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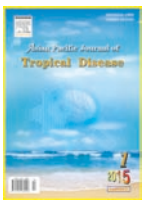

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Ebola virus: bioterrorism for humans

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ABSTRACT

Ebola virus disease is a severe, often fatal, zoonotic infection caused by a virus of the Filoviridae family (genus *Ebolavirus*). Ebola virus (EBOV) spreads by human to human transmission through contacts with body fluids from infected patients. Initial stages of EBOV are non-specific which makes the differential diagnosis broad. Here in this review article we focused on to show the details of EBOV, from its first case right up to the possible targets to cure this lethal disease. In this study we have shown the statistical survey, epidemiology, disease ontology, different genes coding for different proteins in EBOV and future aspects of it.

1. Introduction

Ebola virus disease (EVD) is a severe viral disease that presents with fever and an ensuing bleeding diathesis that is marked by high mortality in human and nonhuman primates. Ebola hemorrhagic fever is one of numerous. Maintaining strategies employed in nature by the various pathogenic agents, and we know much less about the resulting diseases, their pathogenesis, and detailed concepts of the respective agents. Populations unrestricted imagination is captured by the deadliest outbreaks of these viruses and reinforced by the spectre of bioterrorism. As research purpose has gained its pace, it has been keenly observed that Filoviruses use new path to puzzle and fight the immune system[1]. Due to its deadliest nature the death rates are between 50% and 100% and hence Filoviruses are categorized as a biological class four pathogen.

The natural reservoir of the virus is fruit bat, chimpanzees, Gorilla, Porcupines, Bush meat. As a result, little is understood about how Ebola virus (EBOV) is transmitted or how it replicates in its host[2]. Though the key source of infection is unknown, the epidemiological mode of transmission is well defined. A variety of examinations have proven to be precise and very useful for EBOV identification[2]. Yet no Food and Drug Administration approved anti-viral treatment/therapy is launched, where the incubation period of EBOV ranges from 2 to 21 days with no natural immunity existence.

Patients who are competent to an immune response against the EBOV infection will commence to recover in 7 to 10 days and continues for a prolonged improvement.

Since there is no specific treatment for this lethal infection, the effective and supportive supervision and management should be carried out in maintenance of circulatory volume, blood pressure and provision of supplemental oxygen is prime necessity[2]. In almost all outbreaks of EVD, the fatality rate among the residents of Africa is much higher than any other dreadful disease.

2. EVD: first known dreadful disease

EVD caused by EBOV originated from Congo River since 1976. In the year 1995 the case reported 254 deaths out of 315 people affected. Mortality rate was approximately 81%. This case was in village situated near the Ebola River, from which the disease take its name. The virus was first discovered in the year 1976. It is an RNA virus and hence called a negative-sense genome and falls under family Filoviridae[3]. When it infects a cell of the host it makes more virus particles and leads to viral hemorrhagic fevers and is described by a feverish episode, general depression, and nausea that gradually progress into loss of blood and shock that characterizes the deadliest and final disease state and may lead to death[3]. It is a brutal, often fatal disease in human and for other mammals too including (monkeys, gorillas, and chimpanzees) causes' hemorrhagic fever and massive damage to the internal organs[4,5] (Figure 1).

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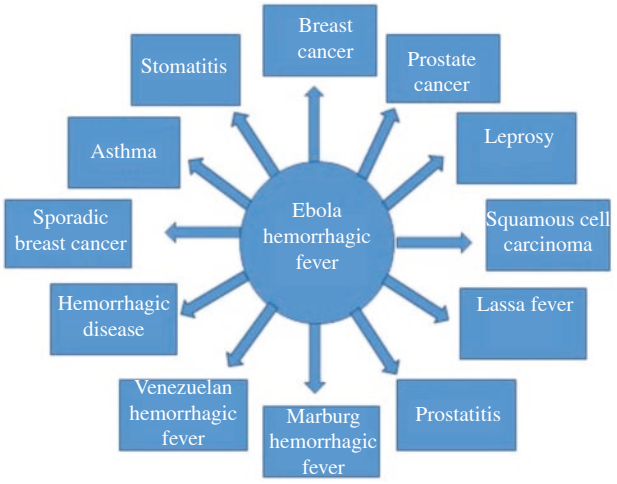

Figure1. Effects of EVD.

Till date five kinds of EBOV: Bundibugyo EBOV, Zaire EBOV, Reston EBOV, Sudan EBOV and Tai Forest EBOV have been reported, off these five only Reston virus affects human body by entering in to cell by host cell interaction mechanism. Recently reported research in gorillas that were affected by an EBOV outbreak shows the disease influences on the reproductive potential, immigration and social dynamics activity[4]. The essential understanding is that this is a zoonosis, with the reservoir being proposed as nonhuman primates (particularly gorillas, chimpanzees, monkeys, and forest duikers), and also porcupines. Finding unusual numbers of these species dead in the forest is a harbinger of an outbreak. In 2007 African fruit bats were found to carry antibody and the Marburg strain RNA genome. The suggestion is that these and perhaps other bats may better fit the definition of 'reservoir', rather than the apes, (because the latter succumb to the infection whereas bats appear not to)[6].

2.1. Statistical analysis

The EVD outbreak reported in February 2014 in parts of Guinea, West Africa and widened its infection reaching to Liberia, Sierra Leone and Nigeria until late July[7] shows an exponential growth rate of 34.8 days. Since, 19th August 2014, 2 240 cases and 1 290 deaths have been recorded, with majorly affected cities of Conakry (Guinea), Freetown (Sierra Leone), Monrovia (Liberia), and Lagos (Nigeria) alarming the distribution of disease at local and international boundaries[8]. The World Health Organization (WHO) reports 4 033 death so far, with 8 399 probable or suspected cases[9,10] where in realty this could be many fold higher due to the under reporting of the disease. A total of 8 033 cases were globally recorded by health map on October 10th, 2014 and suspected to rise 18 391 within the next four weeks[11] (Table 1[12]).

Table 1

Confirmed, probable and suspect cases and deaths from EVD in Guinea, Liberia and Sierra Leone as of 23rd July 2014[12].

	New	Confirmed	Probable	Suspect	Totals by country
Guinea cases	12	311	99	17	427
deaths	05	208	99	12	319
Liberia cases	25	84	84	81	249
deaths	02	60	50	19	129
Sierra Leone cases	71	419	56	50	525
deaths	05	188	33	03	224

2.2. Recent outbreak

1) On October 10th a healthcare worker was tested positive for Ebola at Texas Presbyterian Hospital, was providing a care for index patient reported at hospital[13,14].

2) A second healthcare worker was reported Ebola infection at Texas Presbyterian Hospital on October 14th who provided care for the index patient and reported to the hospital with a low-grade fever and was isolated[14].

3) Entry screening by Center for Disease Control at five US airports travelers from Guinea, Liberia, and Sierra Leone[15].

4) First traveler associated Ebola case was identified on 30th September 2014 in the United States and patient passed away on 8th October 2014[16].

5) Apart from Guinea, Liberia, and Sierra Leone. Nigeria and Senegal did not reported any new case since 5th September 2014,

Table 2

Analysis of Mortality and Affected rates[17].

Month / Year	Epidemiology
September 2014	By September 9, 62 cases and 35 deaths have been reported in the Democratic Republic of Congo.
September 2014	By August 31, 2014 WHO (World Health Organization), reported following mortality. Liberia – 871 deaths out of 1698 cases, Sierra Leone - 476 deaths out of 1216 cases, Guinea – 494 deaths out of 771 cases, Nigeria – 07 deaths out of 21 cases, Senegal – no death reported and even only 01 case is reported
September 2014	Nationwide lock down of Sierra Leone in order to stop the spread of Ebola infection
August 2014	The President of Liberia announces a high alert on a nation-wide and quarantined two community with no movement with respect to their specific area or zone.
August 2014	World Health Organization declares Ebola as an epidemic. The high risk and mostly affected nation Africa is declared as an international health emergency with the deadliest outbreak in the last four decade and needs a global support and approach to fight against it.
July 2014	Withdrawal of health aid workers and volunteers from Liberia, Sierra Leone and Guinea announced by Peace Corps.
July 2014	Death of an American and other aid workers in Liberia, reported positive for Ebola. The victims were infected while treating Ebola patients in Liberia.
July 2014	Death of a first American as a top government official in the Liberian Ministry of Finance dies at a local Nigerian hospital by deadliest Ebola infection.
April 2014	The New England Journal of Medicine published a child died on December 6, 2013, followed by his mother, sister and grandmother over the next month.
March 2014	Pasteur Institute Lyon, France concludes Zaire Ebola virus for 59 deaths out of 86 suspected cases in Guinea

and 29th August 2014[16] (Table 2[17]).

3. Epidemiology

The epidemiological pathways are theoretically considered to be communicable through the following aspects:

1) Any contact with dead or alive infected bats[16].

2) Any contact with dead or living infected primates including dried, cooked, or smoked pieces of meat (bush meat), scientific dissection, or living specimens destined for research[16].

3) Any contact with infected human cases, including nursing, emergency care, all contact with fluids (including blood, tears, semen) infected needles, syringes and contact with the deceased as in autopsy or burial preparation[16].

4) Sexual contact with a recovered case as the virus can be recovered for 7-weeks in semen[16].

4. Disease ontology

A viral infectious disease that is hemorrhagic fever and has material basis in all forms of EBOV such as Zaire, Sudan, cote d'ivoire and Bundibugyo EBOV, where transmission takes place by contact with the body fluids of an infected animal or person, contaminated fomites, or by infected medical equipment[18]. The infection follows with a symptom like fever, headache, joints pain, muscle ache, sore throat, weakness, diarrhea, vomiting, pain in stomach, rashes, redness of eyes, hiccups, internal and external bleeding.

5. Viral genome

Filoviruses are single-stranded, negative-sense, nonsegmented RNA genomes of approximately 19 kb (18.9 kb for EBOV). The gene order is as follows: 3′ leader-nucleoprotein (NP)-viral protein 35 (VP35)-VP40-glycoprotein (GP)-VP30-VP24-RNA-dependent RNA polymerase (L)-5′ trailer. Four of the virion structural proteins, NP, VP30 (transcription factor), VP35 (polymerase cofactor), and L (RNA-dependent RNA polymerase), are associated with the viral genomic RNA to form the ribonucleoprotein complex. These proteins have been shown to be necessary and sufficient for EBOV transcription and replication[19,20] (Figure 2).

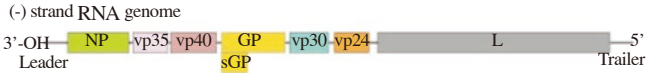

Figure 2. Viral genome[20].

5.1. Nucleoprotein (NP)

The nucleoprotein contains a total length of 739 amino acids which termed it as a largest nucleoprotein among the nonsegmented negative strand viruses. Like other viruses nucleoprotein Ebola nucleoprotein possess same functions in the virus replication process[21].

5.2. Viral protein 35 (VP35)

VP35 is multi purposeful protein, helping as a constituent of the viral RNA polymerase complex, this complex assist as a structural/gathering factor, and holds a function in suppressing of host IFN responses. Since the VP35 is the sole responsible in the process of viral replication, multiplication and pathogenesis knockdown of VP35 in infected mice model reduced its viral multiplication and lethality[22].

5.3. Viral protein 40 (VP40)

The most abundant virion and primary matrix protein found in Ebola is known as VP 40. The maturation takes place at the plasma membrane of infected cells followed by the clustering of viral matrix protein at the assembly site and carries out interaction with the lipid bi-layer[23].

5.4. Secretory glycoprotein (sGP)

It is the fourth gene of the EBOV genome that encodes 2 glycoproteins: the secretory glycoprotein (sGP) and the virion envelope glycoprotein (GP). The molecular proportion of sGP : GP~3:1 (73% *vs* 27%) signifying that sGP is produced extensively with respect to GP. sGP is supposed as nonstructural, secretory glycoprotein, the N-terminal shares 295 amino acids with GP_1 and GP_2. In Zaire ebolvirus sGP substitute for GP_1 and makes a complex with GP_2 which serves as a structural protein[24].

5.5. Glyco protein (GP)

GP is a predicted hair pin loop encoded by GP gene with seven adenosine nucleotides. The common 295 amino acid at N-terminus found on both GP and sGP but shows a distinct C – termini draws a unique model of disulfide bonds and different structure.The dimer form of sGP interacts with GP to form a trimeric structure. This trimeric complex is vital component in pathogenic attachment, fusion and for the purpose of entry in the host[25].

5.6. VP30

VP30 from EBOV acts as an necessary activator role in viral transcription process. In viral particles assembly, VP30 is closely associated with the nucleocapsid complex[26].

5.7. Viral protein 24 (VP24)

VP24 is a secondary matrix protein of EBOV and minor component of virions. It is identified in plasma membrane and perinuclear region in transfected as well as in EBOV infected cells and tightly associated to lipid membranes. VP24 shares a very common structural feature to viral matrix proteins hence it may have a role in virus assembly and budding too[27].

5.8. Polymerase L

A large 250 kDa molecule residing all the required apparatus to synthesize a full c-pped viral mRNA is known as L-polymerase/RNA polymerase L. With the help of host cell machinery/ribosomes inside the viral body RNA polymerase L initiates to copy the (-) strand RNA to make (+) strand copy that imitates the structure of mRNA and completes the translation process[28,29].

5.9. Human protein (NPC1)

NPC1 protein was found in human and encoded by the NPC1 gene and localized on chromosome 18q11, mutation to this gene NPC1 or NPC2 causes Niemann-Pick disease type C, is a exceptional neurovisceral disease in an individual that interrupt intracellular lipid transport, hence increase of lipid products in the late endosomes and lysosomes. Around 95% of people suffering from NPC are found to have mutations in the NPC1 gene[30,31].

5.10. Infection mechanism

The reported expression study of nucleoprotein (NP) from Ebola virus in mammalian cells forms a helical structure and serves a scaffold for the nucleocapsid. The recent finding reports the binding of NP to VP40 is essential for incorporation of nucleocapsid into the virions[32]. The process starts with the binding of GP on to the surface of the lipid–enveloped virions that helps the virion to interact with cellular receptors and fusion of the viral and cellular lipid bilayers[33]. VP40 a viral matrix protein holds a most important structural part of the virion and is critical for virion formation, whereas VP24 a minor member associated protein is involved in nucleocapsis formation also act as interferon antagonist[34,35]. The other proteins which are responsible or act as a facilitator/regulator for replication and transcription process such as nucleoprotein NP, polymerase protein L, and VP30 and VP35, exist in the long axis of the filamentous virion RNA-protein complex[35-37]. The oligomerization of matrix protein and negative sense RNA viruses associated with lipid bilayers tends to form macromolecular complexes. Formation of these macromolecular complexes helps matrix protein to release themselves and further on expressing themselves in mammalian cells promotes to form virus-like particles (VLPs) enveloped in lipid bilayers.

Such form of RNA virus known as negative–sense genome and possess an ability to make more virus particle of its own type on infecting to a particular host cell. Hence when a human is infected by such kind of viruses the tremendous multiplication of virus particle causes a haemorrhagic fever and massive damage to the internal organs[38-47].

5.11. Role of GP

EBOV basically encodes two forms of glycoprotein sGP and GP where sGP is directly transcribed from the viral RNA and second is resulted from transcriptional editing of the glycoprotein ORF which encodes a trimeric membrane bound form glyco protein (GP). The former has unknown function and later is expressed at the cell surface and gets integrated into the virion and traffics/drives the viral attachment and membrane fusion[48]. The expression study reveals the functions of GP and its effects in cell culture on host surface proteins is similar to those observed during viral infection and hence proposed to be the vital determinant of viral pathogenesis.

The effects of GP are due to the highly glycosylated mucin domain region of GP_1 with 150 amino acid in length and contains numerous –N and –O linked glycosylation sites[38,49-55]. Where GP is the sole responsible for the attachment to the host cells and membrane fusion by expressing itself on the virion surface body. Hence, the EBOV GP is a vital protein that can be targeted in vaccine and inhibitor development. Recently a crystal structure of trimeric GP is identified in complex with neutralizing antibody fragment derived from a human survivor of the 1995 Kikwit outbreak. The results outbreak in an experiment where, staining by flow cytometry has linked Ebola infection with a decrease in membrane levels of β1 integrin, a receptor protein that intercede link with the extracellular matrix, as well as major histocompatibility complex 1 (MHC1). Francica *et al.* propose that identification of GP mediated loss of surface protein through steric shielding of surface epitopes[56-58]. This down regulation is neutralized by enzymatic elimination of carbohydrate modification advices that the steric occlusion is mediated by N- and O-linked modification of GP. O-linked glycosylation of the mucin domain may support to an extended conformation of this domain to provide a 150 amino acid residue ling flexible rod that can mask domains in the immediate vicinity. Innate mechanism verifies that GP must localize in closeness to the affected proteins and explaining the types of cell receptors synchronized by this viral protein GP[48,59-61]. This overall mechanism helps the virus in escaping the immune system of the host, whereas the masking of MHC1 by GP may be a strategic condition for evading CD8 T cell-mediated killing of Ebola infected cells[25,62,63]. The natural response to virus infection is a sensational, when encounters with an invading microbe, it responds rapidly by creating interferons and other cytokines which establish an antiviral state. Its effectiveness is highlighted by the fact that every viral genome must encode defensiveness that transforms its activity. Tetherin is a cellular protein whose transcription is induced by interferon and known as interferon-induced gene (ISG) is found on the plasma membrane and in perinuclear compartment of cell, several research groups conformed that presence of Tethrinin cells causes retention retrovirus particle at the cell surface, where Tethered particles are taken into the cell and reducing the viral infection[63,64].

5.12. Mechanism of infection in humans

In humans the infection entry is carried out by the interaction of EBOV GP with human NPC1 protein and hence it is known to be the essential protein for viral entry mechanism. NPC1 is critical filovirus receptor protein and serves a fundamental property of viral receptors: it confer susceptibility to filovirus infection when expressed in non-permissive reptilian cells. NPC1 exclusively binds to viral GP, GP and synthetic single-pass membrane protein by its second luminal domain that holds a viral receptor activity. Experiments confirm that the purified form of NPC1 interacts and binds merely to cleaved form of GP. The cleaved form of GP is produced only inside the cells during entry and virus with cleaved GP can be retargeted as a receptor on to the cell surface. Thus, GP–NPC1 interaction is vital step in viral release into the cytoplasm[65,66]. The EBOV particles first adheres to the attachment factors on the skin and then gets attached to the receptor (TIM-1) possibly, so as to start the process of internalization via macropinocytosis, where the virus enters first in the early endosomes. In the late endosomes the EBOV undergoes cleavage of GP by Cathespin B/L and cleaved GP bind to NPC-1 (Domain C) to further allow the virus to get into cytoplasm. Hence, carrying out replication process[66]. A recent study publish in 2014 reveals that a protein of the EBOV can transform into three different shapes, each with a separate and desire function that is critical to the virus's survival. Each conformation offers a potential target for developing drugs against EVD. VP40 is well known for its creating and releasing of new viral copies from the infected host cells and is also known as one protein three structures. VP40 can alter its shape, causing multiple copies of the protein to come together and make three different assemblies: a butterfly shape composed of two, a ring formed by eight, and a linear structure built from six VP40 molecules. VP40 assumes the butterfly shape inside the infected cells. In the early phase of infection VP40 assembles and transforms its molecular structure in ring form near the cell nucleus, regulating how virus's genetic information is copied. Later this VP40 molecule changes its conformation into the linear structure and plays a crucial role in creation of new copies of the virus[67].

6. Potential drug targets

As early with respect to GP is already discussed as it is the major VP that initially binds to the host protein (NPC1), hence it is one of the prime target in the vaccine and inhibitor development. As VP40 has 3 functions such as travelling inside the cells, regulation of

genetic material and making new viruses are necessary to the EBOV life cycle. Disturbing to any of the corresponding structures or their alterations would severely affect it. Therefore the multi role of VP40 provides and important evidence and draws a path in design and development of novel and potent antiviral drugs.

Currently VP40 is being used in test strips to screen the patients affected by the outbreaks in West Africa. The research team included scientist from the Scripps Research Institute, University of Wisconsin-Madison, University of Tokyo, Research for Advanced Technology Program in Japan. Part of the research was performed at the Stanford Synchrotron Radiation Lightsource's micro beam facility for crystallography. The Stanford Synchrotron Radiation Lightsource Structural Molecular Biology Program is supported by the DOE Office of Biological and Environmental Research, and by the National Institutes of Health, National Institute of General Medical Sciences[68].

7. Information for the future

The actual mechanism of infection to mammals and in humans is not yet identified in depth and its pathogenicity is as much higher compared to other viruses. Till date the performed structural studies and resulted data about the virus pathogenesis has yielded functional of some of the unmapped genes. Additional knowledge of the three-dimensional structure of the virus and its proteins could be helpful in designing inhibitors.

8. Information for now: structural aspects

Most of the known information on ebola viral proteins can be found http://www.pdb.org/pdb/101/motm as it is recently published with the known structural aspect of the virus. Further in future aspect there may be possibility of pharamacological approaches to invade the pathogenesis of ebola viral disease based on the information provided till date.

Conflict of interest statement

We declare that we have no conflict of interest.

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