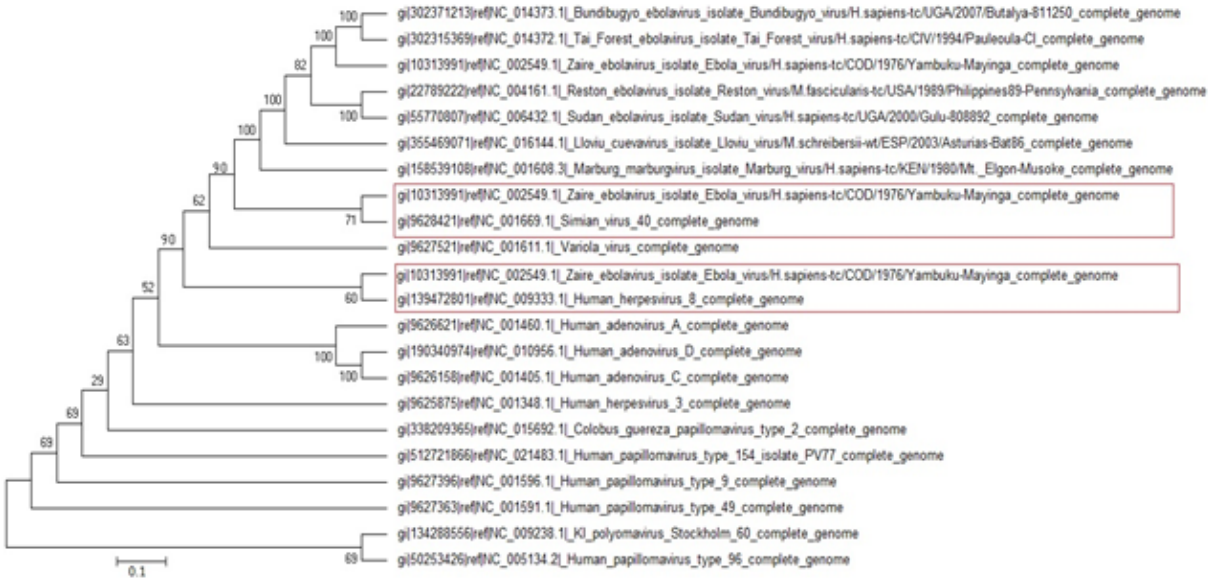

Figure 4: Phylogenetic tree of DSDNA viruses with EBOV & MARV

After the analysis of phylogeny, reference sequences which showed evolutionary relationship were taken to analyze genomic integration as clearly mentioned in Tables (S1 to S6). From this research work we found that ZAIRE strain all over (NP, VP35, VP40, VP30, VP24, & L) integrated with other viruses genomes.

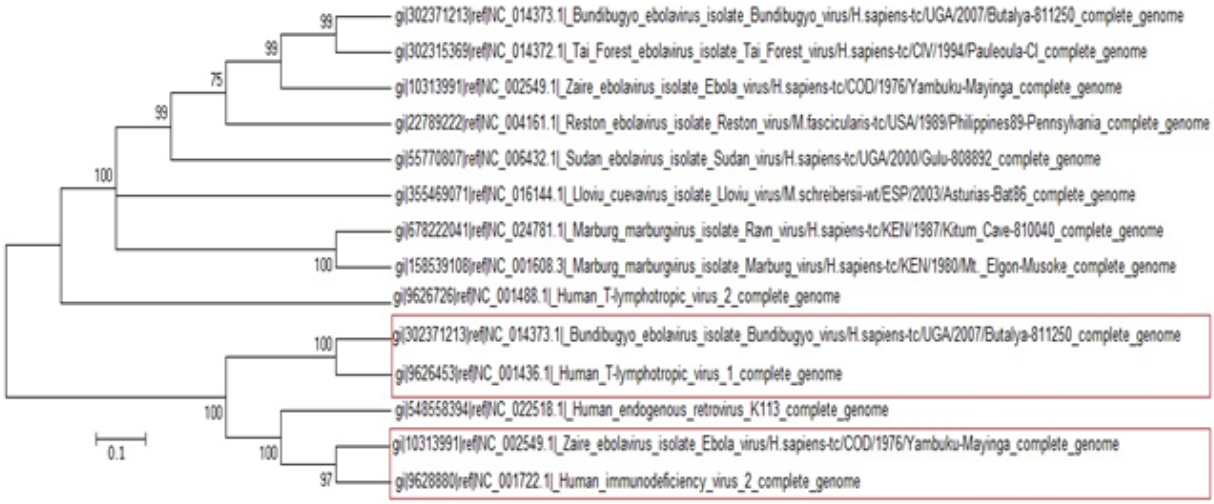

Figure 5: Phylogenetic tree of Retroviruses with EBOV & MARV

DISCUSSION

This research has uncovered a fossil report for currently circulating lethal viral diseases of all virus families that stretch returned that somehow viruses have evolute from their entire related virus world. Consequently, the viruses genome includes a more accurate document of the archival genes of viral infection with DNA/RNA genomes than the associated present-day viruses. Considering the notably high charge of mutation in viruses, and the stringent criteria we utilized to become aware of homologies, what has reported here need to be taken as an underestimate of such viral gene integration events. However, the results of this

search are as fascinating for what was once now not located as what was once found. The endogenous viral sequence ofZAIRE EV that has identified with highest self-assurance to all related currently circulating viruses as mentioned in Tables S1 to S6. The integral part of genome of ZAIRE EV mostly NP, VP40, VP24. These genomic viral proteins play a vital role in drug designing to eradicate of this deadly virus. ZAIRE EV is conservative at a macro level at present, the nucleotide sequences of the Zaire EBOV change little, and the base order is considered conservative during self-replication, as it nevertheless has tremendously pathogenic rate, highly infection rate, highly mortality. The symptoms of Ebola virus similar to almost all references relative viruses such as simian virus, human herpes virus, Boca virus, Diarrheal rotavirus, Influenza C virus, Measles virus, Dengue virus, Japanese encephalitis, Zika virus, SARS corona virus and Chikungunya. Most of viruses till now have no drugs to cure like boca virus, zika virus etc.In Figure S5, unclassified genomes have not showed any relationship EBOV & MARV that means they have fall as outgroups.

At the time of this writing, it's appropriate news that the EVHF epidemic situation is below the control step-by-step in worldwide. On the other hand, the virus can also not be seen stably at a molecular level in present and future, because of error rate of replication and mutations. Hence, the virus is required to remain situation in future research.

CONCLUSION

In summary, our research has made it clear that these present viruses evolved from ancient relatives viruses. This research work is noteworthy important for the evolution of EV and MARV. Phylogenetic and genomic level analysis tell us about that which viruses are nearest to EV and MARV and which region of genome integrated like GP, NP matrix protein &L. This integrated part targeted as to design of new drug molecules. From phylogenetic analysis we know about that howmuch EV/MARV show identity with other viruses. In many viral diseases we still don't know exact treatments and medicines. If we know that medicines this may be tested as for Ebola treatment. Hence this researchis very important for future analysis. Through this work, we will have two types of benefits: one, we will try present viral drugs for the symptomatic treatment of EHF such as amantidine, oseltavir, Favipiravir, ribavirin, naproxen, PCM, aspirin etc.and second is designing of new drugs. These designed new drugs may be eradicate those viruses which have no medicines yet, they can also carry these medicines like simian virus, boca virus, torque teno virus1, human T lymphotropic virus1,hendra virus, nipah virus, zerdali virus, zika virus, mamastro virus.

FUTURE PERSPECTIVE

Ebola virus is use as a weapon of terrorism and some scientists gave the name of bioterrorism. At the time of research work EV in controlled condition in all over world but in future what is the guarantee that this virus will not attack human being again? Till now we don't know what the exact treatment is. How long we continue to offer symptomatic treatment without know the cause. We should be aware of this and prepare to fight it in the future. From the previous outbreaks from 1976 to 2014 and 2014 -1016 its show exponential growth rate in mortality rate.

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FOOTNOTE

We declare that absence of any type of conflict to others.

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