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Demographic and Clinical Characteristics of Scrub Typhus Cases detected by Nested Polymerase Chain Reaction among Febrile Patients in Mymensingh, Bangladesh

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Abstract

Background & objectives: Scrub typhus is a mite borne rickettsial illness, caused by the bacterium Orientia tsutsugamushi, which is endemic to Asia pacific region. Due to wide spectrum of non-specific manifestations and inaccessibility of accurate diagnostic tools, it remains underdiagnosed in many countries of Southeast Asia including Bangladesh, resulting in severe life-threatening complications. Therefore, this study was aimed to diagnose scrub typhus through molecular detection of O. tsutsugamushi by 'Nested Polymerase Chain Reaction (N-PCR)' among febrile patients in Mymensingh, Bangladesh & to study the socio-demographic as well as clinical characteristics of the diagnosed cases. **Methods:** It was a cross-sectional type of study, conducted at department of Microbiology, Mymensingh Medical College between March 2018 and February 2019. Blood samples were taken from 453 febrile patients of suspected rickettsial illness, referred from both inpatient and outpatient facility of Department of Medicine and Department of Pediatrics, Mymensingh Medical college Hospital (MMCH). Then DNA was extracted from the whole blood, following Phenol-Chloroform extraction & ethanol precipitation method. Finally, N-PCR was performed on extracted DNA, targeting 47Kda antigen gene, to detect O. tsutsugamushi. **Results:** Out of 453, this study documented [78 (17.21%)] PCR positive scrub typhus cases, among which majority [42 (53.84%)] were female & maximum [26 (33.33%)] were in age group >15-30 years. Higher number of PCR positive patients [51 (65.39%)] were from rural areas. Among the scrub typhus cases, myalgia (48; 61.53%) was the most common manifestation, followed by headache (44; 56.41%) and cough (44; 56.41%). Eschar was present only in 14 (17.94%) cases and 8 (10.25 %) patients had skin rashes. Leukocytosis and leukopenia were documented in 13 (16.66%) and 5 (6.41%) cases respectively. Decreased Hb% was recorded in 14(17.9%) & 16 (20.5%) cases had thrombocytopenia. **Conclusion:** This study recorded a high number of scrub typhus cases in Mymensingh region with non-specific febrile manifestations, hindering early & accurate clinical diagnosis. Complications like - renal & hepatic impairment, CNS involvement, ARDS as well as hematological

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abnormalities were documented also. So, scrub typhus should be considered in the differential diagnosis of undifferentiated febrile illness in Bangladesh. Further molecular studies on scrub typhus might be done throughout the countries to create awareness among physicians & to evaluate actual disease burden.

Keywords: Scrub typhus, Nested PCR, Orientia tsutsugamushi.

Introduction

Scrub typhus or 'Tsutsugamushi disease' is a potentially life-threatening but easily treatable acute febrile illness, caused by a gram negative obligate intracellular bacterium- Orientia tsutsugamushi.[1] It is the world's most important rickettsial illness in terms of disease burden, which threatens one billion people globally and causes illness in one million people each year.[2,3] According to WHO, scrub typhus is probably one of the most underdiagnosed febrile illness, that often requires hospitalization.[4] In 1931, Dr. Norio Ogata successfully isolated the agent of scrub typhus and named it- "Rickettsia tsutsugamushi", which belonged to the genus 'Rickettsia'. Later on, due to significant phenotypic difference and phylogenetic diversity on the basis of 16s rRNA sequences, it was reclassified into a new genus- 'Orientia' and renamed as- 'Orientia tsutsugamushi'.[5]

Scrub typhus is endemic to a geographically distinct region, known as- "Tsutsugamushi Triangle", covering 13,000,000 km^2 land area which includes Asia-Pacific rim extending from Afghanistan to Japan, Taiwan, China, South Korea, Thailand and northern Australia.[6] It also occurs in Nepal, northern Pakistan, Sri-Lanka, Queensland and all over the India including both southern and northern region.[7] Actually, scrub typhus is very common in countries of Southeast Asia with an average seroprevalence ranging from 9.3%–27.9%.[8] The burden of the disease is very high in rural Asia, where scrub typhus causing up to 20% of all febrile hospital admissions.[1] In Southeast Asia alone, it causes 50,000–80,000 deaths annually. 23.7% seropositivity was reported from Bangladesh according to a study, covering Dhaka, Comilla, Sylhet and Chittagong division.[9] Recently, a molecular and serologic confirmation of O. tsutsugamushi with a detection rate of 16.8% was also reported from Chittagong, Bangladesh.[10]

Scrub typhus is vectored to human hosts by the bite of different larval trombiculid mites (Leptotrombidium deliense, L. akamushi etc). Tiny larval mites (chiggers) feed usually on rodents but, accidentally on humans and can transmit the infection.[11] The word "Scrub" comes from "Scrub growth" consisting of low-lying trees and bushes, where larval mites commonly inhabit.[12] River banks, rice fields, abandoned plantations and kitchen gardens can also be inhabited by those mites. Incidence of scrub typhus is higher among rural population. Cases are more likely to have exposure to rodents, and to occupational (farming) or recreational activities, which further expose them to risk of encountering larval mites.[13] Seasonal variations of scrub typhus can be found, with increased incidence at late rainy season and beginning of winter months (July- November) in South Asia.[14]

Clinical pictures of scrub typhus vary in severity from a mild febrile illness to a potentially fatal disease with Multi-organ Dysfunction Syndrome/-MODS.[15] Non-specific febrile illness is typically accompanied with myalgia, headache, generalized lymphadenopathy, rash, vomiting and conjunctival suffusion.[16] Eschar is a cigarette-burn like, black necrotic lesion in skin, variably seen in 50% of cases, and may be diagnostic.[17] Complications usually develop after first week of illness like- renal failure, hepatic failure, pneumonitis, acute respiratory distress syndrome (ARDS), septic shock, myocarditis, meningoencephalitis and hearing loss.[18] Several genotypic variants are recognized among O. tsutsugamushi, on the basis of their "type specific 56-kDa protein". Gilliam, Karp, and Kato types are well known genotypes, but Shimokoshi, Kawasaki, TA763 and Kuroki types have also been described. Bacterial virulence, disease severity and prognosis may vary among infecting genotypes. Genotypic and antigenic variation of Orientia may be the reason for frequent outbreaks and reinfection of scrub typhus.[19]

Early and accurate laboratory diagnosis is very essential to prevent complications of scrub typhus.[20] The mainstay in diagnostics remains serology, but all the available methods have many limitations. Weil-Felix test is the cheapest & oldest method but it greatly lacks sensitivity & specificity.21 The indirect immunofluorescence (IFA) assay is gold standard, but test result greatly varies between different centers due to variation of antigen preparation.[22] Thus Molecular method, like polymerase chain reaction (PCR) has been developed to diagnose scrub typhus, especially during the phase of early bacteremia (up to 15 days of infection), when most of the serological tests remain negative. For PCR, most common target is the 47 KDa antigen gene & whole blood, buffy coat, serum as well as eschar materials can be used as specimen.[23] Nested PCR (N-PCR) is widely used to improve the sensitivity of conventional PCR (C PCR) because, C-PCR requires 20 ng of DNA for detection, whereas N-PCR requires only 200 pg of DNA which makes N-PCR 100 times more sensitive.[24]

Aim of the present study was to diagnose scrub typhus by Nested PCR (N-PCR) among febrile patients in Mymensingh region as well as to analyze the socio-demographic & clinical characteristics of the diagnosed patients. Bangladesh resides within the endemic triangle of scrub typhus but, there is very limited data regarding detection of O. tsutsugamushi by PCR & subsequent study on diagnosed scrub typhus cases. Thus, this study was undertaken to overcome some of the gap in the existing knowledge.

Methodology

This was a cross-sectional study, conducted at department of microbiology, Mymensingh Medical College from the period of March 2018 to February 2019. A total of 453 Febrile patients (fever for more than 5 days) of suspected rickettsial illness, irrespective of age and sex, referred from outpatient and inpatient facilities of department of Medicine and department of Pediatrics, Mymensingh Medical College Hospital (MMCH) were included in the study. Febrile patients with already established causes of their illness other than rickettsial disease, were excluded from this study. The approval of the institutional ethical committee was obtained before beginning of the study. Before collecting blood sample, informed consent was taken from all patients & socio-demographic data of all the subjects were collected using pre-tested, printed questionnaire. Following all universal safety precautions, 2ml of venous blood was collected with a sterile disposable syringe & was transferred into a clean sterile EDTA tube to perform PCR & stored at -20°C until further use.

From whole blood, using standard protocol with specific primers, O. tsutsugamushi DNA was detected by N-PCR, targeting 47 kDa antigen gene. 3 major steps of PCR were performed DNA extraction from whole blood, DNA amplification in thermal cycler and Agarose gel electrophoresis of PCR products followed by visualization/ documentation under UV light.

DNA was extracted from whole blood by using manual method. After thawing the frozen samples at room temp. & heating them at 55° for 45 minutes, all of them were digested (lysed) by using Fresh proteinase K (PK) and SDS (sodium dodecyl sulfate). Then DNA was extracted from those digested samples using buffer saturated phenol & Chloroform (1:1).

Then extracted as well as purified DNA materials was obtained at the bottom of the microcentrifuge tube as pellets, through Ethanol precipitation technique (using two volumes of 100% chilled ethanol and 10µl of 5M NaCl). Finally, the pellets were diluted with 25µl of DDW to be used in PCR.[25] DNA from some blood samples was also extracted by using the QIAamp DNA Mini Kit (Qiagen, Australia), following manufacturer's protocol. Then using the extracted DNA materials & 47kDa gene specific forward and reverse primers (OtsuFP555 and OtsuRP771)[26] (Table 02), master mixtures were prepared for 1st round of nested PCR. (Table 01)

Table 01: Materials used for preparation of each PCR reaction mixture (25 µl)

Mixture materials	Each reaction
Nuclease free water	14.25 µl
10x buffer (Takara, Japan)	2.5 µl
dNTP (2.5 mM each) (Takara, BIO INC, Japan)	2 µl
Primer mixture (30 pmol of each: OtsuFP555 and OtsuRP771)	1 µl
Taq polymerase (250U) (Takara, BIO INC, Japan)	0.25 µl
Extracted DNA samples as template DNA	5 µl
Total	25 µl

Then each of the reaction mixtures in a separate microcentrifuge tube are placed in an automated DNA thermal cycler (D Lin, China) for DNA amplification. After initial denaturation at 94°C for 4 minutes, 30 cycles each of which consisted of denaturation (94°C for 30 seconds), primer annealing (56°C for 30 seconds) and extension (72°C for 1 minute) were repeated. Final extension was done at 72°C for 5 minutes to get amplified product of first PCR. In 2nd round PCR, the amplified product of the 1st set PCR was used as template. In 2nd PCR amplification, a second set of 47 KDa gene specific forward and reverse primers: (OtsuFP630 and OtsuRP747)[26] (Table-02) were used. Thermal cycling condition for 2nd round of Nested PCR was same to the first round except the primer annealing, which was done at 60°C, instead of 56°C.

Table 02: Descriptions of Primers specific for O. tsutsugamushi 47KDa antigen gene

PCR round	Primer name and Sequences	Product size
1st PCR	Forward primer-- OtsuFP555 5′-TCCTTTCGGTTTAAGAGGAACA-3′	238 bp
	Reverse primer--OtsuRP771 5′-GCATTCAACTGCTTCAAGTACA-3′	
2nd PCR	Forward primer--OtsuFP630 5′-AACTGATTTTATTCAACTAATGCTGCT-3′	118 bp
	Reverse primer--OtsuRP747 5′-TATGCCTGAGTAAGATACRTGAATRGAATT-3′	

The amplified PCR products was visualized by electrophoresis in 2% agarose gel. Then after viewing in UV light, photographs were taken by digital camera.

Results

Among 453 febrile patients enrolled in this study, 145 were from inpatient and 308 from outpatient facility of Mymensingh medical college hospital. Out of 453 samples, 78 (17.21%) were positive by Nested PCR, and remaining 375 (82.79%) were negative (Figure 01). Among the 78 PCR positive cases, 42 (53.84%) were female and 36 (46.16%) were male. Age analysis of the PCR positive cases showed, maximum [26 (33.33%)] were in age group >15-30 years, followed by [22 (28.20%)] were in age group >30-45 years. Age group 0-15 years accounted for 19 (24.35%) cases also (Figure 02). This study documented that majority of the PCR positive cases [51 (65.39%)] were from rural areas and [27 (34.61%)] were from urban areas (Figure 03). Among the 78 scrub typhus cases, maximum [24 (30.76%)] were student followed by [19 (24.35%)] were housewife. 17 (21.79%) cases were farmer and only 2 (2.56 %) were teacher also (Table 03).

Study on the clinical presentations of the scrub typhus cases revealed that myalgia (48; 61.53%) was the most common manifestation, followed by headache (44; 56.41%) and cough (44; 56.41%). Eschar was present only in 14 (17.94%) cases and 8 (10.25 %) patients had skin rashes. 7 (8.97%) cases had jaundice. Oliguria and neck rigidity were documented also in 8 (10.25%) and 4 (5.12%) cases respectively (Table 04). Among the scrub typhus cases leukocytosis and leukopenia was documented in [13 (16.66%)] and [5 (6.41%)] cases respectively. Decreased Hb% was recorded in [14 (17.9%)] cases. [12 (15.3%)] cases were having an elevated bilirubin level and [16 (20.5%)] cases had thrombocytopenia. Serum creatinine was increased in [9 (11.53%)] patients also (Table 05)

Analyzing the month wise distribution of PCR positive scrub typhus cases, it was evident that number of positive cases begun to rise from August (9; 11.53%) and reached to its maximum during the month of September (15; 19.23%).

The case number remained high during the month of October (11; 14.10%) and November (10; 12.82%) also. December accounted for the least number (4; 5.12%) of PCR positive cases (Figure 04).

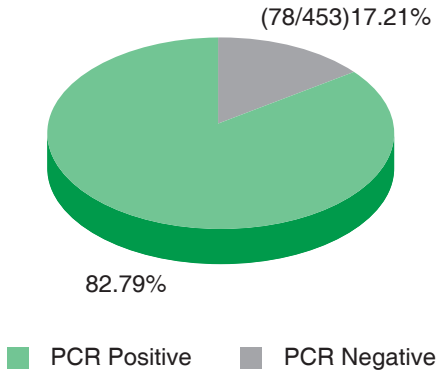

Figure 01: Result of Nested PCR (targeting 47 kDa gene) among total study population

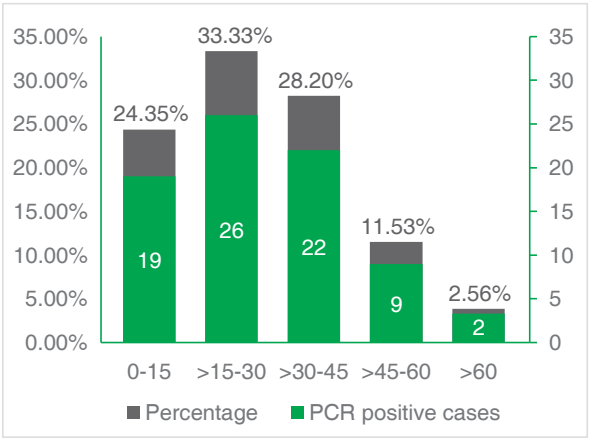

Figure 02: Distribution of PCR positive cases according to age group

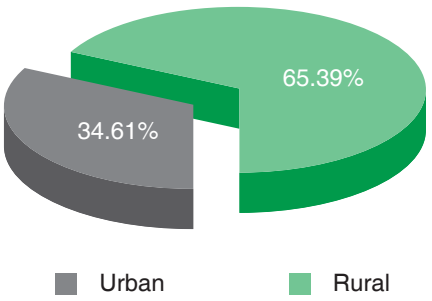

Figure 03: Distribution of PCR positive cases according to locality

Table 03: Distribution of PCR positive cases according to occupation (n= 78)

Occupation	No. of cases	Percentage (%)
Student	24	30.76
Housewife	19	24.35
Farmer	17	21.79
Day laborer	8	10.25
Unemployed	8	10.25
Teacher	2	2.56
Total	**78**	**100**

Table 04: Clinical features of Scrub typhus among PCR positive cases (n=78)

Clinical features	No. of cases	Percentage (%)
Myalgia	48	61.53
Headache	44	56.41
Cough	44	56.41
Eschar	14	17.94
Vomiting	14	17.94
Respiratory distress	12	15.38
Anemia	9	11.53
Skin Rash	8	10.25
Oliguria	8	10.25
Jaundice	7	8.97
Hepatomegaly	15	19.2
Neck rigidity	4	5.12

Table 05: Abnormal laboratory parameters associated with scrub typhus cases (n= 78)

Abnormal laboratory parameters	No. of cases	Percentage (%)
Leukocytosis	13	16.66
Leukopenia	5	6.41
Decreased Hb %	14	17.9
Increased bilirubin	12	15.3
Decreased platelet (Thrombocytopenia)	16	20.5
Increased serum creatinine	9	11.53

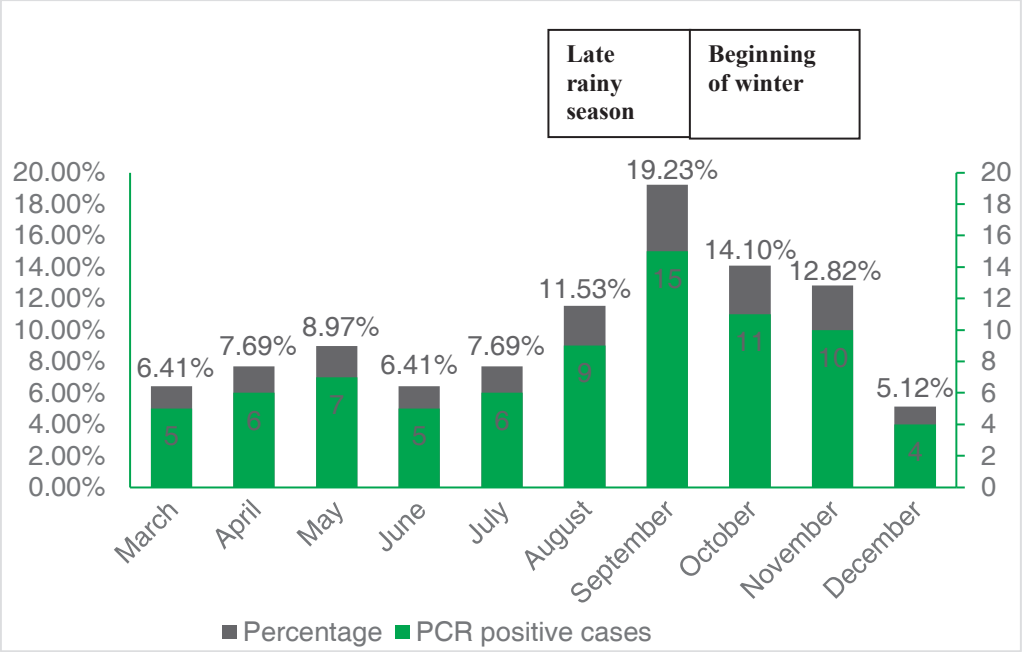

Figure 04: Month wise distribution (seasonal variation) of PCR positive cases

Discussion

We documented a high detection rate of O. tsutsugamushi- 17.21% (78/453) by nested PCR, targeting it's 47 KDa antigen gene. A study over 6 years period of time, conducted by Yang et al. in Eastern Taiwan reported about 15.66% (505/3223 cases) positivity for O. tsutsugamushi by Nested PCR.[27] This was nearly very similar to the present finding. A recent study in Chittagong, Bangladesh, reported 16.8% (70/414) positivity for O. tsutsugamushi by both molecular and serological assay, where 10.9% (45/414) cases were positive by Nested PCR[10], which was considerably lower than the present study. However, exclusion of pediatric age group (below 12 years) may be the possible reason for low PCR positive rate in Chittagong. Kumar et al. reported 24.37% (49/201) detection rate of Orientia by Nested PCR, from Chandigarh (India)[28] which was slightly higher than the present record. Usha et al. also reported a much higher PCR positive rate of 34.69% (230/663) from a study conducted in Andhra Pradesh, India.[19] This variation in Nested PCR detection rate may be due to difference in study design, target study population as well as geographical variation of circulating Orientia genotypes,which alters the sensitivity of molecular diagnostic tests also.[29]

The present study demonstrated slight female predominance (42/78; 53.84%) over male (36/78; 46.16%) among the scrub typhus cases. Vivekanandan et al. also reported the occurrence of increased number of female cases (28/50; 56%) than male (22/50; 44%), during an outbreak of scrub typhus in Pondicherry, India.[30] From Uttarakhand, India, 52.5% female and 47.5% male scrub typhus cases were reported according to a hospital study.[31] These reports were similar enough to the pattern of sex distribution of this study. In contrast to the present study, Usha et al. reported increased number of male (65.30%) than female (34.69%) among scrub typhus cases from Andhra Pradesh, India.[19] Gender variations of scrub typhus cases according to different studies are most likely due to occupational pattern and differing role of men and women in different study population.[32] However, no or very negligible gender variations among scrub typhus cases were documented in many studies in Japan and China.[3]

Present study showed that, most of the PCR positive scrub typhus cases were in age group >15-30 years (26/78; 33.33%), followed by age group >30-45 years (22/78; 28.20%). According to a study in Uttarakhand, India, majority of the

diagnosed scrub typhus cases were in between 20-45 years with a mean age of 35.5 years.[31] Anitha et al. also reported about 65.5% of the scrub typhus cases in Puducherry, India was young adult.[29] These findings were very much in accordance with the present study. Intense outdoor and field exposure due to occupational or recreational activity, may be the possible reason of more scrub typhus cases among this active young adult population, because outfield activity makes them vulnerable to encounter vector mites.[13] In this study, a good number of cases (19/78; 24.35%) were also documented in age group 0-15 years. Kalal et al. reported as high as 51.45% (53/105) seropositivity for scrub typhus among age group <18 years, from Karnataka, India.[33] So, scrub typhus in pediatric age population is not very uncommon.

Scrub typhus was known as a rural variety of typhus from late 1920s, when it was clearly differentiated from urban or endemic typhus.[34] The present study, also proved this statement true, because most of the scrub typhus cases (51/78; 65.39%) were from different rural areas of Mymensingh region in contrast to urban areas (27/78; 34.6%). Bhargava et al. also reported, majority [60.91% (173/284)] scrub typhus cases were from rural areas of Uttar Pradesh, India.[31] Studies from China and Indochina suggest that, a closer proximity of a person's home to the paddy field, scrub vegetations, ditches or wood piles within a village area may increase the risk of scrub typhus, due to more chance of encountering the vector mites. Close proximity to animals, both domestic and rodents, may also play a role in acquisition of scrub typhus.[32] These described scenario are very much common in various rural areas of Bangladesh, which explains the occurrence of higher number of cases from rural regions in the present study.

Farmer or persons related to any on-field agricultural activity are usually considered as the risk group of scrub typhus,[13] but this study documented that though a considerable number [17(21.79%)] were farmer, maximum [24(30.76%)] were student, followed by [19(24.35%)] were housewife and 8(10.25%) cases were day laborer also. Actually, not only farmers but also any person who has exposure to scrub vegetation as part of their daily life may contract scrub typhus due to an encounter with trombiculid mites.[35] According to a case control study in Korea, a significant number of persons including housewife, those were not directly related to farming, also suffered from scrub typhus as they all had outdoor exposure due to recreational activities or due to daily activities like- cutting grass, gathering herbs, collecting fruits, fishing and rearing domestic animals.[36] Housewives and students in Mymensingh region, particularly in rural areas have frequent outdoor exposure to such activities also. So, they are not free from the risk of contracting scrub typhus.

This study demonstrated that, all the PCR positive scrub typhus cases had fever (100%) and many other non-specific signs and symptoms were associated with the febrile episode. Myalgia was the most common of all the features (61.53%), followed by headache (56.41%), cough (56.41%), vomiting (17.94%) and respiratory distress (15.38%). During an outbreak of scrub typhus in Pondicherry, India, it was evident that- all (100%) diagnosed cases had fever, 56% had myalgia, 52% had headache, 40% had dry cough and 26% patients were suffering from breathlessness.[30] This finding was very much consistent with the present data. The present study also found anemia and jaundice on clinical examination in 11.53% and 8.97% scrub typhus cases respectively. Anitha et al. reported, 14.4% scrub typhus cases with anemia from Puducherry, India30 and Sharma et al. demonstrated 8.88% (4/45) scrub typhus patients with jaundice from Himalayan region, India.[37] Again this was nearly very similar to the present findings.

This study documented 10.25% scrub typhus patients had maculopapular skin rash. From Andhra Pradesh, India, 15.11% scrub typhus cases with skin rash were reported.[19] Only 4.08% cases with rash were documented by Kumar et al. from Chandigarh, India,28 whereas as high as 52.9% cases with rash was reported by Zhang et al. from Northwestern China.[38] These differences in the frequency of rashes may be due to variation of the infecting genotypes having variable virulence in and around the study region.[26]

Eschar is a black necrotic skin lesion, diagnostic of scrub typhus but not present in all cases.[17] It's prevalence is highly variable ranging from 7% - 80% among the diagnosed cases in an endemic area.[39] 17.94% cases with eschar were documented in this study which was nearly very similar to the finding of Viswanathan et al. who recorded 20% scrub typhus cases with eschar in Pondicherry, India.[40] In contrast, Zhang et al. documented 67.3% cases with eschar in Anhui province, China.[38] Difficulty in detecting small eschars in dark skinned persons, variable eschar inducing capacity of different infecting genotypes and atypical eschars may be the reason for this difference in documented eschar prevalence.[39]

This study also documented 5.12% cases with neck rigidity, which is a sign of meningitis. 26% cases with clinical evidence of meningitis (neck rigidity) were reported from a study in Pondicherry, India.[40] 10.25% cases with oliguria were also found in this study, which indicates the possibility of acute kidney injury (AKI). A study in Chandigarh, India demonstrated 26.53% of scrub typhus cases had oliguria and they were all diagnosed to have AKI.[28] So, scrub typhus should be regarded as an emerging cause of meningitis & acute kidney injury in clinical practices. From above discussion it is very clear that, scrub typhus usually manifests as a non-specific febrile illness, but disease spectrum can be extended into fatal multi organ involvement also.[15]

Analyzing the reports of complete blood count of scrub typhus cases, it was evident that 13 (16.6%) patients had leukocytosis and 5 (6.4%) had leukopenia. Similarly, a study from Puducherry, India, reported 15.8% scrub typhus patients had leukocytosis & 6.2% had leukopenia.[29] Present study also documented 14 (17.9%) scrub typhus cases had decreased hemoglobin level (anemia) & thrombocytopenia was present in 16 (20.5%) patients. In contrast, Anitha et. al reported a much higher percentage of scrub typhus patient had anemia (40.6%) & thrombocytopenia (30.3%).[29] This study found 15.3% & 11.5% scrub typhus cases had elevated serum bilirubin & serum creatinine respectively, which was fairly in consistent with the findings of Vivekanandan et al. who recorded 20.5% patients with increased bilirubin & 13% had increased serum creatinine during an outbreak of scrub typhus in Pondicherry, India.[30] These data give us the insight that anemia, thrombocytopenia, acute kidney injury as well as hepatic impairment are commonly associated with scrub typhus.

This study found a definite seasonal pattern regarding the incidence of scrub typhus. From the month of August, case number begun to rise & September accounted for the maximum (19.2%) of cases, followed by October (14.10%) and November (12.8%) cases. So, most of the scrub typhus cases were in between August-November, which represents the late rainy season and beginning of the winter months. During an outbreak of scrub typhus in Himachal Pradesh, India, nearly half of the diagnosed cases (45/96; 46.5%) occurred during September-November.37 This finding was consistent with the present record. The increased incidence of scrub typhus after the rainy season (Post monsoon) is expected, as rich growth of secondary or transitional vegetation favors the growth of larval trombiculid mites, leading to increased man-vector contact.[29]

Limitations

Due to lack of logistic facilities, patients from other health care centers in Mymensingh region, could not be included in this study. So, this detection rate of scrub typhus may not reflect the actual disease burden in this region. Moreover, this study couldn't document some other important laboratory parameters (like- LFT, CSF study in case of neurological symptoms) of scrub typhus cases. Treatment response as well as clinical outcomes of the patients was not monitored also.

Conclusion

This study documented a considerable burden of scrub typhus (17.21%) among febrile patients in Mymensingh region. At the early stage of the illness, patients usually present with non-specific febrile manifestations, making it very difficult for the physicians to pinpoint a clinical diagnosis. So, it should be considered in the differential diagnosis of patients with acute febrile illnesses, particularly those with anemia, thrombocytopenia, renal impairment, LFT abnormalities, altered sensorium,

hepatomegaly, pneumonitis, or ARDS. Nested PCR (N-PCR) was very useful for diagnosing scrub typhus in very early as well as late stage of illness. So, N-PCR or Real Time PCR may be considered as an effective tool, at least in tertiary care centers for early detection of scrub typhus to avoid life-threatening complications. Further molecular studies on scrub typhus should be undertaken throughout the country to detect different genotypes of O. tsutsugamushi, which will help to formulate vaccines & appropriate diagnostic tools.

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