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Investigation of Malaria among Patients of Febrile Illness and Co-Infection with Leptospirosis in Andaman and Nicobar Islands, India

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ABSTRACT

Malaria and leptospirosis are endemic in Andaman and Nicobar Islands. Malaria is common in a tribal area called Nicobar district and leptospirosis is mostly confined to South, North and Middle Andaman district. There has been an outbreak of febrile illness in Nancowry groups of Island under Nicobar district suspecting malaria. Clinically there were cases which also resemble leptospirosis. Malaria and leptospirosis are the common infection of tropics and their co-infection occurs. Blood samples were collected from patients with the history of febrile illness attending Community Health Centre (CHC), Nancowry, Nicobar district. A total of 132 blood samples were collected in between the age group of 18 to 53 years. The clinical specimens were subjected to rapid test malaria and Microscopic Agglutination Test (MAT) for leptospirosis following standard procedure and manufacturer instructions. The subjects were labourers from West Bengal who has come to Nancowry groups of Island for re-habitation. Ninety four (71%) samples were malaria positive among 132 patients. *Plasmodium falciparum* infection was seen in 34 (36%) cases followed by *plasmodium vivax* in 12 (13%) cases. Among 132 samples anti-leptospiral antibody by MAT was observed in 12 (9%) patients. In eight (6%) samples malaria and leptospirosis co-infection was observed and vomiting was found to be the common symptom in both the infection. In the present study it has been observed malaria and leptospirosis co-infection exists in malaria endemic zone. This gives an idea that in malaria endemic zone a malaria positive case cannot be the sole cause of the infection and differential diagnosis can be done for other febrile illness cases.

Key words: Malaria, leptospirosis, *Plasmodium falciparum*, *Plasmodium vivax*

INTRODUCTION

Andaman and Nicobar Islands, an Indian union territory situated in the Bay of Bengal east of Indian subcontinent (Shanks *et al.*, 2008). This is an archipelago of 572 tropical Islands in the territory having an area of 7,950 km² (3,070 mile²). Of these, 34 Islands are permanently inhabited by mainlander and tribal population. Nicobar district is situated 143 miles south of capital city Port Blair and it is mainly occupied by mongoloid tribe called Nicobari given in a website of District Administration. There has been increase in malaria cases in Nicobar groups of Island especially in Nancowry after December 26, 2004 tsunami. The Nicobar group of island is mainly

a habitat of a mongoloid tribal population called Nicobarese. The tropical climate in Nancowry favours mosquito proliferation and breeding of *Anopheles Sundaicus* playing a predominant vector throughout the year. The worst effected district after tsunami hit the Andaman and Nicobar Islands was Nicobar district. After this natural disaster in Nicobar groups of islands the complete ecology was disturb and there was an upsurge of malaria cases. Leptospirosis is one of most wide spread zoonotic disease in the world because of the involment of wide variety of animal species (Faine *et al.*, 1999). Malaria and leptospirosis are common infection in every part of the world especially in the tropics. Their co-infection has been suggested by a sero-epidemiologic study in the Peruvian Amazon (Sulzer *et al.*, 1978). But not much study has been conducted in this aspect earlier. There have been studies on co-infection of malaria with filariasis (Ravindran *et al.*, 1998), Dengue (Levett *et al.*, 2000) etc. Differential diagnosis of malaria and leptospirosis becomes difficult to diagnose the disease. Diagnosis of leptospirosis is mainly based on clinical signs, as laboratory test is generally not possible at field level and no diagnostic facility available in the peripheral hospitals. In the present study a total of 132 cases with clinical and laboratory evidence to understand the presence of co-infection with malaria and leptospirosis in Nancowry Island.

MATERIALS AND METHODS

Study area: The study was conducted during the year 2009 for the period of two months (February and March 2009). This study was conducted in the jurisdiction of Community Health Centre (CHC), Nancowry situated at Kamorta. Nancowry, the central group of Islands in Nicobar district with hilly terrain covered with grass, forming undulating meadows. It consists of 12 villages, many are Islands connected only by sea route. The population of the CHC during 2009 was 4875, inhabited by aboriginal Nicobaries tribe.

Study population: The study was conducted among the labourers from the district of West Bengal, who were engaged in construction of permanent shelters after tsunami hit the Island. They were living in camps provided by their contractors. During the study period the labours suffered with febrile illness suspected of malaria. Patients = 18 years of age presenting with acute fever admitted or attended to the CHC, Nancowry were eligible for the study. Evaluation included present history, general examination, routine clinical laboratory test for malaria and leptospirosis, isolation for leptospire.

Rapid test for malaria: Antigen based one step Malaria Pf/Pv test (BIO STANDARD DIAGNOSTICS, India) were used for diagnosis of *Plasmodium vivax* (Pv) and *Plasmodium falciparum* (Pf). *Plasmodium vivax*/*Plasmodium falciparum* test were performed in the laboratory of CHC, Nancowry for the diagnosis of malaria. Microscopic Agglutination Test (MAT) being the gold standard test for leptospirosis was performed at Regional Medical Research Centre (ICMR), Port Blair to find out the co-infection.

Microscopic agglutination test for leptospirosis: Blood sample was collected from 132 patients who were admitted to CHC Nancowry with the history of fever, body ache and head ache. Blood samples were collected at the time of admission (acute samples). Serum was separated and stored at -80°C until processed. Test for Malaria was performed at the CHC, Nancowry and Microscopic Agglutination Test (MAT) was performed at Regional Medical Research centre (Indian Council of Medical Research), Port Blair city for diagnosis of leptospirosis. This centre being WHO

Table 1: List of reference strains used in the panel of MAT antigens

Serogroup	Serovar	Strain
Australis	Australis	Ballico
Autumnalis	Autumnalis	Rachmati
Bataviae	Bataviae	Swart
Canicola	Canicola	Hond utrecht IV
Grippotyphosa	Grippotyphosa	Moskva V
Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
Javanica	Poi	Poi
Ballum	Ballum	Mus127
Pomona	Pomona	Pomona
Sejroe	Hardjo	Hardjoprajitno
Cynopteri	Cynopteri	3522C
Pyrogenes	Pyrogenes	Salinem

Collaborating Centre for leptospirosis for south East Asia and also the National Reference Centre for leptospirosis have the facility to perform the entire test related to leptospirosis. Isolation of leptospira was also performed at the bed side of the patient, where aseptically one and three drops of fresh blood was added in 2 tubes of Ellinghausen-McCullough medium modified by Johnson-Harris (EMJH) semisolid medium simultaneously (Faine *et al.*, 1999). The cultures were screened at every 10 days interval for the period of two months. In MAT a battery of live antigens, which are common circulating serovar in Andaman Islands and in the country were (10 serogroup representing 12 serovars) used in liquid EMJH media. The list of antigen used in MAT is mentioned in (Table 1).

The antigens were 5-7 days old, auto-agglutination free cultures grown in EMJH (DIFCO, USA) liquid medium with approximately 1.2×10^8 organisms per millilitres. MAT was done at double-dilution starting from 1 in 20. A titre of 1 in 80 or more against any of the serovars in MAT was considered as evidence of leptospiral infection (Faine *et al.*, 1999). Clinical case description for leptospirosis is characterized by fever, head ache, chills, myalgia, conjunctival suffusion and rarely meningitis, jaundice, renal failure and pulmonary haemorrhage (Vijayachari *et al.*, 2008). Laboratory criteria for confirmed diagnosis are isolation of leptospira from clinical specimen or demonstration of leptospira in blood or plasma under dark field microscopy (Olympus, Japan).

Information on risk factors was collected from the patients on a predesigned questionnaire. These factors were house hold characteristics, occupation, presence of domestic animals in the house and behavioural factors.

RESULTS

A total of 132 blood samples were collected and subjected to antigen based malaria test and antibody based test for leptospirosis. Out of 132 samples collected malaria positives were seen in 94 patients (71%). *Plasmodium Vivax* (Pv) was positive among 12 samples (13%) and *Plasmodium falciparum* (Pf) was seen in 34 (36%) samples. Forty eight (51%) samples showed mixed positives (both Pv and Pf). Twelve (9%) samples showed anti-leptospiral antibody by MAT among the labourers. The commonest serovar among the labourers were found to be Icterohaemorrhagiae and Hebdomadis (33%) followed by Grippotyphosa and canicola (17%) (Table 2).

Table 2: Serogroup and titre distribution among the positives

Serogroup	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640
Grippotyphosa	0	2	0	0	0
Icterohaemorrhgiae	0	2	0	0	2
Pomona	0	0	0	0	0
Australis	0	0	0	0	0
Autumnalis	0	0	0	0	0
Ballum	0	0	0	0	0
Pyrogenes	0	0	0	0	0
Hebdomadis	0	2	0	0	2
Canicola	0	0	0	2	0

Table 3: Laboratory findings and treatment regimens for patients with malaria and leptospirosis infection among positives

Only malaria positives both (pf/pv)	Treatment
82 cases (n = 94)	Chloroquine 150 mg (4 tablets for 1st and 2nd day) 150 mg (2 tablets for 3rd day)
Only leptospirosis positives	
4 cases (n = 94)	Crystalline penicillin injection (600 mg at 6 h interval for 5-7 days)
Co- infection of malaria and leptospirosis positives	
8 cases (n = 94)	Chloroquine and Crystalline penicillin

n is the total number of malaria and leptospirosis positives among 132 cases

Table 4: Age wise positives of malaria and leptospirosis among labourers

Age groups	Malaria positive	Pv	Pf	Pv and Pf	Leptospirosis positive	Leptospirosis and malaria negative	Leptospirosis positive and malaria positive
18-25	42 (45%)	08	14	22	04 (33%)	02	02
26-32	26 (28%)	02	12	12	04 (33%)	04	-
33-39	12 (13%)	-	02	08	04 (33%)	02	02
40-46	08 (9.0%)	-	04	04	-	-	-
47-53	06 (6.0%)	02	02	02	-	-	-
	*N = 94	12	34	48	°N = 12	08	04

*N is the total no. of malaria positives, °N is the total no. of leptospirosis positives

Eight patients showed co-infection of malaria and leptospirosis with a median age of 34 years (range 18-39). Eighty two patients were found to be malaria positives with a median age of 26 years (range 18-53). Among the 82 malaria positives four of them were women and rest were male. All the suspected cases of malaria and leptospirosis were treated with anti-malarial drug and antibiotics, respectively (Table 3).

Maximum positives of malaria cases were observed in 18-25 (42%) age group followed by 26-32 (28%) year old. The detail of age wise positives is given in Table 4. Fever, chill, body ache, headache, yellow urine and jaundice were found to be common symptoms among all the patients admitted to the CHC (Community Health Centre) for suspected malaria and leptospirosis. Weakness, loss of appetite and chest pain were common symptoms observed in malaria cases and was not observed in leptospirosis positive cases. In case of leptospirosis severe anaemia, diarrhoea and cough were the common symptoms which were not seen in malaria positive cases. Apart from common symptoms of malaria and leptospirosis mentioned earlier, vomiting was seen in all the 8 cases of co-infection with malaria and leptospirosis.

Analysis of domestic animals in the house and house surrounding was done on 12 sero-positive cases in leptospirosis. Risk factors analysis for infection with commonest serovar was done and was found that the subjects were living in camps provided by the concerned contractor. Only one person had dog and showed leptospiral antibodies against serovar Icterohaemorrhagiae. Analysis of information about household characteristics showed that rat infestation was 100% in all the houses of the subjects and were at increased risk of acquiring leptospiral infection. Wet house surroundings was found in 83% and was the second most increased risk of acquiring leptospiral infection.

Isolation of leptospira was attempted at the bedside of the patient and they are screened for the period of two months but no cultures turned positive.

DISCUSSION

Leptospirosis is included as one of the most important re-emerging infectious disease of public health importance. There have been frequent post monsoon epidemics of leptospirosis occurring in mainland, India in the recent past. Disease has been endemic in many parts of the country namely Andaman Islands, Tamil Nadu, Kerala, Maharashtra, Gujarat, etc (Vijayachari *et al.*, 2008). Half of the world's population (approximately 3 billion) live in areas that have risk of malaria transmissions. India had an estimated 60% of the Malaria cases (Krishnamoorthy *et al.*, 2005). Andaman and Nicobar Islands a union territory of India as historically been known for high malarial risk (Shanks *et al.*, 2008). Same way leptospirosis is also known to be endemic in Andaman Islands.

There has been an upsurge of malaria cases observed post tsunami in Carnicobar district especially in Nancowry groups of Island. The result showed 71% was malaria positive among 132 blood sample collected in the post tsunami year. The geological and environmental changes due to tsunami played an important role and contributed in the rise of malaria cases. The re-habitation activity and labour inflow in these groups of islands also played a major role in the upsurge of malaria cases (Manimunda *et al.*, 2011). A study conducted at South Andaman after the tsunami has shown increased salinity in the land water due to gushing in of sea water which favoured the breeding of *Anopheles sundaicus* (Krishnamoorthy *et al.*, 2005), vector known in transmission of malaria.

Plasmodium falciparum (Pf) was found in 36% of the cases, which has been seen earlier during 2007 and 2008. In other parts of the country Pf is associated with severe form of malaria especially cerebral malaria, but there are no such reports from this group of Island. The increase in malaria could be due to the increase entry of labour from West Bengal and large scale of construction activity (Manimunda *et al.*, 2011). *Plasmodium vivax* is associated with mild disease and has been seen in 12% of the cases.

Antibodies against leptospirosis were seen in 12 (9%) cases in the present study. Earlier study conducted in animal populations in Nicobar groups of Islands (Sharma *et al.*, 2003) also showed 13% positivity which is similar to that of the present study (Flatau *et al.*, 2000). This also correlates well with seroprevalence rates in human populations in this Island. This could be due to the reason that the soil is sandy with low water retaining capacity and thus there is very little stagnation of water. The chance of survival of leptospira is negligible in this kind of environment. Environmental factors play a major role in maintaining the infection in the environment and transmit the infection to humans and animals and because of the low survivability chance of the organism to stay in the environment there is very low chance of acquiring leptospira infection to other host.

In the present study, Icterohaemorrhagiae, Hebdomadis, Gripotyphosa and Canicola were the commonest serovars found in MAT. This finding correlates well with the earlier study conducted in Andaman and Nicobar Islands in different population groups. There have been isolates of similar serovars of leptospire from animals, humans and rodents from these Islands (Unpublished data). This could be the source of infection to other animals and humans in these islands. A pilot study is required to understand the transmission dynamics of the disease leptospirosis.

It has been confirmed by the present study that co-infection of malaria and leptospirosis exist. There has been an earlier study which proves co-infection of malaria with filariasis (Ravindran *et al.*, 1998), dengue (Levett *et al.*, 2000), Borrelia (Vinetz, 2001) etc. Present study has shown that there has been a rise in the incidence of malaria post tsunami and the trend continued till 2008 -2009. The environmental and geographical changes that took place after tsunami is the reason behind the hike in incidence of malaria in Nancowry group of Islands. Co-infection with leptospirosis causes concern due to increase disease burden to the host because diagnosis of leptospirosis is difficult in the field and diagnostic tools are not available in the field thereby delay in initiation of proper treatment.

CONCLUSION

In a malaria endemic zone if a case is found to be malaria positive, malaria is assumed as the main cause of fever. This could also be the case of leptospirosis. So, It is suggested a thorough investigation of other diseases. In the present study leptospirosis has been found in a malaria cases. So, it has been suggested to diagnose a case of febrile illness for leptospirosis that lives in a close proximately with condition favours transmission of leptospirosis. Because failure to recognize acute leptospirosis co-infection and delay in starting the treatment there by ending up in severe complication such as pulmonary form of the disease (Vinetz, 2001).

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