Survey of clinical features, pathogenesis and therapeutic options for *Ebola* haemorrhagic fever

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ABSTRACT

The genus Ebola virus first was recognized in 1976, when two outbreaks occurred in Zaire and Sudan. Ebola virus disease (EVD) is a highly contagious disease that can affect both human and nonhuman primates: Zaire ebolavirus (ZEBOV), Sudan ebolavirus (SEBOV), Côte d'Ivoire ebolavirus (CEBOV), Bundibugyo ebolavirus (BEBOV) and Reston ebolavirus (REBOV) are five members of the Filoviridae family that can cause haemorrhagic fever. EVD is transmitted by direct contact with contaminated blood or other biological fluids of the infected animals such as chimpanzees, gorillas, fruit bats, monkeys, forest antelope and porcupines found ill or dead or in the rainforest. Ebola is responsible for different clinical futures that can be ranged from fever, headache, arthralgia, myalgia, abdominal pain, anorexia and vomiting to severe respiratory disorders, viral hemorrhagic fever, cardio-vascular disorders and hypovolaemic shock. Although there is no specific treatment for EVD, considerable advances like use of monoclonal antibody, intefron and Favipiravir/T-705 as effective chemotherapeutic agent in treatment of EBV have been made. To date, 25 outbreaks of EVD have been reported. Hence, EVD as a zoonotic disease should be more focused not only in endemic area but also in throughout the world. Awareness of the disease and routes of transmission and also continuous surveillance to combat disease and outbreaks is necessary.

Key words: Ebola virus; Clinical features; Ebola haemorrhagic fever; Therapeutic options**.**

INTRODUCTION

Ebola virus as a causative agent of a severe form of viral haemorrhagic fever has drawn international attention particularly for the largest outbreaks in West African countries. Ebola haemorrhagic fever (EHF) is a zoonosis disease that can affect both human and nonhuman primates and caused by members of the Filoviridae family: Zaire ebolavirus (ZEBOV), Sudan ebolavirus (SEBOV), Côte d'Ivoire ebolavirus (CEBOV), Bundibugyo ebolavirus (BEBOV) and Reston ebolavirus (REBOV) [1]. Ebola virus was first documented in 1976 in Republic of the Congo near the Ebola River [2]; since then, outbreaks have been reported. So far about 25 outbreaks of Ebola have been approximately recognized that all occurring in Africa, with fatality rates of 25% to 90% [3, 4]. Ebola virus disease (EVD) which was known as EHF is a highly contagious anthropozoonotic disease that can cause by direct contact with contaminated blood or other biological fluids of the infected animals such as chimpanzees, gorillas, fruit bats, monkeys, forest antelope and porcupines found ill or dead or in the rainforest [2, 5, 6]. The Ebola virus with a relatively high mortality rate 20-90% (depending on the virus species) and the possibility of misuse as a biological weapon is a serious threat to the worldwide. The natural reservoir host of Ebola virus remains unknown. However, on the basis of evidence and the nature of similar viruses, many researchers believe that this virus is animal-borne and bats are the most likely reservoir [7].

Despite ongoing efforts to recognize the molecular biology and pathogenesis of Ebola virus during the past two decades, there are ambiguities regarding virulence factors, host responses, treatment methods and vaccines of EV. The purpose of this review is to present essential and up-to-date information required to identify, management, prevention and treatment of EVD.

Virology and Pathogenic mechanisms

Ebola virus and Marburg virus are members of the family Filoviridae and is included in the order Mononegavirales[8]. They are similar to families of arenaviruses, bunyaviruses, and flaviviruses which are cause viral hemorrhagic fever. Ebola virus has a lipid envelope derived from the host cell, negative single strand RNA with a 19 kb genome. EV particles have a uniform diameter of 80 nm but can greatly vary in length, with a mean unit length of 12000 nm [9]. The seven genes are ordered in the genome as follows: 3'-UTR- NP (nucleoprotein)– VP35 (virion protein) -VP40-GP (glycoprotein)-VP30-VP24- L (RNA-dependent RNA polymerase)-5'-UTR. All of these genes with the exception of the glycoprotein gene are monocistronic [9, 10]. The glycoprotein or peplomer glycoprotein as a transmembrane surface protein makes up the viral surface spikes in the form of trimers 10 nm in length. The Ebola virus glycoproteins (structural glycoprotein GP1,2) are responsible for binding the virus to target-cells and also aid the virus on immune evasion [10, 11]. The viral RNA polymerase, encoded by the L gene, partially uncoats the nucleocapsid and transcribes the genes into positive-strand mRNAs, which are then translated into structural and nonstructural proteins. The ribonucleoprotein complex of virus composed of the RNA genome, nucleoprotein and four structural proteins VP24 (interferes with interferon signaling), VP30 (virion structural protein 30), VP35 (interferon antagonist) and VP40 (matrix protein and mediates particle formation [10, 12]. The exact mechanism of Ebola virus entry to host cells remains poorly understood but several studies demonstrated clathrin-mediated endocytosis, caveolin-mediated endocytosis and macropinocytosise pathways of entry. Macropinocytosis is the most likely entry mechanism employed by the EV. The early targets of the virus are monocytes and macrophages, dendritic cells, liver cells, fibroblasts, hepatocytes, adrenal cortical cells and endothelial cells. The life cycle of Ebola virus is begun with attaching a virus by GP to specific cell-surface receptors such as C-type lectins, DC-SIGN, or integrins, via fusion of the viral envelope with cellular membranes [13, 14]. Interaction of GP with cell surface receptors can trigger macropinocytosis. Soluble glycoprotein (sGP) is expressed through RNA editing by infected cells and is an important distinction of Ebola virus from other Mononegavirales [15]. Previous studies were reported sGP, but not GP, can bind to neutrophils and inhibit early neutrophil activation while both type of GP can neutralize antibodies. After macropinocytosis, virion traps to early endosomes and fuse with lysosomes then the viral envelope is cleaved. This processing appears to allow the virus to release the viral nucleocapsid within host cytoplasm [13, 15]. Viral transcription is initiated at the leader end of the genome with the binding of the polymerase complex. RNA genome is transcribed into seven monocistronic mRNAs. According to the template, NP mRNA is the most abundant species and the L mRNA is the least abundant. VP30 was shown to be important for transcription reinitiation of subsequent genes and is essential for the viral life cycle while VP24 is an inhibitor for transcription and replication of the Ebola virus genome. The VP24 protein is unique of EV and can influence on genome replication, transcription and nucleocapsid assembly. Replication presumably starts when enough nucleoprotein is present to encapsidate neo-synthetized antigenomes and genomes [16, 17]. Newly synthesized structural proteins and genomes self-assemble and accumulate near the inside of the cell membrane. Virions bud via host ESCRT (endosomal sorting complex required for transport) gaining their envelopes from the cellular membrane which they bud. The mature progeny particles then infect other cells to repeat the cycle. The genetics of the Ebola virus are difficult to study because of EBOV's virulent characteristics [18-21]. As previously mentioned, Ebola viruses have five subtypes including: Zaire (EOBV), Sudan (SUDV), Bundibugyo (BDBV), Tai Forest (TAFV) and Reston (RESTV). Each subtype has specific biological characteristic and virulence [2].

Epidemiology

Ebola virus first was reported in 1976 in two outbreaks that were occurred in Zaire and Sudan. In 1995, an epidemic caused by the Zaire species caused several hundred cases in Kiewit, Democratic Republic of the Congo, and in 2000, the Sudan virus infected more than 400 people in

Gulu, Uganda [3, 4]. To date, more than 25 outbreaks of EVD have been reported [22]. Outbreaks of Ebola virus disease have been confined to Sub-Saharan Africa [23]. The Ebola outbreak in West Africa was first reported by World Health Organization (WHO) in March 2014 is the largest outbreak yet [24]. It started in Guinea in late 2013 then spreading across land borders to Sierra Leone and Liberia, by air to Nigeria (one traveller) and USA (one traveller), and by land to Senegal (one traveller) and Mali (two travellers) [25]. As of October 25, 2014, out of 10,141 suspected cases (5692 laboratory-confirmed cases) and 4922 deaths was reported by WHO and the Centers for Disease Control and Prevention [26]. Twenty seven cases (20 cases from Nigeria, 4 from the United States, and one each from Senegal, Mali, and Spain) were reported as travel-associated cases and localized transmissions [27].

On 24 August, 2014, outbreak occurred in multiple villages in the Democratic Republic of the Congo in Equatorial Africa. The index case was a pregnant woman from Ikanamongo Village who butchered a bush animal. This outbreak had a zoonotic origin different from the current epidemic in West Africa. A total of 66 cases, including 38 laboratory confirmed cases, and a total of 49 deaths were reported. Also EVD was found in United State, Spain, Mali, Senegal and Nigeria due to the mechanisms of travel-related and healthcare transmission [28]. A country can be considered free of Ebola virus transmission when the double maximum incubation period (42 days) has elapsed since the last patient in isolation, became laboratory negative for EVD [29]. According to the World Health Organization update on January 28, 2015, a total of 26290 confirmed, probable, and suspected cases of Ebola and 10890 deaths had been reported from the three West African countries (Guinea, Liberia, and Sierra Leone) where transmission has been widespread and intense. Distribution of cases is including Guinea, 3581 cases and 2381 deaths; Liberia, 10322 cases and 4608 deaths; Sierra Leone: 12387 cases and 3901 deaths [30].

Clinical finding and pathogenesis

As mentioned, EVD is a highly contagious anthropozoonotic disease that caused by exposure with infected blood or body fluids. Disease symptoms start 2 to 21 days after exposure with an average incubation period of 7-10 days [31]. Incubation period may vary according to the route of infection. It has been demonstrated that incubation period for cases of Zaire Ebola virus infection due to injection is 3-6 days; versus 5-9 days for contact exposures however most of patients become symptomatic after 8 to 9 days. EVD is responsible for a spectrum of clinical manifestations that can be ranged from nonspecific symptoms such as flu-like symptoms, sudden high fever, weakness, malaise, headache, diarrhea, and vomiting which can be confused with typhoid fever, malaria, meningococcemia, and other bacterial infections to a multisystem organ failure, viral hemorrhagic fever, hypovolemia, and disseminated intravascular coagulation. In severe course, patients often die within 8 to 9 days. Although patient who survives beyond 2 weeks tends to survive, after 2 weeks may be complicated by arthralgias, ocular disease, tinnitus, pericarditis, orchitis, and suppurative parotitis [31, 32].

In patients with EVD, gastrointestinal abnormalities such as severe watery diarrhea, nausea, vomiting and abdominal pain may develop after about 5 days from the initial non-specific symptoms. Also, the symptoms of respiratory disorders include chest pain, shortness of breath, cough, headache or confusion. Neurological manifestations include prostration, delirium, confusion and coma [33]. The signs of hemorrhagic manifestations in EVD patients can include petechiae, ecchymosis, mucosal hemorrhage (especially in the conjunctiva), oozing from venipuncture sites and post-mortem evidence of visceral hemorrhagic effusions. Several studies indicated that unexplained bleeding manifestations are not always present in EVD while fever (39–40°C) can be as the most common finding in the early phase of the disease and tachypnea as the best predictor of outcome.

Nonpruritic maculopapular rash is usually noted between 5–7 days on the trunk and upper arms, ending with desquamation in the convalescence period. This sign is a valuable diagnostic key and is usually followed by desquamation in survivors [10]. The most common signs and symptoms reported from West Africa during the current outbreak from symptom-onset to the time the case was detected include: fever (87%), fatigue (76%), vomiting (68%), diarrhea (66%), and loss of appetite (65%) [3]. The detailed pathogenesis of the disease is not well known and is sparse [34]. Ebola virus enters through inhalation, parenteral route, mucous membranes and abrasions and breaks in the skin. It seems that the route of infection can be affected the disease course and outcome. Studies on nonhuman primates have shown that Ebola virus has a broad cell tropism and infects many cell types; including monocytes, macrophages, dendritic cells, endothelial cells, fibroblasts, hepatocytes, adrenal cortical cells and epithelial cells. These cells seem to have pivotal roles in dissemination of the virus. However, in vitro studies have also demonstrated that virus can detect in semen, genital secretions fibroblasts, hepatocytes, endothelial cells and adrenal cells of infected patients. Infection can be disseminated via infected monocytes, macrophages and dendritic cells and their migration to the spleen and also, migration of lymph nodes to other tissues [35, 36].

Transmission

Although natural reservoir host of Ebola viruses is unknown, recent studies exhibited that fruit bats may be as a reservoir for Ebola virus species. Animal-to-human transmission may be the result of contact with an infected animal. Ebola virus seems to spread by direct contact infectious secretions with abrasions of the skin or mucosal or by objects such as needles. Therefore, healthcare providers, family and friends who are in close contact with Ebola patients are known as the most groups at risk of transmission. Human-to-human transmission of the virus also may be occurred through direct contact with body fluids of symptomatic patients and/or infected cadavers and leads to outbreaks [32, 37]. It is documented that outbreak of Ebola virus in Sudan and Zaire, Gabon and Democratic Republic of the Congo (DRC) respectively were due to the reuse of contaminated needles, Butchering of a chimpanzee and consumption of infected bats [38, 39]. In general, the role of droplets or airborne transmission in disease is not well known and it was demonstrated among non-human primates in an experimental setting. Laboratory exposure through needle stick and reuse of contaminated needle has been reported [37]. The milestone for controlling of EVD is to interrupt the chain of virus transmission. Early identification and isolation of symptomatic patients and contact tracing and follow-up surveillance of all contacts are important issues for restriction EVD transmission and control the further spread of the infection. In accordance with the maximum incubation period of EVD, suggested time for follow up of contacts is about 21 days. International air travelers and Healthcare workers (HCWs) are important high-risk groups for transmission. Tracking should be done for all of high-risk groups [40, 41].

According to the WHO definition for EVD a suspected case was

1) Any person with abrupt onset of high fever and contact with patient with EVD or contact with a sick/dead animal

2) Any person with abrupt onset of high fever and at least of three of the following symptoms: headache, loss of appetite, swallowing and/or breathing difficulties, anorexia, hiccup, vomiting, diarrhea, unexplained bleeding, aching muscles or joints, lethargy, stomach pain, or any sudden death due to an unknown cause [28].

According to the WHO definition for EVD in category Person under Investigation (PUI) is a person who has both consistent signs or symptoms and risk factors as follows:

1) Elevated body temperature or subjective fever or symptoms, including severe headache, fatigue, muscle pain, vomiting, diarrhea, abdominal pain, or unexplained hemorrhage.

2) An epidemiologic risk factor within 21 days before the initiation of symptoms.

3) Confirm of cases as Ebola virus infection is based on category PUI with a laboratory confirmation of Ebola virus infection [3].

Diagnosis

Early recognition and rapid and accurate diagnosis of Ebola infection is essential to implement appropriate control measures. The initial diagnosis of Ebola infection is based on clinical assessment. Laboratory findings of EVD are classified in 4 classes include:

1) Hematologic findings (thrombocytopenia, leucopenia, granulocytosis, lymphopenia, anemia and coagulation abnormalities)
2) Hepatic findings (transaminitis)
3) Renal findings (elevated creatinine level, proteinuria and hematuria)
4) Metabolic findings (low calcium level) [42, 43].

In general, laboratory detection of EVD is based on two ways measurement of host specific immune responses to infection and detection of viral particles, or particle components in infected individuals. Laboratory diagnosis for EVD is generally done in reference laboratory. Laboratory findings can support the diagnosis of Ebola viral disease but are not specific [32]. The diagnosis of Ebola virus infection is an actual challenge. Due to nonspecific symptoms, particularly early in the course, diagnosis of EVD can often be confused with other more common infectious diseases such as tropical diseases like malaria, typhoid fever, shigellosis, leptospirosis, yellow fever, and Chikungunya fever [44]. Well–equipped laboratory with biosafety level 4 are necessity for laboratory diagnosis of EVD [43]. There are several laboratory techniques developed for diagnosis of EVD including RT-PCR, ELISA, antigen-capture detection test, immunohistochemistry staining, electron microscopy and cell culture. Nowadays, the primary assays for laboratory diagnosis of EVD are RT-PCR and ELISA which can be positive from the third day in blood. RT-PCR is an important method for diagnosis of EVD that can detect virus approximately 10 days after symptoms appear [44]. ELISA is the most generally assay for antibody detection. IgM antibodies can be determined from day 2 after the onset of symptoms and decrease to undetectable levels 30–168 days after infection. Specific IgG antibodies appear after 6–18 days of the onset of the symptoms and stable for years. It is better that all these techniques perform after inactivation of virus. An efficient way to inactivate the virus for antigen and antibody detection is using of heat, gamma irradiation and denaturation of the proteins with guanidinium isothiocyanate [45, 46].

Treatment

There is no specific treatment available for EVD patients despite of considerable advances in chemotherapeutic agents that can be used for increasing survival chance of them. The main therapeutic options are supportive and symptomatic treatment such as preserving fluid and electrolyte balance, blood pressure and maintaining a stable level of oxygen saturation [2, 47]. For as much as this disease may be mistaken with malaria and other tropical diseases, empiric treatment of malaria, broad-spectrum antibiotics, analgesics and antipyretics should be initiated immediately while obtaining the confirmed diagnosis [2]. ZMapp as an experimental monoclonal antibody can be effective in treatment of infected non-human primates. In addition, various chemotherapeutic agents can be used for treatment of EVD such as: recombinant human activated protein C, recombinant nematode anticoagulant protein c2 (rNAPc2), anti-sense phosphorodiamidate morpholino oligomers, broad spectrum nucleoside analog BCX4430, Favipiravir/T-705 and interferons, cyanobacterial lectins cytovirin (SVN), mAbs and Ad-vectored interferon (IFN)-α [13, 15, 48, 49]. Newly developed drugs are including small interfering ribonucleic acids and adenosine analogues that target viral proteins activities. The majority of these therapeutic options exhibited effectiveness in animals but so far, has not been tested in Ebola patients [50].

Prevention

Previously, due to low frequency of Ebola cases the usefulness of an Ebola virus vaccine was disputed but with frequent outbreaks in past decade, vaccine was considered by several companies. In general, there are 3 types of vaccine including live attenuated, killed or inactivated and subunit vaccines. Live attenuated viruses and killed or inactivated in compare with recombinant vaccine have lower immunogenicity. Human adenovirus-type-5, vesicular stomatitis virus, human parainfluenza virus type 3, virus-like particles and recombinant Ebola virus without VP35 are potential vaccine candidates against Ebola virus and also have shown effectiveness in rodents and non-human primates [51, 52]. Human adenovirus-type-5 vectors have been greatest success rate to protect non-human primates from lethal Ebola virus challenge [53, 54].

One of the potential limitations in use of adenovirus based recombinant vaccines is natural host immunity response to adenovirus-type-5 that could potentially interfere in the generation of high titer immunity. There are attempts to utilize CMV and rabies virus as a platform to prepare and test an

Ebola virus vaccine but needs to be further evaluated [55].

CONCLUSION

In conclusion, Ebola virus infection as a zoonotic disease with high mortality rate should be more focused not only in endemic area but also in throughout the world. Unfortunately there is no standard and specific treatment for EVD therefore prevention is the best strategy to control of outbreak and infection caused by this bacterium. Awareness of the disease's symptoms and transmission routes, use of active surveillance systems, rapid identification of cases, contact tracing, isolation of patients, appropriate use of personal protective equipment (PPE) and improvement of community education is essential to combat disease and outbreaks.

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