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EBOLA VIRUS PATHOGENESIS: IMPLICATIONS FOR DIAGNOSIS AND PREVENTION

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ABSTRACT

Ebola virus disease (EVD) declared as "one of the world's most virulent diseases" by WHO was popularly known as Ebola haemorrhagic fever in the past which is usually considered a severe and deadly illness when humans are concerned. It can caused by a virus of the Filoviridae family (genus *Ebolavirus*) and spreads by human to human transmission through contacts with body fluids from infected patients. Common symptoms include fever, profound weakness, diarrhea, abdominal pain, cramping, nausea and vomiting for 3-5 days and maybe persisting for up to a week. Laboratory complications like elevated aminotransferase levels, marked lymphocytopenia, and thrombocytopenia may have occurred. The symptoms progress over the time and patients suffer from various types of diseases like dehydration, stupor, confusion, hypotension, multi-organ failure, leading to fulminant shock and eventually death. Others characteristics of Ebola virus infections are suppression of immune response and a systemic inflammatory response, which further causes impairment of the vascular, coagulation, and immune systems. It can be diagnosed by enzyme-linked immuno-sorbent assay (ELISA), antigen detection tests, serum neutralization test, reverse transcription polymerase chain reaction (RT-PCR) assay, virus isolation by cell culture. Based on above mentioned, the present article aimed to review the pathophysiology, transmission, clinical manifestation, diagnosis, treatment, and prevention of Ebola virus disease.

Keywords: EVD, Risk, Pathophysiology.

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 [1].Ebola virus emerged as a menace for population of West Africa, which has global consequences through risk of imported infections and mishandling for biological terrorism. Fatality rate of African inhabitants is very high, around 90%, due to non-availability of any type of treatment [2]. Ebola hemorrhagic fever (EHF) was first reported in 1976 with two concurrent outbreaks of acute viral hemorrhagic fever centered in Yambuku (near the Ebola River), Democratic Republic of Congo, and also in Nzara, Sudan. There have been almost 20 other outbreaks that involving nearly 2500 cases happened before 2014 [3]. Ebola hemorrhagic fever (EHF) is caused by any of five genetically distinct members of the *Filoviridae* family: *Zaire ebola virus* (ZEBOV), *Sudan ebola virus* (SEBOV), *Côte d'Ivoire ebolavirus* (CEBOV), *Bundibugyo ebolavirus* (BEBOV) and *Reston ebolavirus* (REBOV) [4]. 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. 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 [1].

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Pathophysiology of Ebola Virus Disease:

At the entry site into the body, MARV and EBOV are capable to infect macrophages and other cells of the phagocytes system (Fig.1 and Fig.2). Macrophages *in vitro* are highly susceptible to infection and produce a large number of viral particles, and hence serve as a vehicle to deliver the virus to a variety of organ systems such as liver, endothelium, spleen, lymph nodes, kidney, adrenal gland, and pancreas [6]. *Ebola virus* belongs to the *Filovirus* family, characterized by membrane enveloped filamentous particles in the shape of as hepherd's crook or in the shape of a "U" or a "6". It is comprised of three components namely viral envelope, matrix and nucleocapsid. The viral envelope is derived from cell membrane of the host during the budding process and it is here that the underlying viral encoded glycoprotein's (GP) insert during transcriptional editing [7]. Marked leukopenia with a left shift and atypical lymphocytes can be observed on peripheral smears of infected patients. Since lymphocytes are not assumed to be host targets for the virus, a substantial reduction in the number of lymphocytes is supposed as a result of bystander apoptosis, showing the death of a large number of lymphocytes triggered by mediators which are released from virus infected target cells and/or secretion of viral GP. Impaired production of pro-inflammatory cytokines and impaired stimulation of T cells also play a role in this phenomenon [6].

After entry in the human body, EBOV mainly affects:

Lymph nodes:

Here it leads to infection of macrophages and dendritic cells and results in the lymphocytes depletion and host immune response impairment.

Liver:

In liver, it leads to the infection and necrosis of hepatocytes through the damage of the endothelial cells that are responsible for the formation of the lining of the blood vessels and leads to difficulty in coagulation of the infected individual's blood. As the platelets would not be able to coagulate, this result in hypovolemic shock or decrease in blood pressure and death may also occur.

Adrenal gland:

Causes infection and necrosis of adrenal cortical cells. As a result, synthesis of steroids is impaired [2]. In most outbreaks, Ebola virus is introduced into human populations via the handling of infected animal carcasses. In these cases, the first source of transmission is an animal found dead or hunted in the forest, followed by person-to-person transmission from index case to family members or health-care staff (Fig. 3). Animal-to-human transmission occurs when people come into contact with tissues and bodily fluids of infected animals, especially with infected nonhuman primates [11]. Transmission has been reported in Côte d'Ivoire where an ethologist was infected through handling an infected, dead chimpanzee in the Taï Forest [12]. It was confirmed that the deaths of chimpanzees were indeed due to Ebola virus. In Gabon and the Republic of the Congo, outbreaks in humans were associated with extensive deaths of chimpanzees and gorillas [13]. In contrast, the animal source of infection during the DRC, Uganda and Sudan outbreaks has never been detecte [4].

Ebola is one of the zoonotic viruses that can lead to a high fatal disease in human beings. Humans are also one of the accidental hosts and can be infected through close contact with blood and bodily fluids of another infected case (including humans and animals), either by direct contact or indirectly from a contaminated environment. It seems those mosquitoes and other insects do not play a role in the virus transmission and also it is not spread through the air. Ebola virus has high transmissibility and virulence so that less than 10 viral particles are enough for becoming infected. The incubation period range is from 2-21 days (average 5-6). Fortunately the disease is not transmissible until the patient becomes symptomatic, but it continues to be contagious, even postmortem. Family and healthcare providers caring the Ebola patients are at the highest risk for becoming infected because of their possible contact with contaminated blood or body fluids. So the virus can easily spread if reasonable preventive precautions are not taken [3].

EBOV and MARV are regarded as re-emerging and highly infectious pathogens. Outbreaks have been associated with human sporadic cases, involve high rates of case-fatality and cause social and economic disruption. The substantial clinical appearance of both EBOV and MARV with severe hemorrhaging in most cases has also contributed to the high transmission rate and the fear of epidemic and imported cases. According to the US CDC, EBOV and MARV have been classified as Category A bioterrorism agents due to their highly infectious nature and potential use in biological weapons [6].

DIAGNOSIS

Except Ebola virus, there are other types of viruses that can cause hemorrhagic fever, including Crimean-Congo hemorrhagic fever virus, Marburg virus, Lassa virus, and emerging ones such as Lujo virus. These viruses have particular public health importance because of their spread ability to careers and healthcare workers, difficulties in their rapid recognition, the lack of effective specific therapeutics, and high fatality rate [3]. If EVD is suspected, thorough medical history including travel and work history as well as any exposure to wildlife in recent past is important to investigate. Laboratory tests that may be indicative include basic blood tests – Complete blood count (CBC) with differential, liver enzymes, bilirubin,

creatinine levels, blood urea nitrogen (BUN) and pH. Diagnosis is confirmed by isolating the virus and detecting its RNA and proteins or detecting antibodies against the virus in a person's blood (Fig.4). Isolating virus by tissue culture (to be performed only in high-containment laboratories), detecting viral RNA by polymerase chain reaction (PCR) and antigen detection by enzyme-linked immune-sorbent assay (ELISA) are effective early and in those who have died from the disease. Serologic testing for demonstrating immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against the virus is effective late in the disease and in those who recover [7]. Centers of Disease Control and Prevention (CDC) have performed standardized enzyme-linked immune-sorbent assay (ELISA) for detection of Ebola virus specific antibodies. This test has high sensitivity and can be used for detecting antibodies in human beings even after 10 years exposing to the virus [3].

Management considerations for EVD:

There are eight steps for the management of EVD that are given below:

1. Prepare
2. Assess the patient
3. Gather appropriate consultants and team
4. Determine the appropriate level of infection control measures
5. Provide additional communications
6. Therapeutic approaches

1. Prepare: Occupational:

Health clinics should develop the methods of assessing the need for isolation and laboratory decontamination, exit, and notification procedures for the patients. They should maintain a close relationship with bio-safety office; to be update with the agents emerged for the management of the EVD. It should be decided in advance that where the patient would be kept isolated, observed and where the treatment will have been carried out. One person should always be available there to observe the patients.

2. Assess the patient:

Assess the patient for the symptoms of EVD i.e. fever (104°C), muscle pain, weakness, headache, vomiting, diarrhea, abdominal pain or hemorrhage and also assess if the patient has a potential exposure from travelling to a country with widespread Ebola transmission or having contact with an Ebola patient in the 21 days before illness onset. If fever or compatible Ebola symptoms and an exposure are present, then suspect Ebola.

3. Gather Appropriate Consultants and Team:

A primary physician should be designated to develop the plan of isolation and treatment in consultation with other experts. Another person should be designated to coordinate with other activities such as arranging conferences with external experts, who handles media inquiries, issuing press releases, and interacting with external agencies surrounding a high profile exposure, to free the primary physician for the care of the patient [2].

4. Determine the Appropriate Level of Infection Control Measures:

Initial assessment and management:

- Isolation of infected patient in separate room.
- Notify the suspected person to hospital Infection Control Program and other appropriate staff.
- Evaluate for any risk exposures for Ebola of the Returned Traveler.
- Consider, test for, and treat (when appropriate) other possible infectious causes of symptoms (e.g., malaria, bacterial infections).
- Provide appropriate care including IV fluid resuscitation if needed.
- Assessment of bleeding, hematological and coagulation parameters in patient.

Assessment for High-risk exposures:

- Percutaneous (e.g. needle stick) or mucous membrane exposure to blood or body fluids from an EVD patient.
- Direct contact of skin with skin, blood or body fluids from an EVD patient.
- Processing of blood or body fluids from an EVD patient without appropriate protection
- Direct contact with a dead body with Ebola-affected area without appropriate precaution

Assessment for Low-risk exposures:

- Household members of an EVD patient or others who had brief direct contact (e.g., shaking hands) with an EVD patient without appropriate Personal protective equipment (PPE).
- Facilities of healthcare personnel for EVD patients who have been in care areas of EVD patients without recommended PPE [8].

5. Provide Additional Communications:

As EVD is one of the major infections, so there is need to educate and inform the people about this disease proactively. There is need to develop press releases and arrange conferences in conjunction with a medical or scientific experts for the need of public affairs personnel. We can learn lessons from the tularemia exposures at Boston University and Russian researcher's death from infection with Ebola virus after a delay in disseminating that information.

There is need to maintain the regular communication with the laboratory's work force and to update the modifications in the procedures and infection control practices for medical care personnel [5].

6. Therapeutic approaches:

As there is no specific treatment or vaccine for Ebola, so for the severely ill patients symptomatic treatment and intensive care must be given. Following therapeutic approaches are adopted for the Ebola Virus disease patients: For high grade fever patient should be treated with only paracetamol tablet and avoid aspirin.

Palliative care:

Due to repeated vomiting and diarrhea patient may present with shock and electrolyte imbalance so plenty of oral fluids must be given for the maintenance of electrolyte balance (for example, potassium supplement), kidney and liver function support

Symptomatic treatment:

Painkillers, anti-emetic against vomiting, anxiolytics to combat anxiety, antibiotics, anti-malarial remedies must be provided according to the need of the patient.

Intensive care:

Patient may require ICU support for breathlessness (provide oxygen) due to lung involvement or critical condition

In case of severe bleeding and if intravenous therapy is an option then transfuse blood or previously tested blood components i.e. RBC's, platelet concentrates and fresh frozen plasma.

Proper use of equipments to monitor biochemical and blood values of patients to maintain the electrolyte balance.

EVD patients with high blood pressure, diabetes, coronary artery diseases (CAD) and pregnancy should be carefully treated.

At the state of current knowledge, serotherapy is not recommended for the treatment of Ebola or Marburg [9].

Treatment:

Managing Ebola patients in the African setting was a major challenge because there was no effective antiviral drug and no specific vaccine available. Only supportive care could be administered, to sustain cardiac and renal functions with prudent use of perfusion. Oral rehydration was recommended but sometimes not realistic because of throat pain, vomiting and intense fatigue. The main objective was to provide optimal care to the patient with maximum protection of the medical and nursing staff. For that purpose, medical and nursing staff had been trained in donning and removing personal protective equipment (PPE) and applying barrier–nursing procedures [4]. There is no specific antiviral agent or vaccine against Ebola viruses. Therefore supportive care is the most important aspect of its management.

Aggressive prevention of intravascular volume depletion is critical to avoid life-threatening complications by using proper fluid therapy, oxygen therapy, correcting profound electrolyte abnormalities, and preventing the complications of shock such as acid–base derangements. Treatment of other infections, if they occur, and close monitoring of vital signs and regular biochemical and blood gas check should also be done. These proceedings are considered as the foundation of critical care medicine and should be applied in both resource-rich and resource constrained settings. With improving supportive cares, EHF outcomes may also improve. Symptom control with taking narcotics and benzodiazepines were often reported as the end-of-life therapy in some patients [3].

Therapeutic agents under consideration for treatment or prevention of EVD include the following:

• Estrogen receptor drugs used to treat infertility and breast cancer (clomiphene and toremifene)
• Favipiravir has proved useful in mouse models
• Nucleoside analogue inhibitors of S-adenosylhomocysteine hydrolase (SAH)
• Interferon beta
• Recombinant human interferon alfa-2
• Recombinant inhibitor of factor VIIa / tissue factor
• Activated protein
• Horse- or goat-derived immune globulins
• Human-derived convalescent immune globulin preparations
• Recombinant human monoclonal antibody against the envelope glycoprotein (GP) of Ebola virus
• DNA vaccines expressing either envelope glycoprotein or nucleocapsid protein genes of Ebola virus [7].

Recently, a great attention has been paid to unlicensed treatments and vaccines. A "cocktail" of humanized-mouse antibodies (ZMapp) is among the therapies in development, showing promise in nonhuman primates. Two US citizens who recently evacuated from Liberia to Atlanta were given ZMapp and both patients' demonstrated clinical improvements. Other candidate therapeutics covers RNA polymerase inhibitors and small interfering RNA nano-particles that are inhibitors of protein production. The results obtained from gene-silencing treatment using small interfering RNAs have been good both in guinea pigs and non-human primate models of Ebola infections [6].

Prevention

All possible measures should be undertaken to reduce exposure to virus and prevent transmission in a health care setting.

The following means should be adopted:

Assessment of risk:

Risk assessment of patient is the primary step to determine the level of appropriate protective measures that need to be taken. Higher risk of exposure is present in cases that are convalescent, in later stages of infection, when patient is incapable of self-care and procedures requiring contact with blood and fluids.

Source control:

Place suspected or confirmed Ebola patients' incomplete isolation. Patients should be advised on proper respiratory hygiene, cough etiquettes and hand hygiene after toileting and vomiting. Only essential health care workers (HCW) entry with proper personal protective equipment (PPE) should be permitted in patient area. Trained personnel should be deputed for monitoring proper use, removal and disposal of PPE to avoid spread outside the patient's room. AGMPs should be minimized and if at all needed should follow airborne precautions including use of respirators rather than masks. Strategies should be undertaken to prevent generation of aerosols.

Personal protection measures:

HCWs should be properly trained and educated towards hazards of exposure to blood, bodily fluids and contaminated surfaces. Standard infection control measures including basic hand hygiene measures should be the minimum precautions that are exercised in a health care setting. Level of desired protection is guided by risk assessment and expected contact with potential pathogens and fluids. PPE should be donned properly in a clean place**.** Workers should receive proper instructions, training and supervision in this regard. Removal of PPE is also equally important and should be done correctly to prevent contamination. To prevent self-contamination, mucous membranes of eyes, nose and mouth should not be touched by hands.

Patient transportation:

Transportation of patient in the hospital premises should be kept to a bare minimum and only when essential. Transportation should follow a direct route with entry restricted to other individuals. Before transportation, patient's clothing and bedding should be changed. Transportation personnel should wear proper PPE. Receiving area for the patients should be minimally occupied and all workers here should also use PPE.

Sharps handling:

Restrict the use of needles or sharps. Used needles need not be recapped and should be disposed off immediately in puncture proof containers. Caution should be exercised while disposing to avoid injury.

Precautions in laboratory:

Sample collection and transportation should follow hospital laid down protocols. Samples should be sent to only high containment laboratories where lab personnel are also properly trained to handle the samples. Prior notification should be sent to the laboratory about the probable diagnosis.

Dedicated patient equipment:

Non critical equipment should be dedicated for single patient use and preferably be disposable. Their disposal should be in a container labeled as 'no touch bio hazardous'.

Equipment processing:

Semi critical and critical items should be processed separately following routine sterilization protocols. Handle used linen, cutlery and equipment in a way that minimum exposure to skin and mucous membranes occurs and transfer of pathogens to other patients and surfaces is prevented. Soiled objects should be disposed in a no touch bio hazardous container.

Environment cleaning:

Environment should be thoroughly cleaned and disinfected on a regular basis. Frequently touched surfaces should be disinfected more frequently with a broad spectrum virucidal disinfectant. Like all other personnel, cleaning staff should also get education and hands on training courses and adopt proper PPE protocols.

Handling deceased patients:

Proper PPE should be worn when handling dead bodies as even at this stage contamination is a possibility. Devices like catheters and endotracheal tubes should not be removed but wrapped along with the body in high quality plastic shroud. Double protection by wrapping in leak proof body bag is provided. Its exterior surface is rubbed with a surface disinfectant. Body bag should not be reopened.

Patient education:

Patients should be educated towards the need for prevention, preventive measures undertaken, duration for prevention and a special emphasis on hand and respiratory hygiene.

Visitor education and management:

Visitors should also be educated, monitored and screened for EVD prior to entering the hospital. They should not be permitted into the patients' room rather should be restricted to the patient care area or waiting room. Visitors should also wear PPE in accordance with the hospital guidelines [7].

Figure 1. Pathogenesis of Ebola Virus

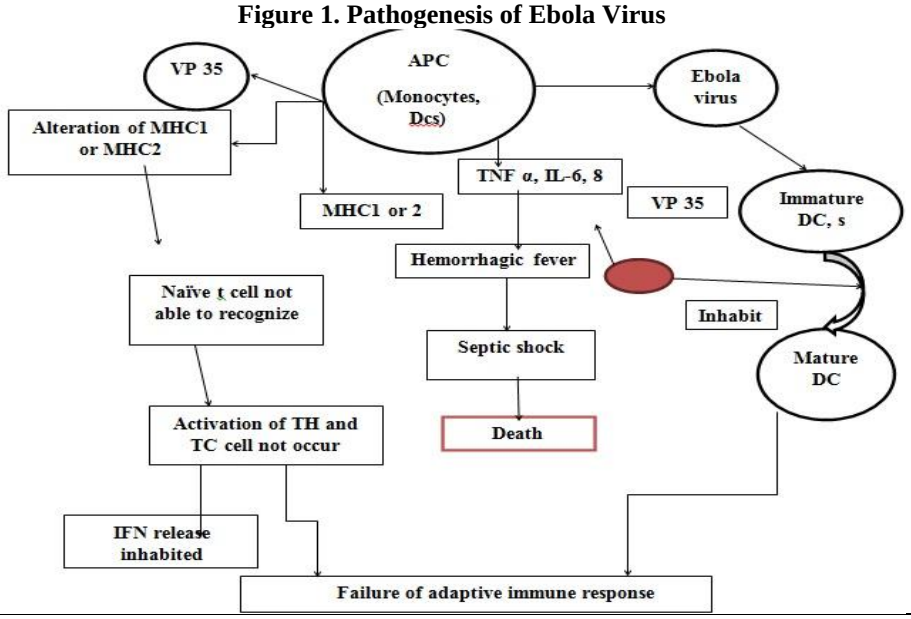


Figure 2. Diagram demonstrating the pathogenesis of Ebola virus infection

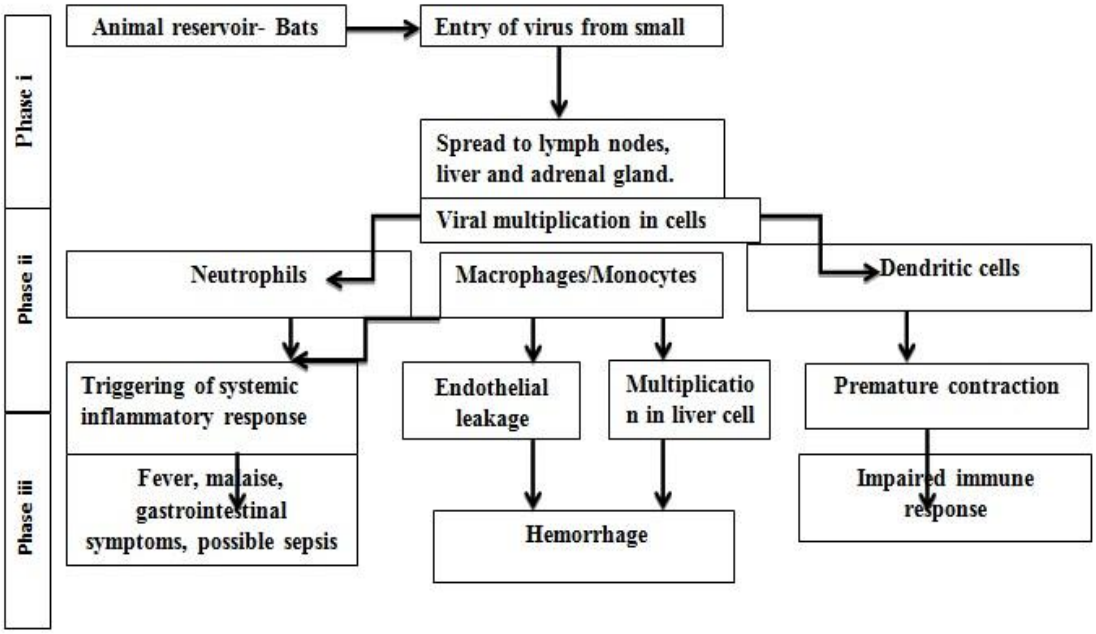


Figure 3. Transmission of Ebola Virus

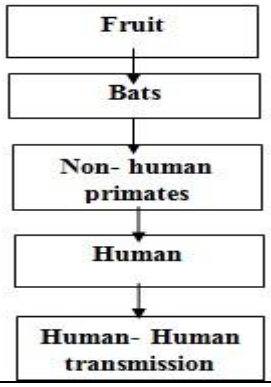
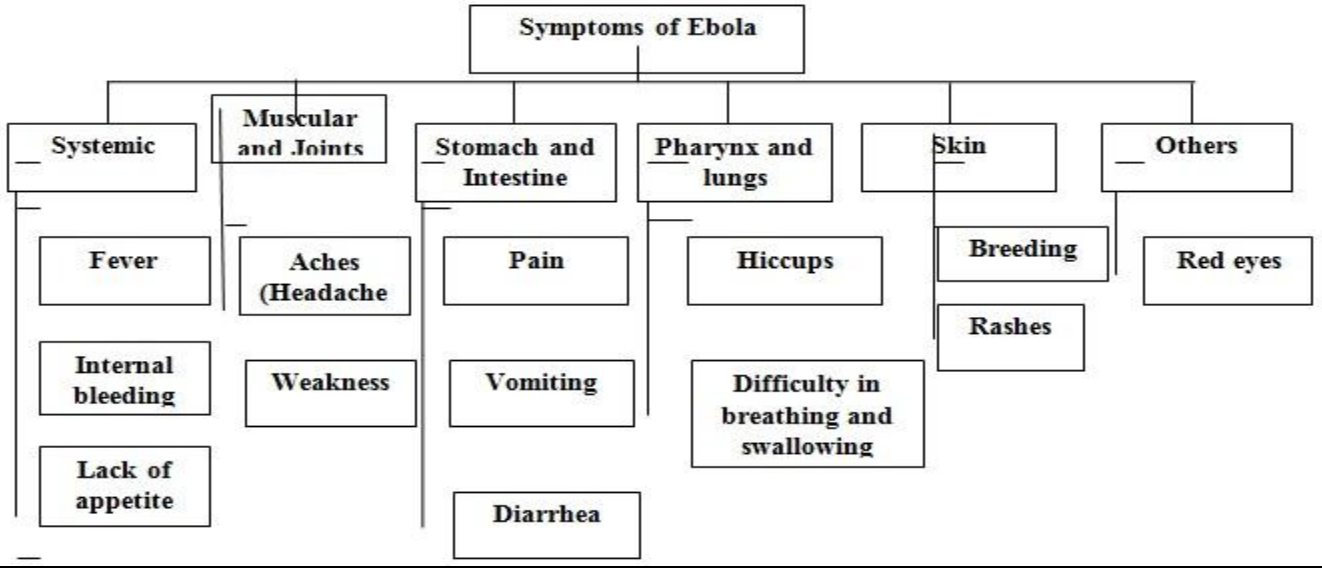


Figure 4. Symptoms of Ebola.



DISCUSSION

A tremendous and rapid research is being carried out in combating the infectious Ebola virus. Till now, we have not come up with any licensed vaccine and medications to fight with this debilitating disease [2]. Ebola virus disease (EVD), a filovirus hemorrhagic fever, is often a devastating disease in humans as it involves the body's vascular system resulting in significant internal bleeding and multi-organ system involvement [7].

The Global Health Security Agenda seeks to enforce public health systems in most affected countries in order to eliminate the spreads before they become emergencies. Although great improvements have been achieved over the past decade, better surveillance, real-time sharing of data and taking rapid action based on the available information remain necessary [6].

Previous epidemics were detected after a long delay, especially because of the remoteness of the epidemic focus, the lack of laboratory facilities and the poor knowledge of the disease by doctors and nurses, who confused Ebola disease with malaria or typhoid fever [4].

CONCLUSION

Ebola virus disease (EVD) is frequently an overwhelming disease in humans as it involves the body's vascular system consequential in significant internal bleeding and multi-organ system dysfunction. Most Ebola outbreaks have been reported in equatorial Africa and have remained a cause of great concern and fear across the world owing to their high lethality, as high as 90% and risk of spread due to transport exportation. Ebola virus is highly infectious, because of its potential aerosol and droplet transmissibility and it is included in the 'category A' of bio- terrorism agents. Till now, no effective prophylaxis, anti-viral treatment, or vaccinations have been invented for this fatal disease. Hence, increasing awareness of risk factors for Ebola infection and understanding protective measures which individuals can take is perhaps one of the most effective ways to reduce human infection and death.

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