Sero-evidence of Rickettsia Infection by ELISA in the Northern-Central Area of Bangladesh

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Detection of rickettsia most commonly done by simple, economical Weil-Felix test which detects IgM antibody. This initial investigation provides limited sound guidance to clinical decisions because of its low specificity and sensitivity. An alternative test, enzyme-linked immunosorbent assay (ELISA) is faster, less complicated, can also be automated. Advancements in molecular method like polymerase chain reaction (PCR) are highly specific, sensitive and rapid assays for detection of *rickettsiales* in many different samples including blood, tissue etc. This study was carried out to diagnose the rickettsial agent in the north-central (Mymensingh division) area of Bangladesh. In laboratory, we performed ELISA and PCR. The agent was diagnosed up to species level by molecular approach. A total of 150 febrile patients were included. All were clinically suspected cases of rickettsial fever attending inpatient and outpatient department of medicine and pediatrics of Mymensingh Medical College Hospital from July 2012 to January 2014. The laboratory tests were performed in Microbiology department of Mymensingh Medical College. Following universal safety precautions blood samples were collected, serum separated and both were stored at -20°C. IgM ELISA and Nested PCR were performed. Several genes by PCR were detected for confirmation of the presence of rickettsial agent in the blood. Among 150 clinically suspected cases 76(50.66%) were positive for ELISA, and 69(46.0%) were positive for PCR. The sensitivity and specificity of ELISA were 92.75% and 85.19% respectively taking PCR as gold standard. The prevalence of *rickettsial* infection found in this study was very much close to other countries of this Sub continent.

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Key words: Rickettsial infection, ELISA, PCR

Introduction

Rickettsioses are the infectious diseases which are caused by obligate intracellular gram- negative bacteria. They belong to the order of Rickettsiales, family Rickettsiaceae[1]. They cause significant amounts of disability and fatalities among both military and civilian populations throughout the world[2]. They are found in arthropod vectors such as fleas, lice, ticks and mites[3,4]. Outbreaks of typhus fever are reported from Burundi, Algeria, Peru and Russia. In 1997 in the refugee camps of Burundi, 1,00,000 cases of typhus fever occurred during a civil war[5]. In Africa, from 2007 to 2008, the rickettsial seroprevalence among febrile patients was 28.0% - 58.0%. In the same duration, it was identified in 8.0% of febrile cases in Tanzania and 6.0% Senegal and Kenya[6]. WHO in 1993, reported that, rickettsial infections are major causes of febrile illnesses throughout the Asia-Pacific region[7]. In India rickettsial disease has been documented from Jammu and Kashmir, Himachal Pradesh, Uttaranchal, Rajasthan, Assam, West Bengal, Maharashtra, Kerala and Tamil Nadu.

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In India, actual prevalence of rickettsial diseases could not be estimated due to lack of community data and laboratory test. In India during 2005-2009, scrub typhus was detected 48.2% followed by spotted fever group 27.5% and typhus group 6.8%[8]. Early sign symptoms of this illness are notoriously non-specific or mimic benign viral illness, making them difficult to diagnose in a clinical setting[9]. Evidence for rickettsial infections in human populations has been identified by using Weil-Felix test, ELISA and PCR. The Weil-Felix test is a serodiagnostic test still serves as a useful and cheap diagnostic tool for detection of rickettsial disease[10] and routinely performed in poor countries for diagnosis of acute cases. It yields false positive results with leptospirosis, malaria, Proteus infections and other febrile illnesses[11]. From India, a study reported the sensitivity and specificity 43.0% and 98.0% respectively[12]. In another study it was found the sensitivity and specificity 49.0% and 96.0% respectively[13]. ELISA (Enzyme linked Immuno-sorbant assay) is a specific and sensitive test, useful for both diagnosis of acute and convalescence case and seroepidemiology[14]. ELISA is faster, automated and less complicated[15]. Molecular technique PCR (Polymerase chain reaction) is highly specific, sensitive and rapid assays for detection of *Rickettsiales* in many different samples including blood, tissue and arthropods[16]. Under diagnosed or misdiagnosed rickettsioses are emerging infectious diseases throughout the world[17] and major causes of febrile illnesses in Asia-Pacific region[7], also in several parts of the Indian subcontinent[18]. Failure of timely diagnosis leads to significant morbidity and mortality[19]. In this study, we developed and evaluated recombinant rickettsial protein IgM (Immunoglobulin M) by ELISAs for serodiagnosis of rickettsia disease and confirmed by molecular technique PCR. Further confirmation and species identification was performed by sequencing in all the PCR positive cases.

Methods

Clinically suspected 150 febrile patients were selected randomly from outpatient and inpatient department of medicine and pediatrics unit of Mymensingh Medical College Hospital (MMCH), Bangladesh. This study was approved by the Institutional Review Board and informed consent was taken from each patient and their guardians before his or her entry into the study. Specimen collection and laboratory work was done in the Department of Microbiology from July 2012 through January 2014. Patients with fever (axillary temperature >99°F) more than 15 days not responding to most of the commonly used antibiotics were included. They were of any age and sex. Patients suffering from fever other than the rickettsial infection were excluded by thick film and immunochromatography.

Collection of specimens

Venous blood was collected aseptically from all cases following universal safety precaution. Wearing sterile disposable gloves, the punctured area was washed with iodine and 70.0% alcohol. With a sterile disposable syringe, 03 ml of blood was collected of which 01 ml was transferred in a sterile test tube with EDTA for PCR and 02 ml of blood was transferred in sterile plain test tube for serum collection for ELISA. Both serum and blood specimens were stored in -20ºC for longer duration.

Laboratory procedure

ELISA (Enzyme-Linked Immunosorbent Assay) to detect IgM antibody of Rickettsia specific antigen[20]

The Typhus Group-Rickettsia EIA IgM Antibody kit was intended for the qualitative detection of IgM class human antibody to Typhus Group Rickettsia. The ELISA module wells in the kit utilize a group-specific lipopolysaccharide (rLPS) antigen extracted from Rickettsia typhi (Epidemic typhus), a member of the Typhus Group. ELISA was done according to the instruction of Manufacturer of ELISA kit (Delta Biologicals S.R.L. via Nicaragua 12-14 00040 Pomezia Italy, DBE-043, 13 ERIC 101-z). Values which were more than cut off value, were accepted as positive result.

Results

Among 150 cases, in ELISA 76(50.66%) were positive and 74(49.34%) were negative and in PCR 69(46.0%) were positive and 81(54.0%) were negative (Table I).

The Table II showing sensitivity and specificity of ELISA were 92.75% and 85.19% respectively taking PCR as gold standard.

Table I: Results of the tests performed among the clinically suspected cases

Name of test	Positive	Negative	Total
	n (%)	n (%)	
ELISA	76 (50.66)	74 (49.34)	150
PCR	69 (46.00)	81 (54.00)	150

Table II: Correlation of ELISA and PCR considering PCR as gold standard

Test	PCR positive	PCR negative	Total	Sensitivity	Specificity
ELISA (Positive)	64	12	76	92.75%	85.19%
ELISA (Negative)	05	69	74		
Total	69	81	150		

Discussion

All 150 suspected cases, Blood and serum sample were examined by ELISA and PCR (Table I). The positive result by ELISA was 76(50.66%) and PCR was 69(46.0%) in 150 cases. This study reflects the sensitivity and the specificity of IgM ELISA are 92.75% and 85.19% respectively (Table II). A similar study carried out in India by Rathi et al. where IgM ELISA had a sensitivity and specificity of 91.0% and 100.0% respectively[20]. This study shows similarity in sensitivity with the study, but the specificity is lower, because we used the ELISA antigen of *R. typhi* as we did not know which species is present in our country. Later we detected the presence of R. felis in our positive cases by PCR and sequencing. In this study 69(46.0%) cases were positive in PCR. Fournier and Raoult from France reported 45.6% positive by PCR[21]. In India 58.6% of the 58 clinically suspected cases of spotted fever rickettsioses were confirmed by nested PCR[21]. The positive result by PCR is almost similar with other mentioned studies. Using PCR method and sequencing technique, rickettsial infection in this area was confirmed to be caused by the species *R. felis.*

Conclusion

The high prevalence of rickettsia infection suggests that this infection is endemic to the north-central area of this country. Weil Felix test can serve as initial but not sole method to recognize and diagnose rickettsial diseases, particularly if no rickettsioses have been previously isolated or detected in the considered area. If it is possible for most microbiology laboratories across the country to start ELISA to assess the burden in that area. Seroservilence study throughout the country is highly recommended to find out the prevalence of this disease.

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