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A prospective study of the effectiveness of clinical RGA (Rathi Goodman Aghai) score in relation with PCR in the diagnosis of scrub typhus

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Abstract
Background: Rickettsial diseases are emerging as an important cause of acute undifferentiated febrile illness throughout the Asia-Pacific region. Rickettsial infections are generally incapacitating and difficult to diagnose; untreated cases have case fatality rates as high as 30-45% with multiple organ dysfunction, if not promptly diagnosed and appropriately treated. Prompt antibiotic therapy, even based on suspicion, shortens the course of the disease and lowers the risk of complications and in turn reduces morbidity and mortality due to rickettsial diseases.
Objectives: To study the clinical RGA score, in relation with PCR in the diagnosis of Scrub Typhus cases admitted in medical college hospital.
Methodology: It is prospective observational study. The present study was conducted at the department of Paediatrics, SVRR Govt. General Hospital, Tirupati for a period of 1 year. A total of 99 cases in the age group of 1 month to 12 years were taken into the study by considering the inclusion and exclusion criteria.
Results: RGA scoring was done for every case and blood samples were drawn for IgM ELISA and PCR. There is higher incidence of scrub typhus in the age group of 1-5 years accounting to56.6%. Males outnumber females. Male: Female ratio is 1.6:1. Majority of the children are from rural area constituting 69.7% of the total cases. Majority of the cases had RGA score ≥14(73.7%). RGA score of ≥14 shows a sensitivity of 92% and specificity of 83.3% when compared with the PCR. There was an increase in the sensitivity when the cut-off was lowered. RGA ≥9 showed a sensitivity of 97%. RGA score of ≥14 showed a sensitivity of 93.8% in those with both IgM ELISA and PCR positive. IgM ELISA shows a sensitivity of 73% when compared with PCR.
Conclusion: Scrub typhus has become a leading infectious disease in India and an important cause of infectious fever. The proposed scoring system can be used for early detection, treatment and prevention of mortality and morbidity from spotted fever group.

Keywords: Scrub typhus fever, PCR, RGA score, IgM ELISA

Introduction
Rickettsial diseases are considered as some of the most covert emerging and re-emerging diseases and are being increasingly recognized in India. Rickettsial diseases have been documented in India since the 1930s with reports of scrub typhus from Kumaon region [1], Assam in soldiers during the second world war [2, 3] of scrub and murine typhus from Jabalpur area in Madhya Pradesh [4] and of murine typhus from Kashmir [5]. Rickettsioses, of which scrub typhus is the commonest, has been clearly reported from several states in India including Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Bihar, West Bengal, Meghalaya, Rajasthan, Maharashtra, Karnataka, Tamil Nadu and Kerala [6-9]. Rickettsial infections are generally incapacitating and difficult to diagnose; untreated cases have case fatality rates as high as 30-45% with multiple organ dysfunction, if not promptly diagnosed and appropriately treated [9]. Prompt antibiotic therapy, even based on suspicion, shortens the course of the disease, lowers the risk of complications and in turn reduces morbidity and mortality due to rickettsial diseases. The clinical manifestations of scrub typhus in children are nonspecific and likely to be misdiagnosed. The delay in administering effective antibiotic treatment of some patient's can lead to enhanced complications or mortality.
Thus, achieving a rapid and precise diagnosis is necessary for the proper medical management of scrub typhus.

The routinely available rapid diagnostic tests for the detection of *O. tsutsugamushi* have low sensitivity and specificity which may lead to improper diagnosis or delay in the diagnosis to differentiate from fever without focus due to other cause. The PCR assay is a useful tool for facilitating the diagnosis of infectious diseases that are caused by fastidious or slowly growing microorganisms. The results of PCR can be available within 24hours, and this can greatly help to guide proper patient management. But PCR is costly and is not available at all centres. Hence, PCR cannot be routinely done to diagnose scrub typhus. The diagnosis of scrub typhus has traditionally been based on the assessment of the antibody titer in the serum samples obtained during the acute and convalescent phases of illness. However, it takes several weeks to confirm the diagnosis through serologic testing (IgM ELISA) for establishing a 4-fold or greater titre increase and cannot have an impact on diagnosis and management. Hence, there is a need of a systematic approach combining both clinical and laboratory criteria which should be cost effective and which can be applied in any resource limited setting so that complicated diseases are diagnosed very early and proper management can be done. Narendra B Rathi *et al*. (2011) [10] proposed RGA (Rathi Goodman Aghai) scoring system for diagnosis of rickettsial infections. RGA score ≥14 is considered as positive. The RGA scoring system can be used for early detection, treatment and prevention of mortality and morbidity from rickettsial diseases [10]. So, this study has been done to compare the effectiveness of RGA scoring in respect to PCR.

Aims and Objectives

The main aim of the present study to identify the effectiveness of clinical RGA (RATHI GOODMAN AGHAI) score in relation with the PCR in the diagnosis of scrub typhus.

Materials and Methods

Study design

Hospital based prospective observational study.

Duration of study

This study was conducted between September 2015 and August 2016 in the department of Paediatrics at Sri Venkateswara Ramanarain Ruia Government General Hospital (SVRRGGH), Tirupati.

Inclusion criteria

All children in the age group of 1 month to 12 years, admitted in the Department of Paediatrics, SVRRGGH, Tirupati during the study period with fever without a focus and one or more of the following features like Rash, Edema, Lymphaedenopathy, Eschar, Hepatosplenomegaly or History of tick bite or exposure and diagnosed as scrub typhus with PCR/IgM ELISA.

Exclusion criteria

All children diagnosed with Dengue, Enteric fever, Leptospirosis, Malaria and Haemato oncological disorders.

Method of collection of data

All children in the age groupof1monthto 12 years, admitted in the hospital during the study period satisfying the inclusion criteria were enrolled in the study. Permission to conduct the study was obtained from institutional ethics committee, Sri Venkateswara Medical College, Tirupati. Detailed history was taken. On examination the site and type of rash was noted and looked for conjunctival congestion, lymphaedenopathy, eschar, facial puffiness/pedal edema/anasarca and hepatosplenomegaly. RGA scoring was done in all children. PCR and IgM ELISA for scrub typhus was done in all children. Investigations were done as per the requirement of fever without focus including Complete blood count, Peripheral smear, Blood culture for enteric fever, NS1 antigen/IgM Dengue antibody, IgM Leptospira, C-reactive protein, Serum electrolytes, Urine analysis, Liver function tests, Serum albumin, etc.

Rathi Goodman Aghai (RGA) scoring was done based on the history, clinical findings and laboratory reports as follows:

Table 1: RGA Scoring System

Clinical features	Score	Laboratory	Score
Rural	1	Hemoglobin ≤9g/dL(%)	1
Pets	1	Platelets <150,000 (cells/L)	1
Tick exposure	2	CRP ≥50 (mg/dL)	2
Tick bite	3	Serum albumin <3g/dL	1
Conjunctival congestion (Non exudative)	2	Urine albumin ≥2+	1
Maculopapular rash	1	SGPT ≥100 (U/L)	2
Purpura	2	Na ≤130 (mEq/L)	2
Palpable purpura/ecchymosis/necrotic rash	3		
Rash appearing 48-96h after fever	2		
Pedal edema	2		
Rash on palms and soles	3		
Hepatomegaly	2		
Lymphadenopathy	1		
Total	25		10

Sample collection and processing for polymerase chain reaction

Sample for Polymerase Chain Reaction was collected at the time of admission or within 5 days of onset of illness whichever was possible. 2ml of venous blood is drawn into an EDTA containing tube (Vacutainer) and mixed gently and stored at-20^{0}C and the collected samples were processed at the laboratory. First DNA extraction was done and then the DNA was subjected to nested Polymerase Chain Reaction.

DNA extraction: Sample stored at -20^{0}C is brought to room temperature before beginning the procedure of DNA extraction. DNA is extracted from 200µl of whole blood using HiPurA^{TM} Blood Genomic DNA Miniprep Purification Kit as per the manufacturer s instructions. The eluate of 50µl containing pure genomic DNA is stored at -20^{0}C for further DNA amplification.

Nested polymerase chain reaction

DNA amplification was performed using a nested format. The primers used in this study were described in1993 by Furuya *et al.* [14] and reported by Saisongkorh and co-workers in 2004 [15]. A 483bp segment of the gene encoding the 56 kDa antigen of *O. tsutsugamushi* was amplified by nested PCR and the primer sequences (Sigma-Aldrich, Bangalore, India) used areas given below:

Outer primer set

P55:5‟-TCAAGCTTATTGCTA GTGCAATGTCTGC- 3‟
P34:5‟–AGGGATCCCTGCTGCTGT GCT TGC TGCG-3‟

Inner primer set

P10:5‟-GATCAAGCT TCCTCAGCCTACTAT AAT GCC-3‟
P11:5‟-CTAGGGATC CCG ACAGATGCACTATTA GGC-3‟

The PCR amplification mixture (50µlvolume) contained 1.25 units of Taqpolymerase, 1.5Mm $MgCl_2$, 50mM KCl, 10mM Tris-HCl (pH 8.3), 200µM each of dATP, dGTP, dCTP, dTTP (Ferments, Glen Burnie, MD, USA)10 pm ol of primers (p55 and p34) and 5µl of the extracted DNA. The second set of primers (p10 and p11) replaced the first set in the second PCR. For both PCR runs, the amplification protocol consisted of denaturation of template at 94^{0}C for 30sec, annealing at 57^{0}C for 2min followed by extension at 70^{0}C for 2min for 35 cycles in a thermal cycler. PCR product was visualized using a gel documentation system (GelDoc XR, BioRadInc, Hercules, CA, USA) after electrophoresis in 1.5% agarose gel containing ethidium bromide (0.5µg/ml).

IgM ELISA

2ml of venous blood was drawn into a vacutainer without any additives after 5days of onset of illness. Sample was sent to the Department of Microbiology, Sri Venkateswara Medical College, Tirupati. There sample was processed using In Bios Scrub Typhus Detecting ELISA kit and results were given.

Analysis of data

The data collected was entered in Microsoft Excel. ROC curve analysis was done to know the sensitivity, specificity of Rathi Goodman Aghai score in relation with the Polymerase Chain Reaction. Statistical analysis was done using epiinfo software 7.1.4.

Results

120 children were enrolled into the study after satisfying inclusion criteria. Out of which 9 showed IgM dengue positive, 4 showed Malaria parasite in the smear and 8 were positive for widal. So, these 21children were excluded from the study. The data of 99 cases was taken and analysed accordingly.

Table 2: Age distribution in cases

Age in Years	No. of. Cases
1 month-1 year	9
1-5 years	56
5-12 years	34

Majority of cases (56.6%) were in the age group of 1-5 years. Minimum age of the child included in the study was 6months.

Table 3: Gender wise Distribution

Sex	No of Cases	Percentage
Male	61	61.6%
Female	38	38.4%

Majority of the cases were males which constituted 61.6% of the total cases.

Table 4: Area wise distribution

Area	No. Of. Cases	Percentage
Rural	69	69.7%
Urban	30	30.3%

Majority of the children were from rural area constituting 69.7% of the total cases.

Table 5: Day wise illness at the time of admission

Day of illness	Cases	Percentage
< 3 days	10	10.1%
3-7 days	76	76.7%
>7 days	13	13.1%

Majority of the children presented to the hospital within 3-7 days of onset of illness. The time of presentation may interfere with the scoring and diagnostic test results.

Table 6: Clinical Features of study patients

Clinical Features	Cases	%
Eschar	31	31.3
Tick exposure	47	47.5
Conjuctival congestion	60	60.6
Purpura	04	4.04
Anasarca	11	11.1
Pedal edema	20	20.2
Hepatomegaly	26	26.3
Lymphoadenopathy	53	53.5

Rash constitutes the major clinical feature. Eschar, the definitive sign of rickettsial infection was seen in 31.3% cases. 7 cases had atypical presentation of which 5 cases presented with meningitis, 1 with ARDS, and 1with hypertension.

Table 7: Showing number of PCR cases

PCR	Cases	%
Positive	75	75.7%
Negative	24	24.3%

In the present study, PCR was positive in 75.7% cases.

Table 8: Showing Number of IgM ELISA positive cases

IgM ELISA	No. of. Cases	%
Positive	89	89.9%
Negative	10	10.1%

The present study showed that IgM ELISA was positive in 89.9% cases.

Table 9: Showing RGA score

RGA Score	No. of. Cases	%
≥14	73	73.7%
<14	26	26.3%

The present results revealed that RGA score was positive in 73.7% of children.

Table 10: Showing RGA score with PCR

RGA score	PCR	
	Positive	Negative
≥14	69	4
<14	6	20%

RGA score ≥14 has a sensitivity of 92% and specificity of 83.3% when compared with the PCR.

Table 11: Showing RGA score with IgM ELISA

RGA score	PCR	
	Positive	Negative
≥14	65	8
<14	24	2

RGA score ≥14 has a sensitivity of 73% when compared with IgM ELISA.

RGA score comparison with IgM ELISA and PCR

65 children have both IgM ELISA and PCR positive. Out of which 61 children showed RGA ≥14. This shows that the sensitivity of RGA with cutoff of14 is 93.8% when compared to IgM ELISA and PCR. Around 92% of children with scrub typhus (diagnosed by PCR) were diagnosed clinically with RGA score cutoff 14. IgM ELISA showed sensitivity of 86.6%% when compared with PCR.

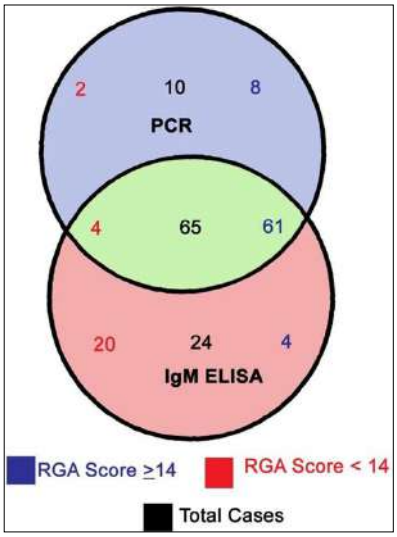

Fig 1: RGA score comparison with IgM ELISA and PCR

Table 12: Showing comparison of IgM ELISA with PCR

IgM ELISA	PCR	
	Positive	Negative
Positive	65	24
Negative	10	0

In the present study, IgM ELISA shows a sensitivity of 86.7% when compared with PCR.

Discussion

Scrub typhus is a rickettsial infection which is caused by *Orientia tsutsugamushi* and transmitted by the bite of the chigger of a mite. An estimated one billion people are at risk for scrub typhus and an estimated one million cases occur annually. Mortality rates in untreated patients range from 0-30% depending on the geographic area and the rickettsial strain and the time of intervention. The clinical manifestations of scrub typhus in children are nonspecific and likely to be misdiagnosed. Due to overlapping of clinical features and hematological manifestations, only clinical diagnosis of scrub typhus is very difficult especially during epidemics of viral infections like Dengue, other viral hemorrhagic fevers and Malaria. The delay in administering effective antibiotic treatment can lead to enhanced complications or mortality. Thus, achieving a rapid and precise diagnosis is necessary for the proper medical management of scrub typhus.

Hence, there is a need of a systematic approach combining both clinical and laboratory criteria which should be cost effective and which can be applied in any resource limited setting, so that complicated diseases are diagnosed very early and proper management can be done. Narendra B Rathi *et al.* [10] proposed RGA (RATHI GOODMAN AGHAI) scoring system for diagnosis of rickettsial infections. RGA score ≥14 is considered as positive.

Hence this study has compared the effectiveness of RGA score in respect to PCR and IgM ELISA. Present study showed that high incidence of scrub typhus was in the children <5 years which is slightly higher (56.6%) when compared to other studies done by Nirmala *et al* (2014) [11] and Ira Shah *et al.* (2010) [12] where the incidence was 46.2% and 54%respectively.

Out of 99 cases included in our study there was male predominance of 59%.Similar male predominance was seen in other study done by Nowneet Kumar *et al.* (2014) [13]. A slightly higher male predominance was seen in the study done by Narendra Rathi *et al.* (2010) [10].

Out of 99 cases in our study, 69% cases had rural background. A slight higher incidence of cases from rural back ground was seen in other studies done by Narendra B Rathi *et al.* (2011) [10]. Nowneet Kumar Bhat *et al.* (2014) [13] which showed 81% and 83% of cases respectively from rural area.

RGA score of ≥14 shows a sensitivity of 92% and specificity of 83.3%. There are no studies correlating RGA score with PCR. Around 92% of cases can be diagnosed clinically as scrub typhus with RGA score cutoff of 14.

RGA score of ≥9 has a sensitivity of 97.3% and specificity of 70.8% when compared with PCR. RGA score ≥14 had sensitivity of 73% in the present study when compared to Narendra B Rathi *et al.* (2011) [10] study which had 96.5% when compared with IgM ELISA.

Conclusion

The overall sensitivity of RGA scoring system in children with scrub typhus at a cut-off of 14 is 74% and have good specificity. Around 3/4th of children with scrub typhus can be diagnosed with RGA scoring system. RGA scoring has shown significant sensitivity and specificity in comparison with PCR in the diagnosis of scrub typhus. Hence, can be applied as reliable alternative diagnostic tool in resource limited settings. Further studies are needed on a large group in different geographical settings for generalization.

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Conflict of interest

The author declares that there is no conflict of interest.

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