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An overview of Crimean- Congo Hemorrhagic Fever in Iran

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ABSTRACT

Crimean- Congo Hemorrhagic Fever (CCHF) is a viral zoonotic tick-born disease with a mortality rate of up to 50% in humans. After a short incubation period, the disease is characterized by sudden fever, chills, severe headache, dizziness, back, and abdominal pain. Additional symptoms can include nausea, vomiting, diarrhea, neuropsychiatric, and cardiovascular changes. In severe cases, hemorrhagic manifestations, ranging from petechiae to large areas of ecchymosis develop. The CCHF Virus (CCHFV) is from the genus Nairovirus and family Bunyaviridae. CCHFV is transmitted to humans by the bite of infected tick and by direct contact with blood or tissue from infected humans and livestock. In addition to zoonotic transmission, CCHFV can be spread from person to person and is one of the rare hemorrhagic fever viruses able to cause nosocomial outbreaks in hospitals. CCHF is a public health problem in many regions of the world e.g Eastern Europe, Asia, Middle East, and Africa. The history of CCHF in Iran shows that the disease has been detected in Iran since 1970. From 1970 to 1978 some scientists worked on serology and epidemiology of this disease in humans and livestock in Iran. Since 1999 , establishment of a surveillance and laboratory detection system on viral hemorrhagic fevers particularly on CCHF has had benefits. One of which is the fact that a mortality rate approaching 20% in the year 2000 remarkably dropped to 6% in the year 2007.

Keywords: CCHF, Arboviruses, Iran.

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) may have been reported as early as 1110 AD. In the thesaurus of the Khwarazm, compiled by the Dzhurzhoni, a disease in Tajikistan similar to CCHF, transmitted by an arthropod was described (1) . The disease was first characterized in the West Crimean region of the former USSR in 1944 (2). In 1956, a virus was isolated from the blood of a patient in the Belgian Congo and became the prototype of the Congo virus (3- 4). In 1969, Casals antigenically demonstrated similarity between the Crimean and the Congo prototypes (5) , and then the name Crimean-Congo Hemorrhagic Fever virus gradually took acceptance.

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Crimean- Congo haemorrhagic fever in Iran was first reported in 1970, where 45 of 100 sheep sera that were sent from the Tehran abattoir to Moscow (Institute of Polio-myelitis and Viral Encephalitides) reacted positively for CCHF virus infection (1). In 1974, a type of typhoid in 60 suspected cases with haemorrhagic syndromes in eastern Azerbaijan was described, and it was suggested the disease had been CCHF (6). In 1974 – 1975 , more clinical cases in this area were reported (7). In 1975 , a large scale serological study was performed in cooperation of Tehran university with Yale University, mostly in the northern half of Iran. In this study, where agar gel diffusion precipitation tests were used, 13% of human sera, 38% of cattle sera and 18% of sheep sera were seropositive for CCHF (8). In 1978, CCHFV was isolated for the first time from the tick *Alveonasus lahorensis* in the north eastern region of Iran (9). Subsequently, there was no report of the disease until 1999 , when several cases were seen in different provinces, mainly Chaharmahal-va-Bakhtiari province south-west of Iran (10 - 11).

In 2000 , CCHF was recognized as a major public health problem and with establishment of the laboratory of Arboviruses and Viral Hemorrhagic Fevers as a National Reference Laboratory in Pasteur Institute of Iran (member of National Expert Committee on Viral Hemorrhagic Fevers) and according to the protocols from the committee, all human, livestock and ticks suspected of having CCHF have been sent to the laboratory, and all provinces of Iran were monitored for CCHF with rapid and free of charge detection. From 2000 till now (October 2008), Chinikar and colleagues demonstrated the prevalence of CCHF infection in Iran and also showed that 24 provinces out of 30 provinces of Iran are infected with CCHF virus, although Sistan-va-Balouchistan, Isfahan, Fars, Khozestan are the most infected provinces of Iran in that order. In 2002, CCHF virus genome was detected in 22.3% of ticks collected from Chaharmahal-va-Bakhtiari province, southwest of Iran. In 2004, after a report of a confirmed human case in Hamadan province, western region of Iran, demonstrated CCHF virus infection in 11.3% of ticks and nearly 30% of the livestock were seropositive. With respect to the 2003 - 2004 study done in Sistan-va- Balouchistan province, among 285 human volunteers, 6.3% appeared seropositive for the CCHF virus infection. A seroepidemiology survey among the livestock population of Isfahan province between the years 2004 - 2005 demonstrated 56% seropositivity. In the years 2003 to 2005 , of the 448 livestock sera collected from Khorasan province, northeast of Iran, 77.5% of 298 sheep samples and 46% of 150 goat samples were sero positive which suggested to be a hyper enzootic region for CCHF. By isolating and analyzing the CCHF virus genome, phylogenetic relationship of the virus strains circulating in Iran were determined on the basis of the (S) segment of the genome in 2004. It was also demonstrated that Iranian strains are very similar to the Matin strain of Pakistan. In 2006, recombinant antigen of CCHF virus was produced by Semliki Forest Virus expression system. The recombinant antigen is used for ELISA serological diagnosis of CCHF. The advantage of which is that its production does not need biosafety level 4. In recent years, some research projects on different aspects of CCHF are progressively being performed in the National Reference Laboratory (10-16).

Crimean- Congo Hemorrhagic Fever (CCHF) , as a viral zoonotic disease, can develop into a severe hemorrhagic fever in humans resulting in death rates of 13-50 %. The disease is caused by CCHF virus (CCHFV) which is a member of the genus Nairovirus and family of *Bunyaviridae* (17 - 20). The virus is enveloped and possesses a tripartite, negative-sense, single- stranded RNA genome. The small (S) segment encodes a nucleocapsid protein (NP) , the medium (M) segment encodes a glycoprotein precursor, resulting in the two envelope glycoproteins, G1 and G2, and the large (L) segment encodes an RNA- dependent RNA polymerase (13, 21- 22).

The virus is transmitted to humans through the bite of *Ixodid* ticks or by contact with blood or tissues from infected livestock (23). Moreover, it has been demonstrated that most statistics about transmission routes in Iran related to people indicate close contact to tissue and blood of affected livestock (13). In addition to zoonotic transmission, CCHFV can be spread from person to person and is one of the rare hemorrhagic fever viruses able to cause nosocomial outbreaks in hospitals (14, 24 - 26). As ticks are efficient in the cycle of CCHFV, the geographical distribution of CCHF cases corresponds most closely with the distribution of *Hyaloma* ticks, suggesting their role as the principal vector. Although other *Ixodid* ticks can be infected, some species of the *Hyaloma*, *Dermacentor*, and *Rhipicephalus* genera have been shown to be capable of transstadial transmission (i.e., passing the virus from larva to nymph to adult) of CCHFV after feeding on a viremic host. Transovarial transmission (i.e., passage of virus to offspring) of CCHFV has also been shown to occur with some of the species in these genera. Although virus can persist in ticks, vertebrates are needed to provide blood meals for the ticks (27 - 30). It is worth mentioning that there was no efficient vaccine against the CCHF virus till now, although there have been some studies on the development of a subunit vaccine (31) .

EPIDEMIOLOGY OF CCHF IN IRAN AND ELSEWHERE

Since CCHF was first discovered in the Crimean region of Russia in the 1940s, the disease has been reported in many regions of Africa, the Middle East, Europe, and Asia. It has also been reported in parts of Europe including southern parts of the former USSR (Moldova, Ukraine and Transcancasus), and in central Asian countries (Tajikistan, Turkmenistan,

Uzbekistan, Kyrgyzstan, and Kazakhstan) Turkey, Bulgaria, Greece, Albania and Kosovo, province of the former Yugoslavia. The initial recognition of hemorrhagic cases in Africa occurred in 1960s, resulting in a series of in-depth studies in South Africa and reports of additional outbreaks from Congo, Mauritania, Burkina Faso, Tanzania, and Senegal (25, 31- 35).

A surveillance and control program of CCHF in Iran was established by three collaborating organizations in 1999 including the Center for Disease Control (CDC) at the Ministry of Health (MOH), Pasteur Institute of Iran (PII) (with the establishment of Arboviruses and viral Hemorrhagic-Fevers Laboratory known as a National Reference Lab) and the Veterinary Organization, all organized at the National Level. These three organizations instituted a National Expert Committee on Viral Hemorrhagic Fevers (NECVHFs). This committee has been dedicated to all activities related to control, awareness, diagnosis, treatment, etc. Universities and public health centers located in different cities, categorized as Level II, have also been dictated lots of health care and administrative activities.

Patients screened as probable cases through the NECVHFs protocol issued by Chinikar et al. , (2008). During the course of the disease, the sera samples are taken three times respectively at the onset of the disease, 5 days after the onset and 10 days after the onset. According to the protocol, the sera are sent to the Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory) , the laboratory informs the CDC of Iran in order for them to treat the CCHF patient with Ribavirin and supportive medication free of charge. Also, the National Reference Laboratory has been conducting several research projects such as serological and molecular epidemiology of the CCHF virus in the country (some of which has been mentioned above) including production of recombinant antigen through a mammalian cell expression system used for ELISA IgM and IgG detection (14), and development of a subunit vaccine against CCHF virus.

Since the outbreak in Chaharmahal- va- Bakhtiari in 1999, the complete distribution map of CCHF in Iran has been extensively identified by the laboratory of Arboviruses and Viral Hemorrhagic Fevers. According to the findings, 24 of 30 provinces of Iran have been shown to be affected by CCHF. Among infected provinces, Sistan- Va- Balouchistan, Isfahan, and Fars provinces ranked the highest rate of infection, respectively. It is necessary to mention that some outbreaks are seen in the country like the recent outbreak in Larestan region of Fars province.

CLINICAL FEATURE AND PATHOGENESIS

Human infection with CCHF virus results in severe hemorrhagic disease. The main course of CCHF has been noted by authors as progressing through four distinct phases including incubation, prehemorrhagic, hemorrhagic, and convalescence (1). The incubation period is variable and influenced by the route of exposure from 1 to 3 days with a maximum of 9 days when infection is caused by the bite of a tick, and from 5 to 6 days with a maximum of 13 days when the infection is due to contact with infected tissue or blood (34 - 35). After the incubation period, the prehemorrhagic period is characterized by a sudden

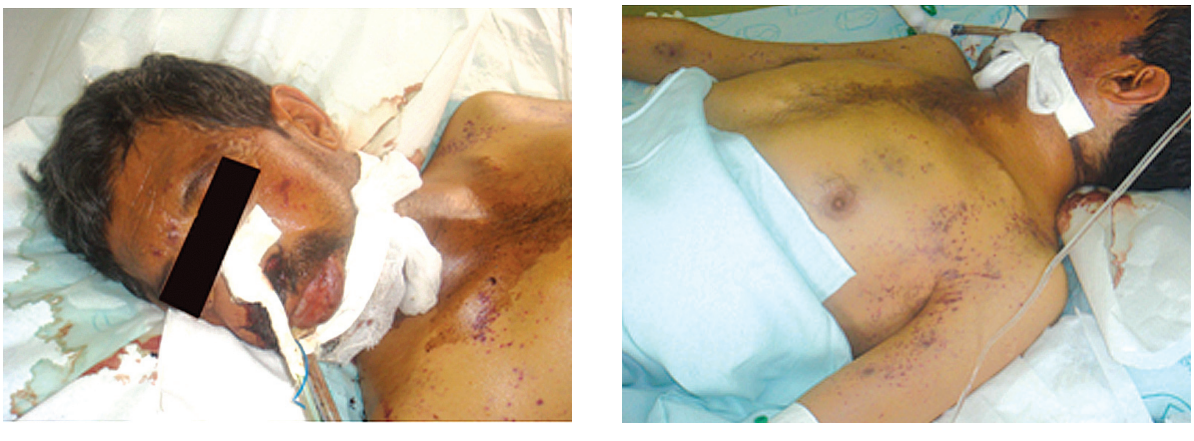

Fig.1. Massive hemorrhages on the upper body of an Iranian patient.

onset of fever, chills, severe headache, dizziness, photophobia, back and abdominal pain (34, 36). Symptoms may last from 1 to 7 days after incubation (32) . In severe cases, 3 - 6 days after onset of disease, hemorrhagic symptoms rapidly manifest. These can range from petechiae to large areas of ecchymosis (Fig. 1) and often appear on the mucus membranes and skin, especially on the upper body and/or extremities (36). The primary cause of bleeding may be due to a cytokine storm that has been demonstrated by some documents (37). Mortality rates for various CCHF outbreaks varied greatly. Convalescence period begins about 15- 20 days after onset of illness. The average fatality rate is often 30 - 50% (1 , 20) but mortality rates of 10% to 80% have been reported in various outbreaks, (e.g. 27.7 % and 80% from the United Arab Emirates and China, respectively. 38 - 39). Mortality rates of nosocomial infections are often much higher than those acquired naturally through tick bite and this may be due to viral load (36).

Pathogenesis of CCHF is not well understood yet. A common pathogenic feature of hemorrhagic fever viruses is their ability to disable the host immune response by attacking and manipulating the cells that initiate the antiviral response (40). This damage is characterized by marked replication of the virus together with dysregulation of the vascular system and lymphoid organs (41). In fatal cases, a fulminant shock-like syndrome occurs. It is suggested that inflammatory mediators may play an important role in the pathogenesis (32, 40) and some research is being done on this aspect of CCHF. The virus mainly infects endothelial cells and monocytes which cause the viremic phase of the disease. Endothelial damage, evidenced in the skin by a rash, contributes to stimulating platelet aggregation and activation of the intrinsic coagulation cascade. Organ lesions cause the release of procoagulants and disruption of the capacity to regenerate the consumed clotting factors (42).

LABORATORY DIAGNOSIS

The laboratory of Arboviruses and Viral Hemorrhagic Fevers has been equipped with advanced molecular and serological techniques for diagnosis and research on the CCHF virus and other arboviruses and viral hemorrhagic fevers like the West Nile, Rift Valley Fever, Chikungunia, Hanta, Pumala, Dengue, Yellow Fever, Alkhorma, Lassa Fever, Tick Born Encephalities, Onyog Nyoung, Sindbis, Mayaro and Ross River Fever.

Serological Assay. Serum samples are analyzed by specific ELISA for IgM and IgG detection. In IgM detection, the ELISA plates are coated with the goat IgG fraction to human IgM (anti μ chain) diluted in PBS 1 X and incubated overnight at 4˚C. Then the serum sample is diluted in PBS containing Tween (PBST) and 3% skim milk (PBSTM) and the plates are incubated for 1 hour at 37˚C. After dilution of the antigen in PBSTM, the plates are incubated for 3 hours at 37˚C . Diluted immuno ascites then is added and the plates are incubated for 1 hour at 37˚C. Peroxidase-labled anti-mouse immunoglobulin is added and the plates are incubated for 1 hour at 37˚C. The plates are then washed 3 times with PBST containing 0.5% Tween. Finally, hydrogen peroxide and TMB (3 , 3′, 5 , 5′ Tetra Methyl Benzedrine) is added and the plates are incubated for 15 minutes at room temperature. The enzymatic reaction is stopped by the addition of 4 N sulfuric acid. The plates are read by ELISA reader at 450 nm. In IgG detection; the ELISA plates are coated with the mouse hyper immune ascetic fluid diluted in PBS 1X and incubated overnight at 4˚C. The native or recombinant antigen (which is produced in this lab) diluted in PBSTM is added and the plates incubated for 3 hours at 37˚C. Diluted serum in PBSTM is added and the plates are incubated for 1hour at 37˚C. After adding the diluted Peroxidase-labled anti-human or animal immunoglobulin in PBSTM, the plates are incubated for 1 hour at 37˚C. The plates then are washed 3 times with phosphate-buffered saline (PBST) containing 0.5% Tween after each incubation. Finally, hydrogen peroxide and TMB is added and the plates are incubated for 15 minutes at room temperature. The enzymatic reaction is stopped by the addition of 4 N sulfuric acid. The plates are read by ELISA reader (Anathos 2020) at 450 nm (11, 12 , 14) .

Molecular Assay. Viral RNA is extracted from 140 μl of serum or from phenol extracted tick suspensions using QIAamp RNA Easy Mini kit according to manufacturers instructions (QIAgen, GmbH, Hilden, Germany). The extracted viral RNA is analyzed subsequently by Real-time RT-PCR using the one-step RT-PCR kit (QIAgen, GmbH, Hilden, Germany) and using specific primers which amplify a 536 bp fragment of the S-segment of the CCHFV genome. The PCR reaction is done in 50 μl of total volume in

sequence of 30 minutes at 50˚C, 15 minutes at 95˚C , and 40 cycles including 30 seconds at 95˚ C, 30 seconds at 50˚C, 45 seconds at 72˚C , and finally 10 minutes in 72˚C as final extension (12) .

CONCLUSION

Most of neighbors of Iran including Pakistan and Afghanistan in the east, and Turkey in the west are endemic regions for CCHF, so Iran is seriously threatened by the danger of CCHF. In addition , CCHF was first discovered in Crimea, (located in northwest of Iran) . Our molecular investigations have demonstrated that the Iranian CCHF virus strains are very similar to the Pakistani Matin Strain (13).

The Iranian National Reference Lab for Arboviruses and Viral Hemorrhagic Fevers Laboratory, at PII performs timely and free of charge molecular and serological diagnosis and research. The CDC supervises timely referral of CCHF probable cases` sera to the National Reference Lab and provides immediate and free of charge treatment of patients. The Iranian Veterinary Organization collects suspected livestock` sera and ticks from high risk regions and sends the samples to the National Reference Lab. With prevention planning and control program for tick populations, the mortality rate of 20% (year 2000) was dramatically decreased to 6% in 2007. It is worth pointing out that the National Reference Lab, in addition to CCHF detection, has been well equipped with several serological and molecular assays for diagnosis and research on a wide variety of arboviruses and viral hemorrhagic fevers such as West Nile, Rift Valley Fever, Chikungunia, Hanta, Pumala, Dengue, and Yellow Fever (12) .

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