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Difference in clinical presentation between the first and second phases of Kyasanur Forest disease: an experience from a teaching hospital in South India

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SUMMARY

Kyasanur forest disease (KFD) is a biphasic tick-borne disease which occurs during the post-monsoon season. The patient may visit the hospital in either of the phases, and it is essential to differentiate between the two phases as the management considerations in both phases are different. This is a retrospective review of patients diagnosed with KFD who were treated by the Infectious Disease Department between September 2019 and May 2020. A total of 14 cases (16 admissions) were diagnosed during the study period by reverse-transcriptase polymerase chain reaction assay. Of these, nine cases came to our hospital during the first phase and seven (including two-readmissions) came to our hospital during the second phase. The manifestations in the first phase included high-grade fever (100%), myalgia (67%), conjunctival suffusion (33%), palatal eruptions (78%), gastrointestinal manifestations (67%), leucopenia (100%), thrombocytopenia (89%), elevated transaminases (89%), elevated creatine phosphokinase (100%) and activated partial thromboplastin time (APTT) (100%). Manifestations in the second phase were fever (57%), headache (100%), blurring of vision (29%), neck signs (71%), leukocytosis (71%), thrombocytopenia (14%), elevated transaminases (40%) and APTT (20%). The clinical symptomatology and laboratory manifestations are different in each of the two phases and can be easily identified by primary care physicians.

Keywords: Acute febrile illness; viral haemorrhagic fever; meningitis.

INTRODUCTION

Kyasanur Forest Disease (KFD) is a tick-borne haemorrhagic disease that is endemic in five states of Southern India [1]. It is transmitted by the bite of a hard tick, *Haemaphysalis spinigera* [2]. It is caused by the KFD virus, which belongs to the Flaviviridae family and is closely related to Alkhurma virus, Omsk haemorrhagic fever and Powassan virus [3]. It is a biphasic illness that occurs during the post-monsoon season when the activity of nymphal ticks is highest [4]. This disease mainly affects the forest dwellers or labourers working in the plantations. The first phase is an acute febrile illness comprising of fever and constitutional symptoms, while the second phase consists of recurrence of fever with neurological symptoms [4]. The second phase is usually observed after one or two weeks in a fraction of the patients [5]. Although there is an overlap between the manifestations of both the phases and the pa-

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tient may sometime come to the hospital for the first time in the second phase, it is crucial to identify which phase the patient is in as the management strategies are different in both the phases. In the first phase, the goal is to control the haemorrhagic manifestation and corresponding dehydration and hypotension. In the second phase, the strategy is to manage and prevent complications that may arise from neurological involvement. Although much has been explored about the epidemiology of this disease, the literature is scarce about the clinical and laboratory manifestations of the disease. Moreover, many of the reports do not differentiate between the clinical and laboratory features of the two phases. In this case series, we report the difference in clinical and laboratory features between the two phases of laboratory-confirmed KFD.

PATIENTS AND METHODS

This is a retrospective review of records after taking permission from the Institute's ethics committee. The case records of all patients admitted in or referred to the Infectious disease department of Kasturba Medical College between September 2019 and May 2020 were screened for the diagnosis of KFD. The diagnosis of the first phase of KFD was made by the presence of febrile illness with/without haemorrhagic manifestations and positive real-time reverse transcriptase-polymerase chain reaction assay (RT-PCR). The second phase was inferred from the reappearance of fever in a patient who was previously diagnosed to have KFD in one month prior to the presentation by positive RT-PCR and was negative for other causes of febrile illness. The RT-PCR targeted NS-5 gene and was performed using a published protocol which has a sensitivity to detect as less as 2.67 pfu/ml of virus [6]. The data of all the eligible patients were recorded using a pre-defined questionnaire. The following variables were recorded: age, sex, place of residence, occupation, clinical and laboratory manifestations. The data were entered into an excel worksheet, and analysis was done using Strata v12. Qualitative variables were expressed as a percentage, and quantitative variables were expressed as mean (with standard deviation) or median (with interquartile range).

RESULTS

Of the 14 patients who were diagnosed with KFD during the study period, 10 (71%) were male. The mean age of the patient was 47.7+/-13.8 years. The patients belonged to the districts of Shimoga (n=11), Uttar Kannada (n=2) and Chikmagalur (n=1). All but one patient presented from January to March. A total of eleven patients had a history of working in agricultural farms (Areca nut farmer, n=8, Coconut plucker, n=2, Coffee plantation, n=1). Out of the 14 cases, nine cases came to the hospital during the first phase while the rest of the five cases came during the second phase. Of the nine patients who were admitted during the first phase, two patients were re-admitted with the second phase. Of the 14 patients, 12 patients were successfully discharged while two patients succumbed to illness. The clinical and laboratory features of the patients during the first and second phase are summarized in Tables 1 and 2.

All the patients who presented during the first phase had a high-grade fever with a mean duration of 6.3+/-3 days. Myalgia, conjunctival suffusion, palatal eruptions and cervical lymphadenopathy were seen in 6 (67%, n=9), 3 (33%, n=9), 7 (78%, n=9) and 4 (44%, n=9) patients respectively (Figure 1). The following gastrointestinal manifestations were seen in six (67%, n=9) patients in the first phase: diarrhoea (n=3), abdominal pain (n=2), upper gastrointestinal (UGI) bleeding (n=2), peritonitis (n=1), appendicitis (1) and pancreatitis (1). All the patients had leucopenia (less than 3000 per cubic millimetre) while thrombocytopenia (less than 100000 per cubic millimetre) was present in all but one patient. Erythrocyte sedimentation rate (ESR) was less than 5 millimetre per hour in all the patients. Aspartate transaminase (AST) and alanine transaminase (ALT) were elevated in all but one of the patients. Kidney function was normal in all the cases except one. Creatine phosphokinase (CPK) was elevated in 100% of the patients with values as high as 47166 units per litre. Activated partial thromboplastin time (APTT) was elevated in all the eight patients with values as high as 50.5 seconds. Prothrombin time (PT) was normal in all nine patients.

The mean interval between the resolution of fever in the first phase and appearance of symptoms (fever or headache) was 6.6+/-2.6 days. Of the seven patients who presented during the second

Table 1 - Summary of clinical and laboratory features of patients presenting with the first phase of Kyasanur forest disease.

P.n	*Clinical features*	*Hb*	*TLC*	*Neu*	*Lym*	*Plt*	*ESR*	*CRP*	*AST*	*ALT*	*CPK*	*APTT*	*Outcome*
1	Fever for 14 days, myalgia, conjunctival suffusion, palatal eruptions, UGI bleed, pancreatitis	13.5	2.9k	37	40	69k	2	0.57	1292	597	14530	37	Second phase
2	Fever for 5 days, conjunctival suffusion, palatal eruptions	14	0.6k	57	29	59k	1		172	193	544	42	Discharged
3	Fever for 5 days, myalgia, palatal eruptions, haematuria	14.6	2.1k	58	34	79k	1	1.22	1307	168	47166	46.5	Discharged
4	Fever for 6 days, myalgia, palatal eruptions, diarrhea	14.8	2.7k	62	30	75k	4	0.46	198	128	8157	43.3	Second phase
5	Fever for 5 days, UGI bleed with jejunal perforation and bacterial sepsis	14.3	1.8k	70	9	74k		115	468	134	2379	42.7	Death
6	Fever for 6 days, conjunctival suffusion, palatal eruptions, diarrhea	16	1.1k	45	27	84k	2	0.56	286	39	309	50.5	Discharged
7	Fever for 7 days, myalgia, palatal eruptions, diarrhoea	13.3	2.3k	61	30	200k	2		621	255	718		Discharged
8	Fever for 5 days, myalgia, palatal eruptions, appendicitis	13.5	0.9k			75k		30.4	41	19		41.6	Discharged
9	Fever for 4 days with myalgia	13.9	0.7k			67k			147	115	1455	39.3	Discharged

*P.n, Patient number; UGI, upper gastrointestinal; Hb-Haemoglobin (gram per decilitre); TLC, total leucocyte count (per cubic millimetre); k- thousand; Neu, Neutrophil (Percentage); Lym, lymphocyte (percentage); plt, platelet (per cubic millimetre); ESR, erythrocyte sedimentation rate (millimeter per hour); CRP, C- reactive protein (milligram per litre); AST, aspartate transaminase (international units/ litre); ALT, alanine transaminase (international units/ litre); CPK, creatine phosphokinase (units per litre); APTT, activated partial thromboplastin time (seconds).

Table 2 - Summary of clinical and laboratory features of patients presenting with the second phase of Kyasanur forest disease.

P.n	*Int*	*Clinical features*	*Hb*	*TLC*	*Plt*	*ESR*	*CRP*	*AST*	*ALT*	*CPK*	*APTT*	*Outcome*
1	7	Headache, neck signs, blurring of vision	13.1	11.4k	313k							Discharged
4	4	Headache, altered sensorium, neck signs	14.3	11.9k	101k	77	77					Discharged
10	5	Fever, headache	17.1	16.5k	358k	5	0.5	31	10	123	24.7	Discharged
11	5	Fever, headache, neck signs, severe encephalopathy, status epilepticus	12.1	3.3k	77k	5		483	121		122	Death
12	12	Fever, headache, blurring of vision	13.3	10k	341k	18	1	13	14	18	24.3	Discharged
13	7	Fever, headache	11.6	12.8k	427k	13	7	26	40	82	28.5	Discharged
14	6	Headache, seizure, encephalopathy	10	19k	287k			75	104		28.2	Discharged

*P.n, Patient number; UGI, upper gastrointestinal; Hb-Haemoglobin (gram per decilitre); TLC, total leucocyte count (per cubic millimetre); k - thousand; Neu, Neutrophil (Percentage); Lym, lymphocyte (percentage); plt, platelet (per cubic millimetre); ESR, erythrocyte sedimentation rate (millimeter per hour); CRP, C - reactive protein (milligram per litre); AST, aspartate transaminase (international units/ litre); ALT, alanine transaminase (international units/litre); CPK, creatine phosphokinase (units per litre); APTT, activated partial thromboplastin time (seconds).

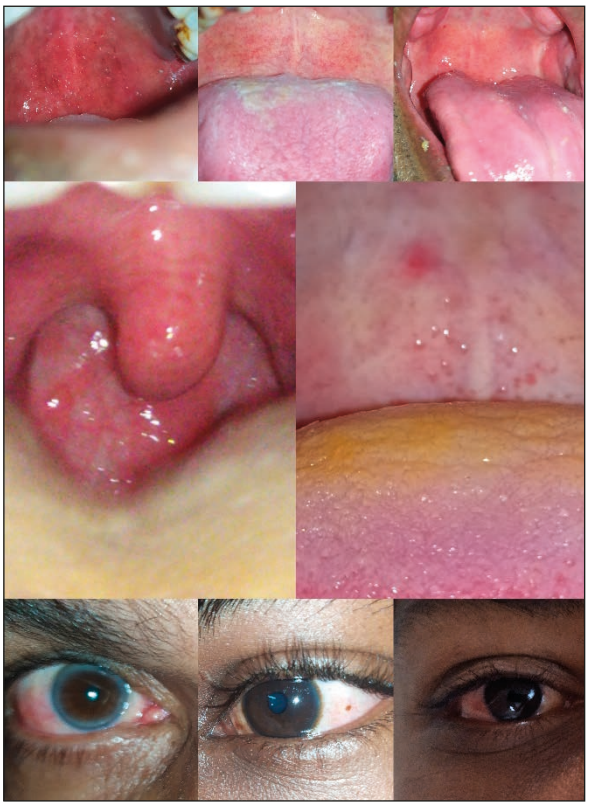

Figure 1 - Palatal eruptions and conjunctival suffusion in patients diagnosed with Kyasanur forest disease.

phase, four patients had low to moderate grade of fever. All the seven patients had a headache while blurring of vision was present in only two patients. Neck signs were present in a total of five patients. A total of two patients presented with multiple episodes of seizures and encephalopathy. Lumbar puncture was done in only one patient, which showed 50 cells (100% lymphocytes). Sugar and protein in cerebrospinal fluid were normal. Instead of leucopenia, leukocytosis (more than 11,000 per cubic millimetre) was seen in all but two patients. Thrombocytopenia was seen in only 1 (14%, n=7) patient. ESR was 5 mm/hour or more in all five patients while CRP was elevated in 2 (50%, n=4) patients. Transaminases were elevated in only two (40%, n=5) patients. CPK was normal in all the three patients in which it was measured. PT and aPTT were normal in four out of the five patients.

The mean haemoglobin, total leucocyte count and platelet count in the first phase were 14.2±0.8 g/dl, 1677±878 per mm^3 and 86888±43021 per mm^3 respectively while it was 13.1±2.2 g/dl, 12128±4997 per mm^3 and 272000±132510 per mm^3 respectively in the second phase. The median AST, ALT and APTT in the first phase was 399 (159-956) IU/l, 168 (121-270) IU/l and 42.3 (39.9- 45.7) seconds respectively while it was 31 (19-279) IU/l, 40 (12-112) IU/l and 28 (24.5- 75.25) seconds respectively in the second phase.

DISCUSSION

KFD was first reported in the Kyasanar forest of Shimoga district, Karnataka, India. It was initially reported in monkeys in whom it is highly fatal. Although for more than fifty years, the human cases were initially restricted to few districts of Karnataka, new cases have been reported from Tamil Nadu, Kerala, Maharashtra, and Goa as well [7-10]. The spread of this disease is probably related to the growing deforestation activities and movement of monkeys to new areas in search of food [11]. Antibodies against the virus have also been demonstrated in individuals hailing from the states of West Bengal, Gujarat and Andaman & Nicobar Islands [12, 13]. In a review by Chakraborty et al., close to ten thousand cases, have been reported between the year 1957 and 2017 [1]. In this period, close to 200 cases were reported every year from the affected areas [1]. The case fatality rate was calculated to be 2.4% [1].

The nymphal forms of the ticks that are primarily responsible for human spread are most prevalent from December to May, which explains the seasonal pattern of this disease [14]. The cases are mostly reported in young and middle-aged adults who are involved in outdoor work and therefore, most likely to be exposed [10]. The predilection of the male sex to KFD in our series can be attributed to higher chances of exposure due to more frequent involvement in outdoor activities such as coconut picking or areca nut farming [15]. This is similar to a previously published report from Karnataka [7]. However, in areas where women venture out to collect fire-woods, a higher number of cases have been reported in females as well [8-10].

The incubation period of KFD ranges from 3 to 8 days. In the first phase, the patient presents with abrupt onset of high-grade fever with myalgia. Localized lymphadenopathy, conjunctival congestion and gastrointestinal manifestations can

also be seen [5]. Bleeding in KFD has been attributed to disseminated intravascular coagulation (DIC) and thrombocytopenia. Haematamesis or the appearance of fresh blood in the stool may also be observed in some cases [7, 8]. Like other viral illnesses, leucopenia is also seen in KFD. In this small series, we report increased APTT but normal PT in almost all the cases of KFD presenting in the first phase. This is a novel observation which the authors could not find in the review of the literature. There is a need to explore the reason for such a finding. Also, the disproportional increase in AST compared to ALT has not been reported widely in the previous reports of KFD.

The following possible differentials were considered in our cases: dengue, chikungunya, scrub typhus, leptospirosis, malaria and enteric fever. All these differentials were ruled out based on the clinical presentation and specific diagnostic tests for each of the illnesses. The clinical and laboratory details that can be used in differentiating KFD from the other differentials have been summarized in Table 3.

The earliest laboratory method used for diagnosis of KFD was intracerebral inoculation of serum into sucking mice, followed by neutralization tests [16]. However, with the advent of nucleic acid amplification tests and serological tests, animal inoculation is rarely done. The method of choice in the first 4 days of illness is PCR as IgM antibodies develop only after Day 4. From Day 4 to Day 24, both PCR and IgM Enzyme-linked immunosorbent assay (ELISA) is done as the sensitivity of PCR falls, and IgM ELISA rises with each passing day in this period [17]. In our study, however, we included only patients who were positive by PCR as serology was not available at our centre.

Although reports summarize the clinical features of KFD, many of these reports fail to distinguish between the first and second phase. We found a significant difference in the manifestations of both the phase. Because the first phase is a self-limiting acute febrile illness, many patients often present during the second phase. It is important to recognize the symptoms of the second phase as they are distinct from the commonly described symptoms

Table 3 - Clinical and laboratory parameters of patients with common acute febrile illnesses in India.

Differentials	*Clinical parameters*		*Laboratory parameters*	
	Features similar to KFD	*Features different to KFD*	*Features similar to KFD*	*Features different to KFD*
Dengue	High grade fever, oral petechiae, bleeding	Conjunctival congestion more common in KFD	Leucopenia, thrombocytopenia, Transaminitis	Raised APTT more common in KFD
Chikungunya	High grade fever	Arthralgia, itchy rash more common in Chikungunya	Leukopenia	Raised APTT and transaminitis more common in KFD
Leptospirosis	Fever, conjunctival suffusion	Jaundice, AKI more common in leptospirosis. Oral petechiae more common in KFD	Thrombocytopenia seen in both but more common in KFD	Lecucocytosis and increase in CRP more common in leptospirosis and scrub typhus. Raised APTT and transaminitis more common in KFD
Scrub typhus	Fever	Rash, eschar seen in scrub. Oral petechiae more common in KFD	Thrombocytopenia seen in both but more common in KFD	
Malaria	Fever with chills and rigors, encephalopathy	Splenomegaly more common in malaria	Thrombocytopenia seen in both but more common in KFD	Anaemia, Increase in unconjugated bilirubin, hypoglycaemia more common in malaria. Raised APTT and transaminitis more common in KFD
Enteric fever	Fever, diarrhoea	Splenomegaly, rose spots more common in enteric fever	Thrombocytopenia seen in both but more common in KFD	Increased C-reactive protein more common in enteric fever. Raised APTT and transaminitis more common in KFD

of the first phase. Most patients present with fever and headache with variable features of meningism [5]. Mouse studies have shown that the virus is neurotropic and leads to inflammatory neuropathological changes [18]. Unlike the leucopenia and thrombocytopenia noticed in the first phase, the second phase shows leukocytosis and normal platelet counts [5]. Also, the coagulation parameters are rarely deranged in the second phase.

In conclusion, the clinical presentation in the two phases of KFD is different from each other. In the first phase, patients present with acute haemorrhagic febrile illness with deranged coagulation parameters and liver functions tests while in the second phase patients present with neurological involvement of varying severity.

Conflicts of interest
None to declare

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REFERENCES

[1] Chakraborty S, Andrade FCD, Ghosh S, Uelmen J, Ruiz MO. Historical expansion of Kyasanur forest disease in India from 1957 to 2017: a retrospective analysis. *GeoHealth*. 2019; 3 (2), 44-55.

[2] Naren Babu N, Jayaram A, Hemanth Kumar H, et al. Spatial distribution of Haemaphysalis species ticks and human Kyasanur Forest Disease cases along the Western Ghats of India, 2017-2018. *Exp Appl Acarol*. 2019; 77 (3). 435-47.

[3] Shah Syed Z., Jabbar Bt, Ahmed N, et al. Epidemiology, pathogenesis, and control of a tick-borne disease - Kyasanur forest disease: current status and future directions. *Front Cell Infect Microbiol.* 2018; 8, 149.

[4] Adhikari Prabha MR., Prabhu MG., Raghuveer CV, Bai M, Mala MA. Clinical study of 100 cases of Kyasanur Forest disease with clinicopathological correlation. *Indian J Med Sci.* 1993; 47 (5), 124-30.

[5] Webb H. E., Rao R. L. Kyasanur forest disease: a general clinical study in which some cases with neurological complications were observed. *Trans R Soc Trop Med Hyg.* 1961; 55, 284-98.

[6] Mourya DT, Yadav PD, Mehla R, et al. Diagnosis of Kyasanur forest disease by nested RT-PCR, real-time RT-PCR and IgM capture ELISA. *J Virol Methods.* 2012; 186 (1-2), 49-54.

[7] Kasabi Gudadappa S, Murhekar Manoj V, Yadav Pragya D, et al. Kyasanur Forest Disease, India, 2011-2012. *Emerg Infect Dis.* 2013; 19 (2), 278-81.

[8] Gurav Yogesh K, Yadav Pragya D, Gokhale Mangesh D, et al. Kyasanur Forest Disease prevalence in western ghats proven and confirmed by recent outbreak in Maharashtra, India, 2016. *Vector Borne Zoonotic Dis.* 2018; 18 (3), 164-72.

[9] Sadanandane C, Elango A, Marja Noonu, et al. An outbreak of Kyasanur forest disease in the Wayanad and Malappuram districts of Kerala, India. *Ticks Tick-Borne Dis.* 2017; 8 (1), 25-30.

[10] Oliveira A, Selvaraj K, Tripathy JP, et al. Geospatial clustering, seasonal trend and forecasting of Kyasanur Forest Disease in the state of Goa, India, 2015-2018. *Trop Med Health.* 2020; 48, 27.

[11] Walsh MG., Mor SM., Maity H, Hossain S. Forest loss shapes the landscape suitability of Kyasanur Forest disease in the biodiversity hotspots of the Western Ghats, India. *Int J Epidemiol.* 2019; 48 (6), 1804-14.

[12] Padbidri VS, Wairagkar NS, Joshi GD, et al. A serological survey of arboviral diseases among the human population of the Andaman and Nicobar Islands, India. *Southeast Asian J Trop Med Public Health.* 2002; 33 (4), 794-800.

[13] Rao T. Ramachandra. Immunological surveys of arbovirus infections in South-East Asia, with special reference to dengue, chikungunya, and Kyasanur Forest disease. *Bull World Health Organ.* 1971; 44 (5), 585-91.

[14] Kiran S. K., Pasi Achhelal, Kumar Satish, et al. Kyasanur Forest disease outbreak and vaccination strategy,Shimoga District, India, 2013-2014. *Emerg Infect Dis.* 2015; 21 (1), 146-9.

[15] Patil DY, Yadav PD, Shete AM, et al. Occupational exposure of cashew nut workers to Kyasanur Forest disease in Goa, India. *Int J Infect Dis.* 2017; 61, 67-9.

[16] Chatterjea JB, Swarup S, Pain SK, Laxmana Rao R. Haematological and biochemical studies in Dyasanur Forest disease. *Indian J Med Res.* 1963; 51**,** 419-35.

[17] Yadav Pragya D, Gurav Yogesh K, Shete Anita M, et al. Kinetics of viral RNA, immunoglobulin-M & G antibodies in Kyasanur forest disease. *Indian J Med Res.* 2019; 150 (2), 186-93.

[18] Basu Atanu, Yadav Pragya, Prasad Sharda, et al. An early passage human isolate of Kyasanur Forest Disease virus shows acute neuropathology in experimentally infected CD-1 Mice. *Vector Borne Zoonotic Dis* 2016; 16 (7), 496-8.