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Chapter

Ebola, the Negative Stranded RNA Virus

Aqsa Farman, Syed Lal Badshah, Khalid Khan, Nasir Ahmad and Abdul Naeem

Abstract

Ebola virus (EBV) is a deadly virus that has resulted in a number of deaths during its outbreaks in Africa in 2014–2016 and 2018–2019. This virus causes a hemorrhagic fever like other pathogenic viruses of the Filoviridae family with high mortality rate. The exact reservoir of the ebola virus is not known, but different mammal groups are the source from which it is transferred to the human population. The transmission among the human population is through body fluids of patients and also through aersol droplets in the air. The role of different glycoproteins in the budding formation has helped a lot in understanding the physiology of the ebola virus. Most of these viral glycoproteins synthesis and the replication enzymes offer a good inhibitory target for drug design against the ebola virus. Recently, different groups have claimed the development of a successful vaccine for the ebola virus. However, the availability of the vaccines to the poor population of Africa and other parts of the world is still not practical.

Keywords: Ebola virus, hemorrhagic fever, vaccine, glycoproteins, molecular docking

1. Introduction and background

The Ebola virus was first discovered back in 1976 when its breakout occurred simultaneously in South Sudan and in the area of Ebola River in Yambuku city of Democratic Republic of Congo (**Figure 1**) [1]. It reemerges in 1990s, 2000s, peaked in 2014 and 2018–2019 (**Figure 1**). This RNA viral disease caused hemorrhagic fever in humans and non-human primates [2]. The African originated species of Ebola virus fatality rate in humans is nearly 90% [2]. Ebola, Marburg, and Cueva virus are the three infectious viruses with a common ancestor of the family Filoviridae [2]. The word "filo" in Filoviridae is derived from the Latin word film, which means thread like, as these virions appear thread-like under the electron microscope [3]. The Filoviridae family is part of the order Mononegavirales, class Monjiviricetes and phylum Negarnaviricota in the virus taxonomic classification [4, 5]. Ebola, also known as Ebola virus disease (EVD/EBOV) and Ebola hemorrhagic fever (EHF) in the past, is a filamentous, enveloped, non-segmented, single stranded negative sense ribonucleic acid (RNA) virus [4]. The replication of Filoviridae family of viruses takes place in the cytoplasm of the host cell [6, 7]. The genomic RNA is non-contagious alone because it is not able to serve as a template for protein synthesis [6, 7]. To start the transcription of positive-sense messenger RNA (mRNA) the viral protein must connect with the genomic RNA [6, 7]. The

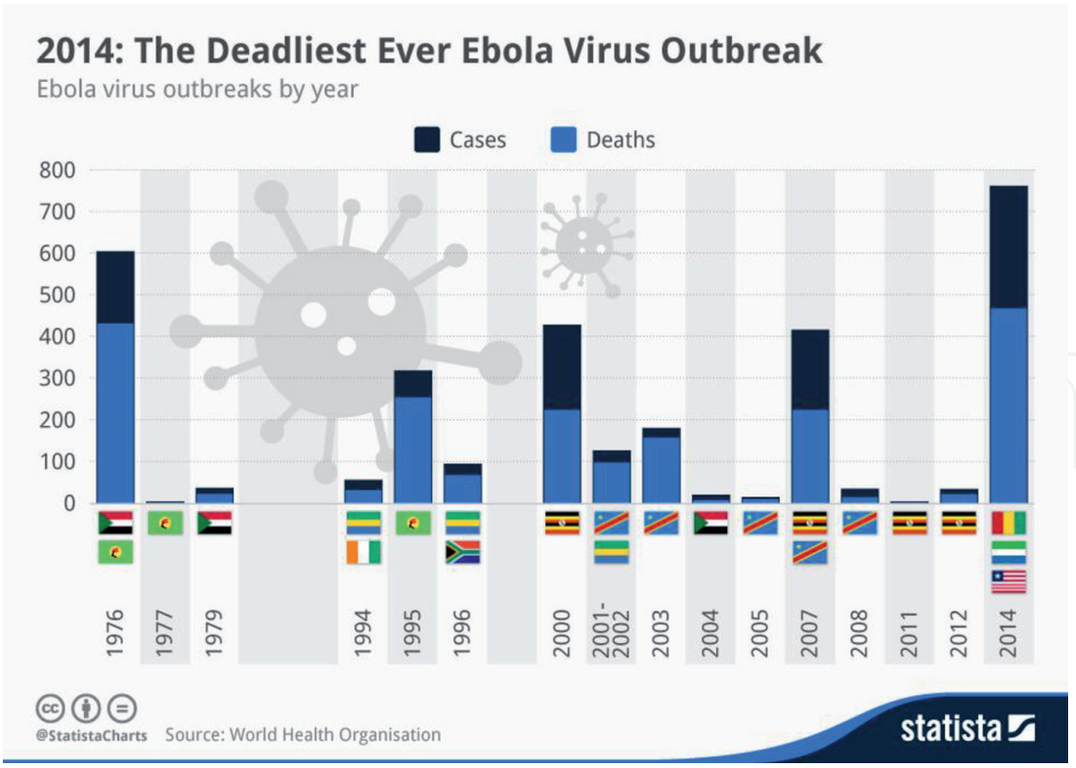

Figure 1.
Reported cases and deaths from EBOV in different countries from 1976 to 2014 according to WHO [8].

objective of this work is to provide firsthand information regarding the EBOV origin, infection, control and progress in vaccine development.

1.1 Origin

The Ebola virus is taxonomically classified into five species. These species includes Zaire Ebola virus (EBOV), Reston Ebola virus (RESTV), Taï Forest Ebola virus (TAFV) or Coted'Ivoire Ebola virus (CIEBOV) [9], Sudan Ebola virus (SUDV), and Bundibugyo Ebola virus (BDBV) [10]. Four of these species are responsible to cause disease in humans and one in nonhuman primates [10]. The species of Ebola viruses are named from where they originated for the first time [11]. The BDBV was first found in 2007 in Bundibugyo district of Uganda with 116 cases and fatality rate of 25% [12, 13]. RESTV emerged in 1989 in Reston (Virginia), USA, causing diseases in nonhuman primates [14, 15]. SUDV was first discovered in 1976 in a cotton factory in South Sudan (Nzara). Its mortality rate varies from 53 to 68%. The mortality rate is the death rate due to certain disease in a particular population. CIEBOV (Tai forest virus) was first reported in 1994. The virus appeared in chimpanzees in Africa in Tai forest [16]. ZEBOV has the highest lethality rate up to 90%, which includes the first appeared virus and the most current outbreak [17].

1.2 History

The Ebola virus is named after a small river, Ebola in Zaire and this viral infection first appeared in 1976 in both South Sudan and Zaire in the African continent [18–20]. The international teams of WHO reached the affected area but were unsuccessful in saving more lives and collecting vital information due to lack of medical facilities and illiteracy in rural areas [21]. This virus family becomes genetically more advanced and spread into other areas, and appeared in the united states in 1989 [22]. EBOV did not appear in Africa for 15 years but as the natural reservoir

was not completely eliminated and due to this reason the virus showed itself again in 1994–1996 [23]. During 1994–1996, the new subtype of Ebola Coted'Ivoire was discovered, while the old subtypes were also present. The people infected were living near the tropical forest area. The ethnologist's discovered that the fifth subtype was found in the chimpanzee group with a high fatality rate. They were transferred to Switzerland for suitable facilities and medical care by a group of scientists [24]. The outbreak in 2014 has the highest fatality rates in the history of EBOV [25].

1.3 Transmission, symptoms, and treatment

EBOV is a rare and deadly disease caused by infection with one of the Ebola virus strains. Humans and non-human primates are the primary hosts of Ebola [26]. The different types of insectivorous bat genus, especially *Mops condylurus* and frugivorous bat species are also the host of EBOV [27–29]. The fruits eating bat is also a common reservoir of ebola virus [30, 31]. The virus is transmitted through contaminated blood and bodily fluids of infected person or animal. Fever, muscle ache, loss of appetite, and fatigue are the common symptoms after onset of EBOV, while in fatal cases, severe vomiting, diarrhea, hemorrhage, septic shock, and multi-organ failure may occur that may lead to death in 30–90% patients [32]. The patients can be treated as the symptoms of EBOV appear in them. Currently, there is no proper Federal Drug Authority (FDA) approved medicines or vaccines. The following are some of the interventions and precautions for survival against EBOV;

a. Intravenous fluids (IV) and body salts should be regularly provided to the EBOV infected patients.

b. The blood pressure and oxygen status of patients should be monitored and maintained to the normal level.

c. The blood lost during the illness should be replaced and medicines should be provided to control the blood loss.

d. The patient should increase the intake of fluids.

e. The other infections which develop during treatment should also be treated simultaneously [33].

1.4 EBOV genomic organizations

The EBOV genome has nucleotide sequences which are extragenic. The sequences combine to produce secondary structures that contain 3′ and 5′ ends. The genome serves to initiate transcription and genome replication [34, 35]. The negative-stranded RNA genome of EBOV contains seven important genes that are arranged in a linear fashion and has a total length of approximately 19 kilobases and it translated into eight proteins [36]. Its sequence is as follows 3′-NP, VP35, VP40, GP, VP30, VP24, L-5′as shown in **Figure 2**. The VP (35) is RNA-dependent RNA polymerase cofactor, VP(40) is a matrix protein, GP(1,2) is spike glycoprotein, VP(30) is a transcriptional activator, VP (24) is the second protein matrix and (L) is RNA polymerase enzyme [6]. The RNA genome of the virus is coated by a complex of ribonuclear protein (RNP). It is also known as nucleocapsid. The nucleocapsid is composed of major nucleoprotein (NP), minor nucleoproteins that include VP(30), VP(35), and polymerase (L) protein as shown in **Figure 3** [37, 38]. The above complex is then enveloped in a layer of matrix. The matrix consists of

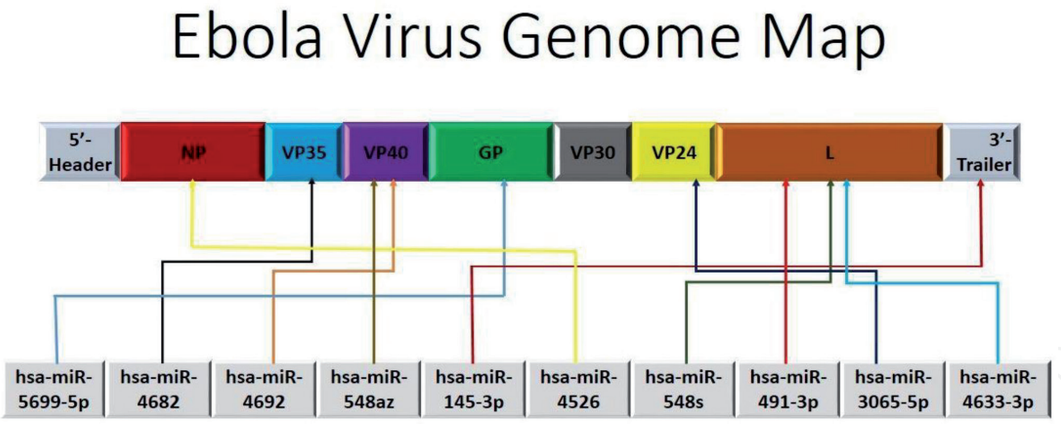

Figure 2.
Important proteins in the RNA genome of EBOV [43].

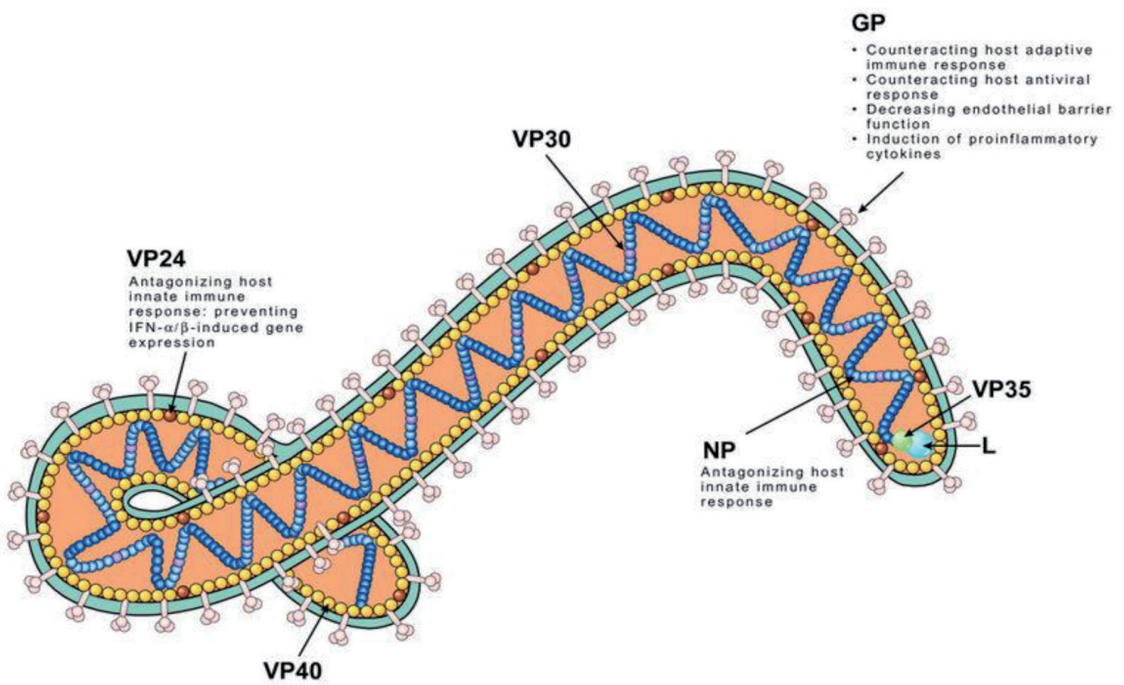

Figure 3.
The virion structure showing the nuclear material and glycoproteins [44].

the major and minor matrix protein VP40, VP24, respectively. Then, the matrix proteins are encapsulated by a double layer of lipid which is then peppered with the viral glycoprotein (GP) that help in binding to the host cell [39, 40]. All five EBOV species discovered so far has the same GP gene organization which is divided into sGP, GP1, GP2, and ssGP [41, 42].

1.5 Structure of glycoproteins

It has been observed that most of the viral components play an active role in the pathogenesis and infection of this viral disease, but the major role is played by the glycoproteins of the EBOV [45]. Thus, they possess a significant importance in the virulence of EBOV and targeting its synthesis is one of important step for controlling this infection [45]. EBOV includes different glycoproteins each of which plays their roles in different aspects of viral life cycle. Each gene product has distinct biochemical and biological properties [46, 47]. The fourth gene, GP

is formed by a process known as glycosylation in which carbohydrate are linked covalently to the polypeptide chain [48].

1.6 Production of glycoproteins

The biosynthesis of glycoproteins occurs by transcription and expression strategies. The precursor of the secreted glycoproteins and full length glycoproteins are the initial products of EBOV glycoprotein. Pre-sGP is transcripted and translated to soluble products sGP and Δ-peptide while glycoprotein GP1, 2 is cleaved to GP1 and GP2. These two fragments are linked together by disulfide-bonding (S—S). The GP1 helps in binding to the host cell and has a crucial role of EBOV entry across the host cell membrane [49]. The flowchart is shown in **Figure 4** [45].

1.7 Classification and functions of EBOV glycoproteins

The Ebola virus actually produces the following soluble glycoproteins during infection:

1.7.1 Small secreted glycoprotein of EBOV

Small secreted glycoprotein (ssGP) is translated from part of mRNA. It is nonstructural and generated when two adenosines are combined or one is deleted during transcription [50]. The ssGP has monomeric structure which has identical 295 amino acids from starting point with secreted GP and full length GP but they vary at the C-terminus of the full length glycoprotein [51]. The host ADAM17 metalloprotease enzyme is responsible to generate ssGP. The antibodies that neutralize the viral glycoproteins are quickly blocked by the secreted complex GP [14]. This complex plays a key function in the virus pathogenesis. In spite of extensive necrosis and massive virus production, it therefore contributes to less inflammatory reaction seen during the infection [14]. When the EBOV enters the host cell, the

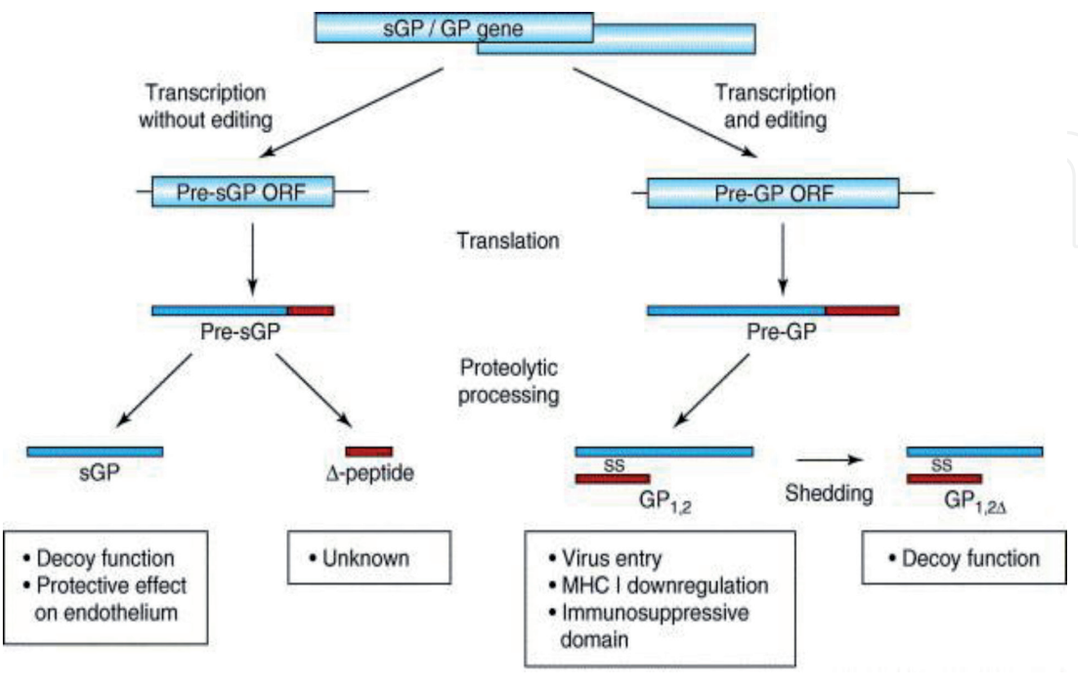

Figure 4.
Biosynthesis of glycoproteins of EBOV [45].

small secretary glycoprotein neutralizes the host cell neutrophils through CD16b and resulting in lymphocyte death and further vascular dysregulation [52].

1.7.2 Secreted or soluble glycoprotein and delta peptide of EBOV

The soluble glycoprotein (sGP) is a non-structural protein with a single frame and has total 364-372 residues. At the N-terminal, 295 residues which also include the signal sequence are similar with full length GP. It differs in the length of C-terminal sequences [46, 50]. The formation of sGP is from Precursor (pre-sGP). Unedited mRNA is frequently produced from infected cells which results in the formation of precursor (pre-sGP). Precursor (pre-sGP) is cleaved by the furin protein to produce sGP and delta peptide [46, 50]. The orientation of sGP is antiparallel and linked together by disulfide bonding. It possesses dimerization between amino and carboxy groups as shown in **Figure 5**. The sGP plays a role in the evasion of humoral immune response by absorbing elicited antibodies. It also predicted to be involved in interaction with neutrophils of the host cells and their neutralization [53].

1.7.3 Envelope or full length glycoprotein of EBOV

It is generated on the surface of mature infectious virions and formed when RNA is edited by the process of transcription. The GP protein has 676 amino acids and is a structural polypeptide chain. It is encoded in two frames and functions in attachment and entry of virus into the defenseless cells of the host [48]. The GP is sub-divided into two glycoproteins; GP1 and GP2. They are membrane associated proteins and are linked with each other through disulfide bonding as shown in **Figure 6**. The GP1 is 130 kDa and GP2 is 24 kDa in size [47]. The GP1 helps in attachment to the receptors which are present on target cells [54]. The interactions allow the binding of viral particles to the dendritic cells and thus facilitates virus into cell entry [48]. GP2 is known as a class I viral fusion protein. Some characteristics of GP2 subunit protein of EBOV are as follows: towards the C-termini is the transmembrane helix while the "fusion- peptide" is found at the N-terminus [55]. The formation of this structure drives to combine the cell membranes of target cells and EBOV. It also helps the particles to enter into the cytoplasm of a healthy cell. The penetration of virus is mediated by the fusion of the membranes of endosomes with the endocytosed virus particle [56].

Survey of the literature showed that currently there are no crystal structures of both secreted and envelope glycoproteins available which are important for designing novel drugs that can inhibit the attachment of these glycoprotein to the host cells receptors and also to halt the activity of the secretary and soluble glycoproteins. In one of our study, we have constructed 3D structures through homology modeling [57]. The molecular dynamics simulation study of the obtained homology models and several drug docking results showed that these proteins can be targeted with small

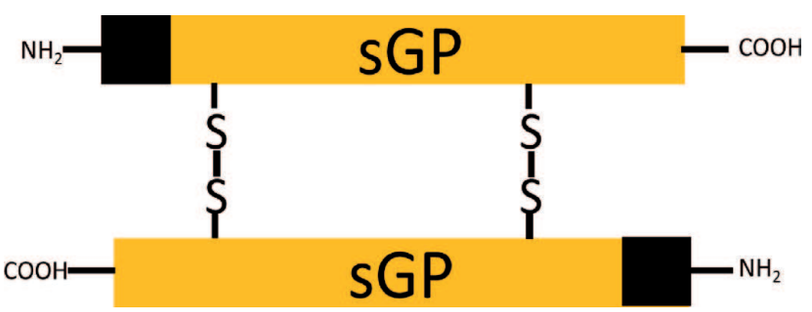

Figure 5.
EBOV soluble glycoprotein (sGP) involved in host neutrophil neutralization and immune system evasion.

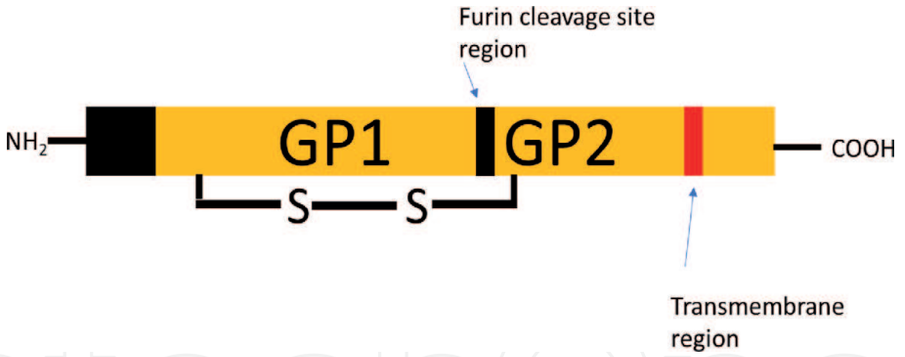

Figure 6.
EBOV envelope glycoprotein.

ligand molecules [57]. Further computation studies and experimental work will prove the usefulness of these small molecules as inhibitor of the EBOV important proteins [57]. The current advancement in cryo-electron microscopy should be utilized for the elucidation of EBOV structure like that of influenza, dengue, Zika and other arboviral diseases [57–60]. The availability of protein structure will help in molecular dynamics simulation and docking of these important viral proteins with suitable ligands and it will enhance the exploration of novel antiviral drugs [59, 60].

1.8 Progress in vaccines development for EBOV

A number of researchers have developed vaccines in their laboratories which are in initial stages and have some minor side effects [61]. A recent investigation showed the development of a vaccine when the EBOV glycoproteins like GP and VP40 were injected in the guinea pigs and a protective immune response was observed [62]. The constructed vaccine recovers the infected rhesus macaque in a single dose [62]. Similarly the glycoproteins along with the matrix proteins VP24 and VP40 and in their absence were also tested in guinea pigs [63]. The antibodies produced against these antigens completely cure the tested subjects from EBOV [63]. Several other vaccine of EBOV was developed recently and they showed promising results in murine models with high success rate [64]. Thus, a success in the development of vaccine has been achieved but passing the clinical trials and its availability in the market will take time.

1.8.1 Prevention and control

A number of steps can be taken for the control and prevention of EBOV break-out and its spread. It is always better to avoid traveling to areas of EBOV outbreak and strict quarantine should be imposed on the people of that particular towns. Only medical teams with proper safety masks, cloths should be allowed for the care of patients infected with EBOV [65]. The damaged fruits and vegetables eaten by bat should not be consumed. Bats, monkeys, pangolins, and other wild animals' meat and their utilization in food, medicine, and soaps should be avoided [66]. Contacting these animals should be discouraged. Proper washing of hands and use of sanitizers should be encouraged in schools, airports, shopping malls, trains, and all public areas. It is always necessary for hospitals and health working organizations to share and spread information related to viral diseases outbreak to the general public on time and awareness campaigns should be launched at regular intervals of time. The advancement in cryo-electron microscopy should be utilized for structural resolution of the important enzymes of the EBOV and other fatal viruses so that novel drugs can be synthesized for their inhibition and control [67].

2. Conclusions

With the 2018 outbreak of EBOV and high probability of future outbreaks and spread, it is highly important to expediate the production of effective vaccine and immunization of vulnerable population across different countries in Africa, that will help in the control of this viral disease. Joint efforts are also needed by the local public health departments and scientific community across the globe for information sharing on different viral outbreaks, vaccine development, and easy access of immunization and medicines.

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Competing interests

All the authors declared that they have no competing interests.

Note

Most part of this chapter is the introduction part of Ms. Aqsa Farman M. Phil (MS) thesis.

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References

[1] Rajak H, Jain DK, Singh A, Sharma AK, Dixit A. Ebola virus disease: Past, present and future. Asian Pacific Journal of Tropical Biomedicine. 2015;**5**:337-343. DOI: 10.1016/S2221-1691(15)30365-8

[2] Feldmann H, Geisbert TW. Ebola haemorrhagic fever. Lancet. 2011;**377**:849-862. DOI: 10.1016/S0140-6736(10)60667-8

[3] Mühlberger E. Chapter 1. Genome organization, replication, and transcription of filoviruses. In: Klenk H-D, Feldmann H, editors. Ebola and Marburg Viruses: Molecular and Cellular Biology. Horizon Bioscience. Norfolk, UK. 2004:1-20

[4] Burk R, Bollinger L, Johnson JC, Wada J, Radoshitzky SR, Palacios G, et al. Neglected filoviruses. FEMS Microbiology Reviews. 2016;**40**: 494-519. DOI: 10.1093/femsre/fuw010

[5] Payne S. Family Filoviridae. Viruses. From Understanding to Investigation. Chapter 21; 2017. pp. 183-190. DOI: 10.1016/B978-0-12-803109-4.00021-00

[6] Sanchez A, Khan AS, Zaki SR, Nabel GJ, Ksiazek TG, Peters CJ. Filoviridae: Marburg and Ebola viruses. Fields Virology. 2001;**1**:1279-1304. DOI: 10.1007/978-1-4612-3900-0_30

[7] Peters CJ, Sanchez A, Feldmann H, Rollin PE, Nichol S, Ksiazek TG. Filoviruses as emerging pathogens. Seminars in Virology. 1994;**5**:147-154. DOI: 10.1006/smvy.1994.1015

[8] WHO, WHO Ebola Situation Reports, WHO. 2016. Available from: http://www.who.int/csr/disease/ebola/situation-reports/archive/en/

[9] Kuhn JH, Andersen KG, Bào Y, Bavari S, Becker S, Bennett RS, et al. Nichol, Filovirus refseq entries: Evaluation and selection of filovirus type variants, type sequences, and names. Viruses. 2014;**6**:3663-3682. DOI: 10.3390/v6093663

[10] Towner JS, Sealy TK, Khristova ML, Albariño CG, Conlan S, Reeder SA, et al. Newly discovered Ebola virus associated with hemorrhagic fever outbreak in Uganda. 2008;**4**: PLoS Pathogens. DOI: 10.1371/journal.ppat.1000212

[11] Gatherer D. The 2014 Ebola virus disease outbreak in West Africa. The Journal of General Virology. 2014;**95**:1619-1624. DOI: 10.1099/vir.0.067199-0

[12] Clark DV, Kibuuka H, Millard M, Wakabi S, Lukwago L, Taylor A, et al. Long-term sequelae after Ebola virus disease in Bundibugyo, Uganda: A retrospective cohort study. The Lancet Infectious Diseases. 2015;**15**:905-912. DOI: 10.1016/S1473-3099(15)70152-0

[13] Roddy P, Howard N, van Kerkhove MD, Lutwama J, Wamala J, Yoti Z, et al. Clinical manifestations and case management of Ebola haemorrhagic fever caused by a newly identified virus strain, Bundibugyo, Uganda, 2007-2008. PLoS One. 2012;**7**. DOI: 10.1371/journal.pone.0052986

[14] Boehmann Y, Enterlein S, Randolf A, Mhlberger E. A reconstituted replication and transcription system for Ebola virus Reston and comparison with Ebola virus Zaire. Virology. 2005;**332**:406-417. DOI: 10.1016/j.virol.2004.11.018

[15] Rollin PE, Williams RJ, Bressler DS, Pearson S, Cottingham M, Pucak G, et al. Ebola (subtype Reston) virus among quarantined nonhuman primates recently imported from the Philippines to the United States. The Journal of Infectious

Diseases. 1999;**179**:S108-S114. DOI: 10.1086/514303

[16] Kuhn JH, Becker S, Ebihara H, Geisbert TW, Johnson KM, Kawaoka Y, et al. Proposal for a revised taxonomy of the family Filoviridae: Classification, names of taxa and viruses, and virus abbreviations. Archives of Virology. 2010;**155**:2083-2103. DOI: 10.1007/s00705-010-0814-x

[17] Chiappelli F, Bakhordarian A, Thames AD, Du AM, Jan AL, Nahcivan M, et al. Ebola: Translational science considerations. Journal of Translational Medicine. 2015;**13**:11. DOI: 10.1186/s12967-014-0362-3

[18] Bitekyerezo M, Kyobutungi C, Kizza R, Mugeni J, Munyarugero E, Tirwomwe F, et al. The outbreak and control of Ebola viral haemorrhagic fever in a Ugandan medical school. Tropical Doctor. 2002;**32**:10-15. DOI: 10.1177/004947550203200107

[19] Okware SI, Omaswa FG, Zaramba S, Opio A, Lutwama JJ, Kamugisha J, et al. An outbreak of Ebola in Uganda. Tropical Medicine & International Health. 2002;**7**:1068-1075. DOI: 10.1046/j.1365-3156.2002.00944.x

[20] Green A. Uganda battles Marburg fever outbreak. Lancet. 2012;**380**:1726. DOI: 10.1016/S0140-6736(12)61973-4

[21] Pattyn SR. Ebola virus haemorrhagic fever. In: Proceedings of an International Colloquium on Ebola Virus Infection and Other Haemorrhagic Fevers held in Antwerp, Belgium, 6-8 December, 1977; 1978. xii

[22] Peters CJ, JW LD. An introduction to Ebola: The virus and the disease. National Center for infectious diseases, Centers for Disease Control and Prevention. The Journal of Infectious Diseases. 1999;**179**:ix-xvi. DOI: 10.1086/514322

[23] Busico KM, Marshall KL, Ksiazek TG, Roels TH, Fleerackers Y, Feldmann H, et al. Prevalence of IgG antibodies to Ebola virus in individuals during an Ebola outbreak, Democratic Republic of the Congo, 1995. The Journal of Infectious Diseases. 1999;**179**(Suppl):S102-S107. DOI: 10.1086/514309

[24] Le Guenno B, Formentry P, Wyers M, Gounon P, Walker F, Boesch C. Isolation and partial characterisation of a new strain of Ebola virus. Lancet. 1995;**345**:1271-1274. DOI: 10.1016/S0140-6736(95)90925-7

[25] Africa S. Outbreak of Ebola hemorrhagic fever Uganda, August 2000-January 2001. MMWR. Morbidity and Mortality Weekly Report. 2001;**50**:73-77. DOI: 10.1001/jama.285.8.1010

[26] Farrar J, Hotez PJ, Junghanss T, Kang G, Lalloo D, White NJ. Chapter 16. Viral hemorrhagic fever. In: Blumberg L, Enria D, Bausch D, editors. Manson's Tropical Diseases. 23rd ed. Elsevier. 2013:171-194.e2

[27] Olival KJ, Hayman DTS. Filoviruses in bats: Current knowledge and future directions. Viruses. 2014;**6**:1759-1788. DOI: 10.3390/v6041759

[28] Schuh AJ, Amman BR, Towner JS. Filoviruses and bats. Microbioloogy Australia. 2017:12-16. DOI: 10.1071/MA17005

[29] Pigott DM, Golding N, Mylne A, Huang Z, Henry AJ, Weiss DJ, et al. Mapping the zoonotic niche of Ebola virus disease in Africa. eLife. 2014;**3**:e04395. DOI: 10.7554/eLife.04395

[30] Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, et al. Fruit bats as reservoirs of Ebola virus. Nature. 2005;**438**:575-576. DOI: 10.1038/438575a

[31] Olival KJ, Islam A, Yu M, Anthony SJ, Epstein JH, Khan SA, et al. Ebola virus antibodies in fruit bats, Bangladesh. Emerging Infectious Diseases. 2013;**19**:270-273. DOI: 10.3201/eid1902.120524

[32] Dixon MG, Schafer IJ. Ebola viral disease outbreak--West Africa, 2014. MMWR. Morbidity and Mortality Weekly Report. 2014;**63**:548-551. DOI: 10.1016/j.annemergmed.2014.10.010

[33] Nakayama E, Takada A. Ebola and Marburg viruses. Journal of Disaster Research. 2011;**6**:381-389

[34] Volchkov VE, Volchkova VA, Chepurnov AA, Blinov VM, Dolnik O, Netesov SV, et al. Characterization of the L gene and 5′ trailer region of Ebola virus. The Journal of General Virology. 1999;**80**:355-362. DOI: 10.1099/0022-1317-80-2-355

[35] Weik M, Enterlein S, Schlenz K, Muhlberger E. The Ebola virus genomic replication promoter is bipartite and follows the rule of six. Journal of Virology. 2005;**79**:10660-10671. DOI: 10.1128/JVI.79.16.10660-10671.2005

[36] Klenk H-D, Feldmann H. Ebola and Marburg viruses: Molecular and cellular biology. In: Klenk HFH-D, editor. Ebola Marbg. Molecular and Cellular Biology of Viruses. Norfolk, UK: Garland Science; 2004. p. 369

[37] Möller P, Pariente N, Klenk H-D, Becker S. Homo-Oligomerization of Marburgvirus VP35 is essential for its function in replication and transcription. Journal of Virology. 2005;**79**:14876-14886. DOI: 10.1128/JVI.79.23.14876-14886.2005

[38] Hlberger EM, Weik M, Volchkov VE, Klenk H-D, Becker S. Comparison of the transcription and replication strategies of Marburg virus and Ebola virus by using artificial replication systems. Journal of Virology. 1999;**73**:2333-2342

[39] Noda T, Sagara H, Suzuki E, Takada A, Kida H, Kawaoka Y. Ebola virus VP40 drives the formation of virus-like filamentous particles along with GP. Journal of Virology. 2002;**76**:4855-4865. DOI: 10.1128/JVI.76.10.4855-4865.2002

[40] Han Z, Boshra H, Sunyer JO, Zwiers SH, Paragas J, Harty RN. Biochemical and functional characterization of the Ebola virus VP24 protein: Implications for a role in virus assembly and budding. Journal of Virology. 2003;**77**:1793. DOI: 10.1128/JVI.77.3.1793

[41] Falzarano D, Krokhin O, Wahl-Jensen V, Seebach J, Wolf K, Schnittler HJ, et al. Structure-function analysis of the soluble glycoprotein, sGP, of ebola virus. ChemBioChem. 2006;**7**:1605-1611. DOI: 10.1002/cbic.200600223

[42] Li YH, Chen SP. Evolutionary history of Ebola virus. Epidemiology and Infection. 2014;**142**:1138-1145. DOI: 10.1017/S0950268813002215

[43] Golkar Z, Pace DG, Bagasra O. Chapter 16: Utility of potent anti-viral MicroRNAs in emerging infectious diseases. In: Biochemistry, Genetics and Molecular Biology. IntechOpen; 2016. DOI: 10.5772/61687

[44] de Wit E, Feldmann H, Munster VJ. Tackling Ebola: New insights into prophylactic and therapeutic intervention strategies. Genome Medicine. 2011;**3**. DOI: 10.1186/gm219

[45] Feldmann H, Volchkov VE, Volchkova VA, Ströher U, Klenk HD. Biosynthesis and role of filoviral glycoproteins. The Journal of General Virology. 2001;**82**:2839-2848. DOI: 10.1099/0022-1317-82-12-2839

[46] Sanchez SG, Trappier BW, Mahy CJ, Peters ST. Nichol, the virion glycoproteins of Ebola viruses are

encoded in two reading frames and are expressed through transcriptional editing. Proceedings of the National Academy of Sciences of the United States of America. 1996;**93**:3602-3607. DOI: 10.1073/pnas.93.8.3602

[47] Sanchez ZY, Yang L, Xu GJ, Crews NT, Peters CJ. Biochemical analysis of the secreted and virion glycoproteins of Ebola virus. Journal of Virology. 1998;**72**:6442-6447

[48] Lee JE, Fusco ML, Hessell AJ, Oswald WB, Burton DR, Saphire EO. Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. Nature. 2008;**454**:177-182. DOI: 10.1038/nature07082

[49] Manicassamy B, Wang J, Jiang H, Rong L. Comprehensive analysis of Ebola virus GP1 in viral entry. Journal of Virology. 2005;**79**:4793-4805. DOI: 10.1128/jvi.79.8.4793-4805.2005

[50] Volchkova VA, Feldmann H, Klenk HD, Volchkov VE. The nonstructural small glycoprotein sGP of Ebola virus is secreted as an antiparallel-orientated homodimer. Virology. 1998;**250**:408-414. DOI: 10.1006/viro.1998.9389

[51] Volchkov VE, Becker S, Volchkova VA, Ternovoj VA, Kotov AN, Netesov SV, et al. GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and Vaccinia virus Polymerases1. Virology. 1995;**214**:421-430. DOI: 10.1006/viro.1995.0052

[52] Khataby K, Kasmi Y, Hammou RA, Laasri FE, Boughribi S, Ennaji MM. Ebola Virus's glycoproteins and entry mechanism. In: Ebola. IntechOpen; 2016. DOI: 10.5772/64032

[53] Towner JS, Rollin PE, Bausch DG, Sanchez A, Crary SM, Vincent M, et al. Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription-PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome. Journal of Virology. 2004;**78**:4330-4341. DOI: 10.1128/JVI.78.8.4330-4341.2004

[54] Sanchez A, Lukwiya M, Bausch D, Mahanty S, Sanchez AJ, Wagoner KD, et al. Analysis of human peripheral blood samples from fatal and nonfatal cases of Ebola (Sudan) hemorrhagic fever: Cellular responses, virus load, and nitric oxide levels. Journal of Virology. 2004;**78**:10370-10377. DOI: 10.1128/JVI.78.19.10370-10377.2004

[55] Volchkov VE, Blinov VM, Netesov SV. The envelope glycoprotein of Ebola virus contains an immunosuppressive-like domain similar to oncogenic retroviruses. FEBS Letters. 1992;**305**:181-184. DOI: 10.1016/0014-5793(92)80662-Z

[56] Groseth A, Ströher U, Theriault S, Feldmann H. Molecular characterization of an isolate from the 1989/90 epizootic of Ebola virus Reston among macaques imported into the United States. Virus Research. 2002;**87**:155-163. DOI: 10.1016/S0168-1702(02)00087-4

[57] Ahmad N, Farman A, Badshah SL, Ur Rahman A, Rashid HU, Khan K. Molecular modeling, simulation and docking study of ebola virus glycoprotein. Journal of Molecular Graphics & Modelling. 2017;**72**:266-271. DOI: 10.1016/j.jmgm.2016.12.010

[58] Syed S, Mabkhot YN, Naeem A, Ahmad N, Badshah SL. Zika virus, microcephaly and its possible global spread. Current Topics in Zika. 2018:1-18. DOI: 10.5772/intechopen.72507

[59] Badshah SL, Ahmad N, Ur Rehman A, Khan K, Ullah A, Alsayari A, et al. Molecular docking and simulation of Zika virus NS3 helicase. BMC

Chemistry. 2019;**13**:67. DOI: 10.1186/s13065-019-0582-y

[60] Ahmad N, Rehman AU, Badshah SL, Ullah A, Mohammad A, Khan K. Molecular dynamics simulation of zika virus NS5 RNA dependent RNA polymerase with selected novel non-nucleoside inhibitors. Journal of Molecular Structure. 2020;**1203**. DOI: 10.1016/j.molstruc.2019.127428

[61] Mire CE, Geisbert TW, Feldmann H, Marzi A. Ebola virus vaccines - reality or fiction? Expert Review of Vaccines. 2016;**15**:1421-1430. DOI: 10.1080/14760584.2016.1178068

[62] Domi A, Feldmann F, Basu R, McCurley N, Shifflett K, Emanuel J, et al. A single dose of modified Vaccinia Ankara expressing Ebola virus like particles protects nonhuman Primates from lethal Ebola virus challenge. Scientific Reports. 2018;**8**:864. DOI: 10.1038/s41598-017-19041-y

[63] Lehrer AT, Wong TAS, Lieberman MM, Johns L, Medina L, Feldmann F, et al. Recombinant subunit vaccines protect Guinea pigs from lethal Ebola virus challenge. Vaccine. 2019;**37**:6942-6950. DOI: 10.1016/j.vaccine.2019.06.035

[64] MME S, KAO M, Steffens JT, Gregory M, Vantongeren SA, Van Hoeven N, et al. Glucopyranosyl lipid adjuvant enhances immune response to Ebola virus-like particle vaccine in mice. Vaccine. 2019;**37**:3902-3910. DOI: 10.1016/j.vaccine.2019.05.026

[65] Rewar S, Mirdha D. Transmission of Ebola virus disease: An overview. Annals of Global Health. 2014:444-445. DOI: 10.1016/j.aogh.2015.02.005

[66] Dhama K, Malik YS, Malik SVS, Singh RK. Ebola from emergence to epidemic: The virus and the disease, global preparedness and perspectives. Journal of Infection in Developing Countries. 2015;**9**:441-455. DOI: 10.3855/jidc.6197

[67] Badshah S, Naeem A, Mabkhot Y. The new high resolution crystal structure of NS2B-NS3 protease of Zika virus. Viruses. 2017;**9**:7. DOI: 10.3390/v9010007