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A STUDY ON THE DIAGNOSTIC METHODS OF SCRUB TYPHUS COMPARITIVELY

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Abstract:

Scrub typhus is one of the causes of acute undifferentiated fever. During the study period from October 2012 to August 2014, a total of 633 cases of suspected Scrub typhus presented to our hospital. Out of these, 95 serum samples were positive for scrub typhus either by Weil Felix or by ELISA (IgM antibody to Orientia tsutsugamushi). Predominant clinical feature was fever with an average of 10 days followed by headache, vomiting, cough, myalgia, breathlessness, loose stools, reduced urine output, abdominalpain and nausea. Eschar was observed in 6 out of the 95 patients (6.3%) in our study. The percentages of patients with Scrub typhus having eschars were variable with different studies citing different results. In our study we observed an almost equal incidence of Scrub typhus in both genders. Other studies showed variable reports with some showing male predominance and others showing female predominance. Maximal incidence of Scrub typhus according to our study was in the age group 41 – 50 yrs with decreasing incidence as one goes further from this peak to either side. Increased numbers of Scrub typhus cases were observed from September to February, which coincided with the cooler months of the year. Scrub Typhus diagnosis is made more complex by the presence of dual infections. Dual infections should be suspected when the patients present with atypical clinical features of either diseaseor when patient responds poorly to treatment. The platelet levels were low in Scrub typhus, but it was lower when the patient was infected with both Scrub typhus and Dengue infection. Main stay of Scrub typhus diagnosis remains serology. The gold standard for the diagnosis is Indirect Fluorescence Antibodyassay. Both Weil Felix and ELISA tests were done and compared withIFA in 100 samples. The sensitivity of Weil Felix OX K at a break point of 1:160 was found to be 40% and specificity 94%, Positive predictive value was 86.9% and negative predictive value 61.0%. The ELISA tests showed sensitivity to be 96% and specificity 88%, Positive predictive value was 88.8% and negative predictive value 95.6 %. All currently available serological methods have theirlimitations. There is an urgent need for newer diagnostic methods. The treatment given is Doxycycline or azithromycin. Most of our cases responded to these drugs except one case where combination therapy was attempted. Scrub typhus responds well to treatment and if not treated intime the patient can go in for complications emphasizing the need for early diagnosis and treatment.

Keywords:- *Doxycycline, azithromycin, Scrub Typhus, ELISA.*

INTRODUCTION

Scrub typhus is an infectious disease that presents as an acute undifferentiated febrile illness and could be life threatening. The agent is Orientia tsutsugamushi which is an obligate intracellular bacteria that is transmitted by larval trombiculid mite, which is the reservoir of the agent and the only life stage that feeds on a vertebrate host[8] . Scrub typhus is endemic to the land mass bound by Japan to the north, Northern Australia to the south and the Arabian Peninsula to the west[59].Recent reports from several parts of India, including south India, indicate there has been a resurgence of the disease[79]. Scrub typhus is a disease which often goes undiagnosed due toits vague clinical symptoms and lack of a definitive protocol for its diagnosis. The clinical syndrome classically consists of fever, rash and eschar and requires laboratory confirmation of diagnosis[59]. It should be considered as a differential diagnosis in patients with acute febrile illness including those with thrombocytopenia, renal impairment, LFT abnormality, altered sensorium, pneumonitis or ARDS. A thorough search for eschar, particularly hidden areas is useful for diagnosis. Eschar may not present in a large number of cases [79].

The main stay in scrub typhus diagnosis remains serology. The oldest test is the Weil-Felix OX K agglutination reaction which is inexpensive, easy to perform and results are available overnight. [90] ELISA for the detection of IgM antibodies against Orientia tsutsugamushi offers advantages of being able to test large number of samples at a time and can be automated.[44]Indirect fluorescent antibody assay is the gold standard assay for the serological detection of antibodies in scrub typhus.

All the currently available serological tests for scrub typhus have some limitations of which the clinician needs to be aware. Serological diagnosis based on a single acute serum sample requires a cut off antibody titer varying from 1:10 to 1:400 depending on the endemic titer. Diagnosis and surveillance of scrub typhus is challenging particularly in the absence of advanced diagnostic techniques. The availability and cost of other serological methods are a major problem in India. The drug of choice for the treatment of scrub typhus is Doxycycline 200mg for 7 days. [69] In children and pregnant women Azithromycin is preferred. Rifampicin is an alternative drug but is not to be given alone to avoid the development of drug resistance.

Materials and Methods

STUDY PERIOD:

The study was done for a period of about 2 years from January 2021 to January 2023.

STUDY TYPE:

Screening test evaluation of Weil Felix and ELISA as compared to the gold standard Indirect Fluorescent Antibody assay (IFA) and a descriptive study of scrub typhus

SAMPLE SIZE:

We tested 633 suspected cases of scrub typhus patient samples by Weil Felix / ELISA. For comparison of Weil Felix and ELISA against the gold standard IFA, 50 IFA scrub typhus positive and 50 IFA negative samples from suspected scrub typhus cases were taken.

STUDY POPULATION:

Among the patients who were visiting or admitted in PSG hospital with fever, those who were clinically suspected of scrub typhus were included in this study.

Inclusion criteria: consecutive blood samples from suspected cases of scrub typhus were included in the study.

Exclusion criteria: Samples that were lysed, icteric or turbid were not included.

Ethics approval: Approval was obtained from the institutional Ethics committee at the start of the study and was renewed periodicallyduring the study period

Data collection method: Details of the patients were entered into the data collection form. The details included the name, age, gender,address and occupation.

SAMPLE COLLECTION:

From the suspected cases of scrub typhus patients, three ml of blood was collected in red (for OP patients) or yellow capped (for ward) BD vacutainer tubes.

CONTROLS:

50 controls were included in this study. Control group comprised of patients with fever but not suffering from scrub typhus.These samples were processed in the same steps as the samples in thestudy group.

SAMPLE PROCESSING

The BD vacutainer tubes with the patients sample were centrifuged at 6000rpm for 15min. One ml of the clear serum on top was transferred to small vials. These were then labeled and numbered and the details were entered into the data collection form. As and when the samples were obtained Weil Felix test was done and ELISA was done weekly. It was then stored at -80^{o}C. When sufficient sample size had been obtained these samples were thawed and IFA was done on the samples.

Weil Felix

Weil Felix test was done using the PROGEN antigen suspensionmanufactured by the Tulip Diagnostics (p) LTD, Goa.

Principle:

The smooth, killed, stained PROGEN antigen suspensions are mixed with the patient's serum. Antibodies produced due to Rickettsia infection if present in the patient serum will react with the stained PROGEN antigen suspension to produce an agglutination reaction. No agglutination indicates the absence of cross reacting antibodies.

Materials Required:

-Test tubes (12 mm x 75 mm), 8 for each test
-Test tube rack
-Pipettes/ Micropipettes,
-Physiological saline
-Incubator (37°C),
-Progen OXK Antigen Suspension
-Serum from patient suspecting scrub typhus.

Procedure:

- In a test tube rack 8 test tubes were taken and arranged in a row, and labeled 1 to 8. The Sample number was written on the first tube. Into the first tube 1.9 ml of physiological saline was added.
- To each of the remaining tubes (2 to 8) 1 ml of physiologicalsaline was added.
- To tube No. 1, 0.1 ml of serum sample to be tested was addedand mixed well.

- From tube No. 1 to tube No.2, 1.0 ml of the diluted serumsample is transferred and mixed well.
- Then 1.0 ml of the diluted serum sample from tube No. 2 is transferred to tube No.3 and mixed well. This serial dilution isthen continued till tube No. 7.
- From Tube No. 7, 1ml of the diluted serum sample wasdiscarded.
- Now the dilutions of the serum sample achieved from tube No. 1to 7 respectively in each set is were follows: 1:20, 1:40, 1:80,
  1:160, 1:320, 1:640, and 1:1280. Tube No. 8 was kept as thenegative control.
- To all the tubes (1 to 8) one drop of well mixed OXK PROGENantigen suspensions from the reagent vials were added and mixed well.
- The tubes were then incubated at 37ºC for approximately 18hours.
- After the appropriate incubation period the tubes were observedfor clearing and granular clumping.

Interpretation of results:

In positive reaction there was clearing and granular agglutination, in negative reaction with physiological saline as negative control a button was formed with no clearing. Titres of ≥1:160 was taken as significant. However lower titres may be seen in the acute phase of the infection and this was noted. Positive reaction can occur due to previous vaccinations, anamnestic response, antibiotic therapy, narcotic addiction and other diseases such as malaria, infectious mononucleosis, typhoid, brucellosis, tuberculosis, liver disease, auto agglutinations as well as urinary infections by Proteus. Therefore, results must be judged on the context of clinical findings.

Enzyme linked Immunosorbent Assay (ELISA)

The ELISA test was done using the Scrub typhus Detect™ IGMELISA system manufactured by InBios International, Inc., USA.

Principle**:**

The Scrub Typhus Detect ELISA system for IgM Test is a qualitative ELISA for the detection of IgM antibodies to O. tsutsugamushi in serum. Wells of each plate have been coated with O. tsutsugamushi derived recombinant antigen mix. During testing, the serum samples are diluted in InBios sample diluent and applied to each well. After incubation and washing, the wells are treated with polyclonal Goat antihuman IgM antibodies labelled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells are incubated with the tetramethylbenzidine (TMB) substrate. An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450nm. The absorbance measured is directly proportional to the concentration of IgM antibodies to OT present. Aset of positive and negative controls are provided as internal controls. These are provided to monitor the integrity of the kit components.

Materials Required**:**

The Scrub Typhus Detect ELISA system for IgM (1 x 96 Wells)contains sufficient reagents for 96 wells. Each kit contains the following reagents:

-Scrub Typhus ELISA Plate with wells coated with O. tsutsugamushiderived recombinant antigen in each well

-Sample Dilution Buffer for Scrub Typhus

-Scrub Typhus IgM Positive Control

-Scrub Typhus Negative Control

-Ready to Use Enzyme Conjugate-HRP for Scrub Typhus IgM
-10X Wash Buffer
-EnWash
-Liquid TMB Substrate
-Stop Solution.
Other materials required but not provided are
-Microtiter plate reader capable of absorbance measurement at 450 nm
-Biological or High-Grade Water
-37^{0}C Incubator
-Pipettes,
-Timer and Human test serum.

Procedure**:**

- The samples were allowed to reach room temperature (~25°C)and mixed thoroughly by gentle inversion before use.
- Positive, negative controls and unknowns were assayed induplicate.
- The number of sera to be tested was determined and organized according to the "Example for Sera Application" provided in thenext page
- Test sera were diluted to 1/100 by using the provided Sample dilution Buffer for Scrub Typhus (such as 4µl of serum plus 396µl of Sample dilution Buffer for Scrub Typhus) and mixedwell.
- To the marked Scrub Typhus ELISA plate 100µl per well of the1/100 diluted test sera and controls were added
- The plate was covered with parafilm and incubated at 37°C for30 minutes in an incubator.

Example for Serum Sample Application

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	S# 7	S# 15	S# 23	S# 31	S# 39	S# 47	S# 55	S# 63	S# 71	S# 79	S# 87
B	NC	S# 8	S# 16	S# 24	S# 32	S# 40	S# 48	S# 56	S# 64	S# 72	S# 80	S# 88
C	S# 1	S# 9	S# 17	S# 25	S# 33	S# 41	S# 49	S# 57	S# 65	S# 73	S# 81	S# 89
D	S# 2	S# 10	S# 18	S# 26	S# 34	S# 42	S# 50	S# 58	S# 66	S# 74	S# 82	S# 90
E	S# 3	S# 11	S# 19	S# 27	S# 35	S# 43	S# 51	S# 59	S# 67	S# 75	S# 83	S# 91
F	S# 4	S# 12	S# 20	S# 28	S# 36	S# 44	S# 52	S# 60	S# 68	S# 76	S# 84	S# 92
G	S# 5	S# 13	S# 21	S# 29	S# 37	S# 45	S# 53	S# 61	S# 69	S# 77	S# 85	S# 93
H	S# 6	S# 14	S# 22	S# 30	S# 38	S# 46	S# 54	S# 62	S# 70	S# 78	S# 86	S# 94

- After the incubation was complete the strips were washed six times with the 1X Wash Buffer using an automatic plate washer.In each wash cycle for all plate washing, 300µl per well of 1X Wash Buffer was used.
- Per well, 100µl of Ready to Use Enzyme-HRP Conjugate for Scrub Typhus IgM was added into all wells by using a pipette.
- The plate was then covered with parafilm and incubated at 37°Cfor 30 minutes in an incubator.
- After the incubation, the plate was again washed 6 times with automatic plate washer using 1X wash buffer, 300 µl per well.
- Into all wells, 150µl Of EnWash was added by using a pipette.
- The plate was incubated at room temperature (20-25°C) for 5minutes without any cover on the plate.
- After the incubation 100 µl per well of Liquid TMB substratewas added into all wells by using a pipette.
- The plate was then incubated at room temperature (20-25°C) in adark place (or container) for 10 minutes without any cover on the plate.
- Lastly 50 µl per well of Stop Solution was added into all wells by a pipette and incubated at room temperature (20-25°C) for 1minute without any cover on the plate.
- After the incubation, the Optical Density (OD) 450nm value wasread with a Microtiter plate reader.

Results**:**

Calculation of the cut off value**:**

Calculation of the cut- off value was determined by the average ODplus three times of the Standard Deviation(SD) of normal human serum and human sera with unrelated infections.

Interpretation of the results**:**

1. Samples with the spectrometric reading > cut – off were considered to be “Reactive” and those below this criterionwere considered to be “Non-Reactive”.

Ensuring Assay performance**:**

The results on the table below were obtained using provided positive and negative control to calculate discrimination capacity of the assay: Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay was then repeated.

Factor	Tolerance
Negative Control(NC) OD	< 0.200
Positive Control(PC) OD	> 0.500
Discrimination Capacity ($R_{PC/NC}$)	≥ 5.0

Indirect fluorescence Antibody assay (IFA)

The IFA test was done using Orientia tsutsugamushi IFA IgM antibodykit manufactured by the Fuller Labs , California , USA.

Principle

The IFA slides in this kit utilize 4 strains (Gilliam, Karp, Kato and Boryong), purified from in vitro propagation and presented as four antigen dots within each slide well. Patient sera are diluted at least 1:64 in an adsorbent suspension and incubated in the individual slide wells to allow reaction of serum antibody with the Orientia. The slides are then washed to remove non-reactive serum proteins, and a fluorescence conjugate is added to label the antigen-antibody complexes. After further incubation, the slides are washed again to remove non-reactive conjugate. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction is seen as small sharply defined fluorescent rod forms dispersed within a red-counterstained background matrix. A negative reaction is seen as either counterstained (red) background or fluorescence different from that seen in the Positive Control wells.

Materials required

The kit contains

-Substrate Slides - 10 x 12-well masked slides containing 4 acetone-fixed antigen strains (Gilliam, Karp, Kato and Boryong) in each well.
-IgM Conjugate, 2.5 mL
-IgM Serum Diluent, 10 mL
-Positive Control, 0.5 mL
-Negative Control, 0.5 mL
-Mounting Medium, 1 mL
-PBS, 1 liter

Materials Required But Not Supplied

- Purified (distilled or deionized) water
-Clean 250 or 500 mL wash bottle for PBS
-Wash bath with slide rack
-Test tubes or microtiter equivalent for manual dilutions
-Precision pipette(s) for making dilutions and delivering exactly 20 µLper slide well
-24 x 50 mm glass cover slips
-Fluorescence microscope with filter system for FITC(maximum excitation wavelength 490 nm, mean emission wavelength 530 nm)and 400X magnification
-37ºC water bath or incubator
-Humidity chamber for slide incubation steps

Procedure

Preparation of samples and reagents

1. Wash Buffer was prepared by adding contents of PBS packet to 1liter purified water and mixing thoroughly.
2. The patient's sample was diluted by adding 10µl of the patient sample to 150µl of the MIF IgM sample diluent giving a dilution of 1:16. This was then diluted further by taking 50µl from the above and adding to 150µl of the Wash buffer giving the dilution of 1:4. The finaldilution of the patient sample was 1:64.It was mixed well and this mixture was allowed to incubate at least 20 minutes at ambient temperature. These treated samples were centrifuged and the supernatant serum was used for testing.
3. This 1:64 dilution was referred to as the screening dilution.

Assay procedure

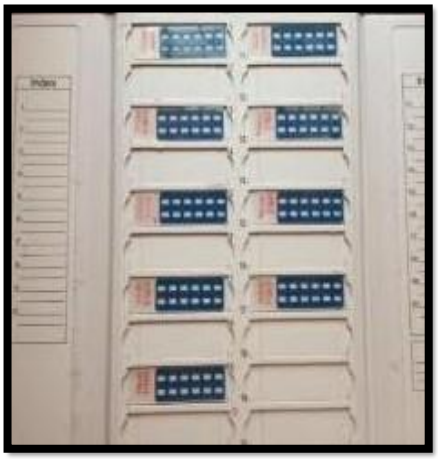
1. Serial 2-fold dilutions in Wash Buffer of the Positive Controls was prepared to include 1 dilution above and 1dilution below the stated endpoint (1:512). All controls are pre-diluted and bottled at 1:64.

2. For each serum sample, 20 µL was added to one slide well and the location is recorded for later reference. For each assay the Positive Control and the serial dilutions of the Positive Control prepared in step 1 were included. Also one drop of Negative Control was added to one well. Samples were applied to the top or bottom edge of the well, avoiding the mid-section containing the antigen microdots.
3. The slides were placed into a humidity chamber and incubated for 30 minutes at 37º± 0.5ºC.
4. The slides were removed from the humidity chamber and rinsedwith a gentle stream of PBS from wash bottle. One row of wells was washed at a time to avoid mixing of specimens.
5. To each slide well 1 drop (20 µL) IgM Conjugate was added, then the slides were placed in the humidity chamber for 30 minutes incubation at 37º± 0.5ºC. Incubation was in the dark to protect the photosensitive conjugate.
6. The slides were washed as in steps 5-6.
7. To each slide 2 – 3 drops of the mounting medium is added and thecover glass is applied.
8. The stained substrate slides were read at 400X magnification under the fluorescent microscope in a dark room, comparing each well to the visual intensity and appearance of the Positive and Negative Control wells. Slides were then stored at 2 - 8ºC in the dark.

Interpretation of results

A positive reaction appeared as bright staining (at least 1+) of short pleomorphic rod forms in any of the 4 antigen areas. The size, appearance, and density of each field were compared with the Positive and Negative Control reactions. Patterns of reactivity different fromthe Positive Controls were considered non-specific. Primary (initial) infection is characterized by a prompt rise in both IgG and IgM class antibody by IFA. IgM levels peak approximately 3 weeks post onset of symptoms and remain detectable for 2-3 months. IgG class antibody peaks in 7-12 weeks, but declines much more slowly than IgM antibody levels and remains elevated for approximately 12 months.

If the slides show positive at 1:64 or greater it suggests recent infectionand if negative at 1:64 it is reported as negative. This assay is not intended to determine strain-specificity ofantibody reactivity but to improve test sensitivity by using 4 differentstrains.



IFA Slides in slide tray

Fluorescent Microscope

RESULTS:

During the study period from October 2012 to August 2014, a totalof 633 cases of suspected scrub typhus presented to our hospital. Out of these, 95 serum samples were positive for scrub typhus either by Weil Felix or by ELISA (IgM antibody to O. tsutsugamushi). From these positive cases the following observations were made.

The patients predominantly presented with the complaints of fever half of which were associated with chills and rigors and the duration of fever was on an average for 10days. Other symptoms seen in decreasing order of frequency were headache, vomiting, cough, myalgia, breathlessness, loose stools, reduced urine output, abdominal pain, nausea, joint pain, eschar and constipation. Eschar was seen and documented in six of the cases.

It was observed that of the 95 cases, males constituted 50.5 %and females constituted 49.4%. It was also observed that about 50 % of these patients were those involved in agriculture.

The age distribution was seen as follows in the descending order. The highest numbers of cases were seen in the age group of 41 –50yrs with 25 cases (26%). This was followed by age group 31 – 40 yrs with 18 cases (18.9%). The age groups 21 – 30 yrs and 51 – 60 yrs
had 15 cases each (15.7%). The age group 61 – 70 had 9 cases (9.4%) and 11 – 20 yrs had 8 cases (8.4%). Lesser number of cases was seenin the age group 2 – 10 with 3 cases (3.1%) and the least number of cases were in the age groups 71 – 80 yrs and 81 – 90 yrs with 1 case each (1.05%). No cases were observed in ages less than 2 and more than 85yrs. (Fig 5.3)

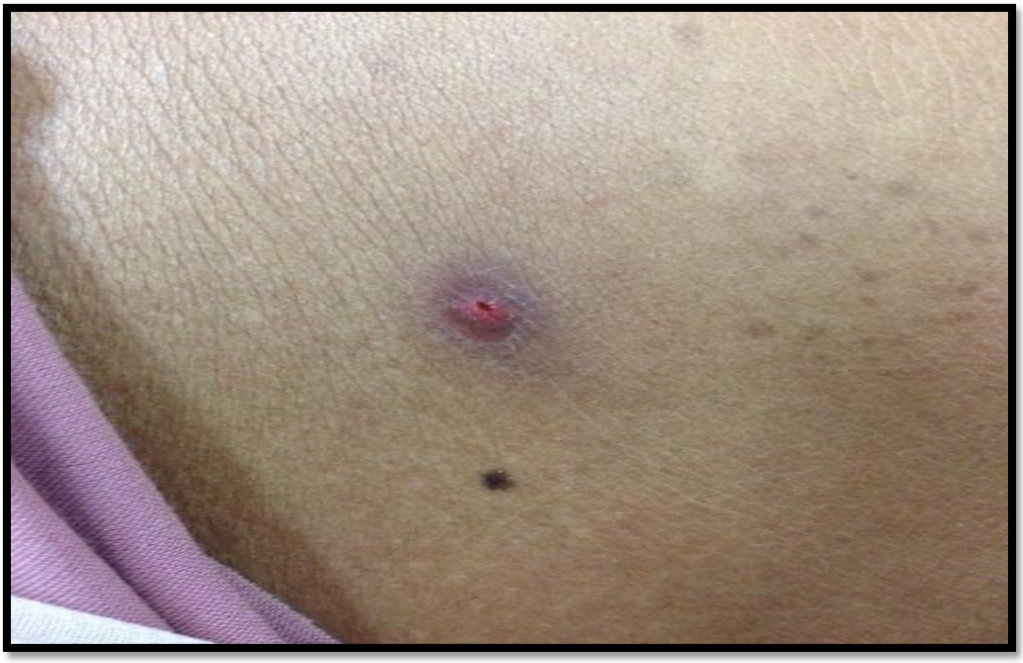
As for the season variation it was observed from the period of September 2012 to August 2014. A total of 41 cases were observed in the months of Sept to November, 40 cases from December to February, 15 cases from March to May and 20 cases from June to August. (Fig 5.4)

With respect to association of scrub typhus with other serologically diagnosable causes of fever with similar presentations, it was observed that of the 95 cases, 63 of them suffered from scrub typhus alone, 20 cases were Dengue and Scrub typhus positive, six cases were Widal and scrub typhus positive, two cases were Malaria and Scrub typhus positive, one was Infectious mononucleosis and Scrub typhus positive and three cases had more than two infectious testpositive.

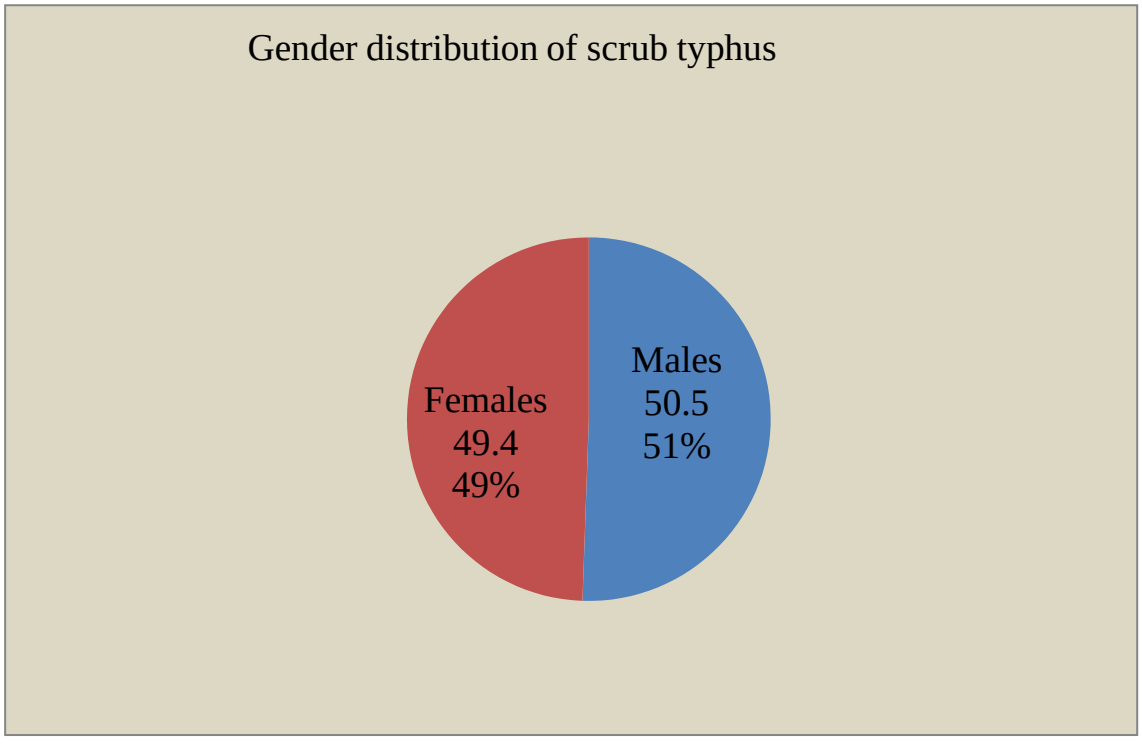
The platelet count was found to be below 150 x 10^3in 30 (47.6%) out of 63 of the patients suffering from scrub typhus alone, in 17(85%) out of 20 of the patients with dengue and scrub typhus, in four out of the six patients with scrub typhus and widal positive and in two out of the two patients with scrub typhus and malaria. The patient who had infectious mononucleosis had normal platelet levels. Of the three patients who had more than two infectious diseases, one had levels less than 1.5 x 10^3, one had normal levels and one patient's platelet level was not available.

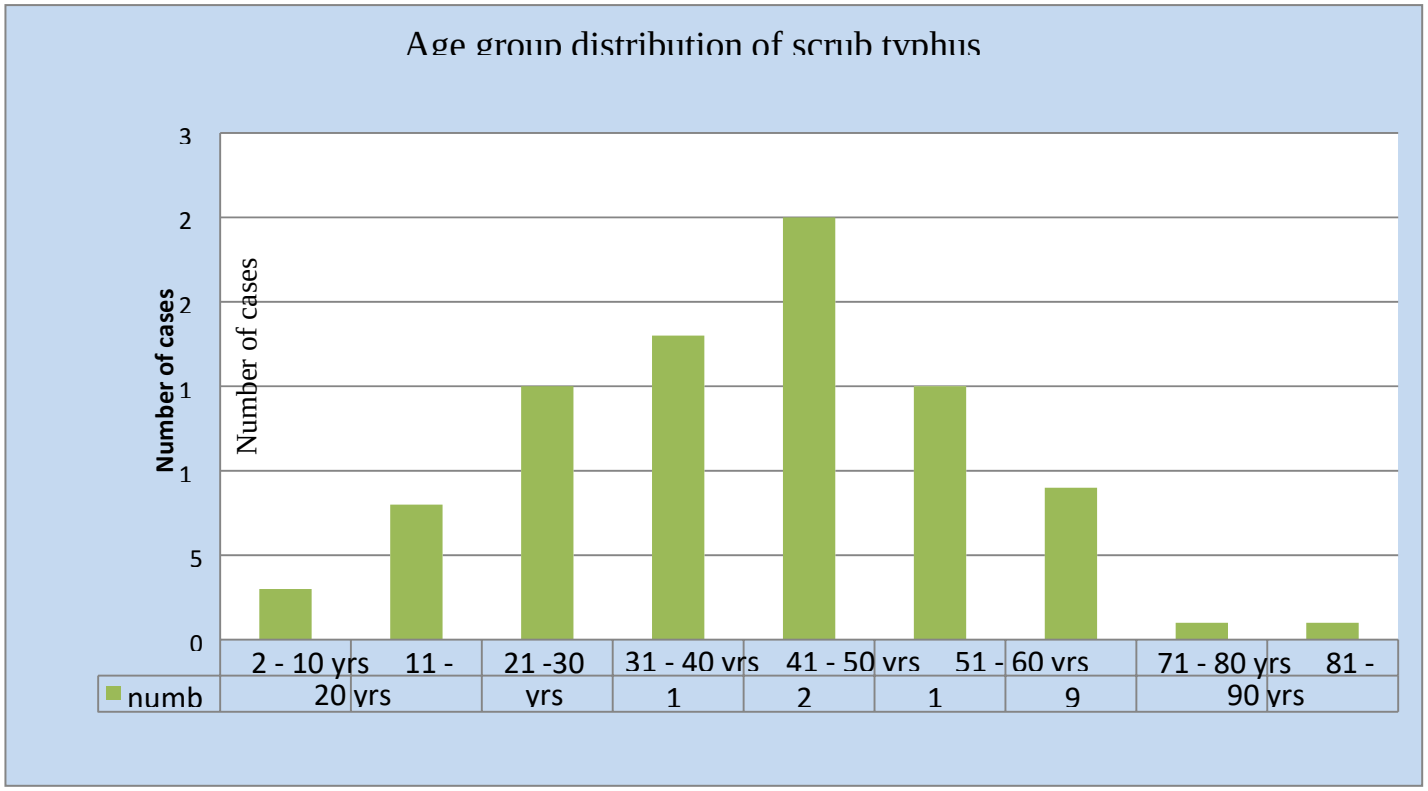
It was observed that out of the 95 cases 64 cases were documented to have been administered doxycycline and 9 cases were administered azithromycin. The remaining 21 cases data was unavailable.

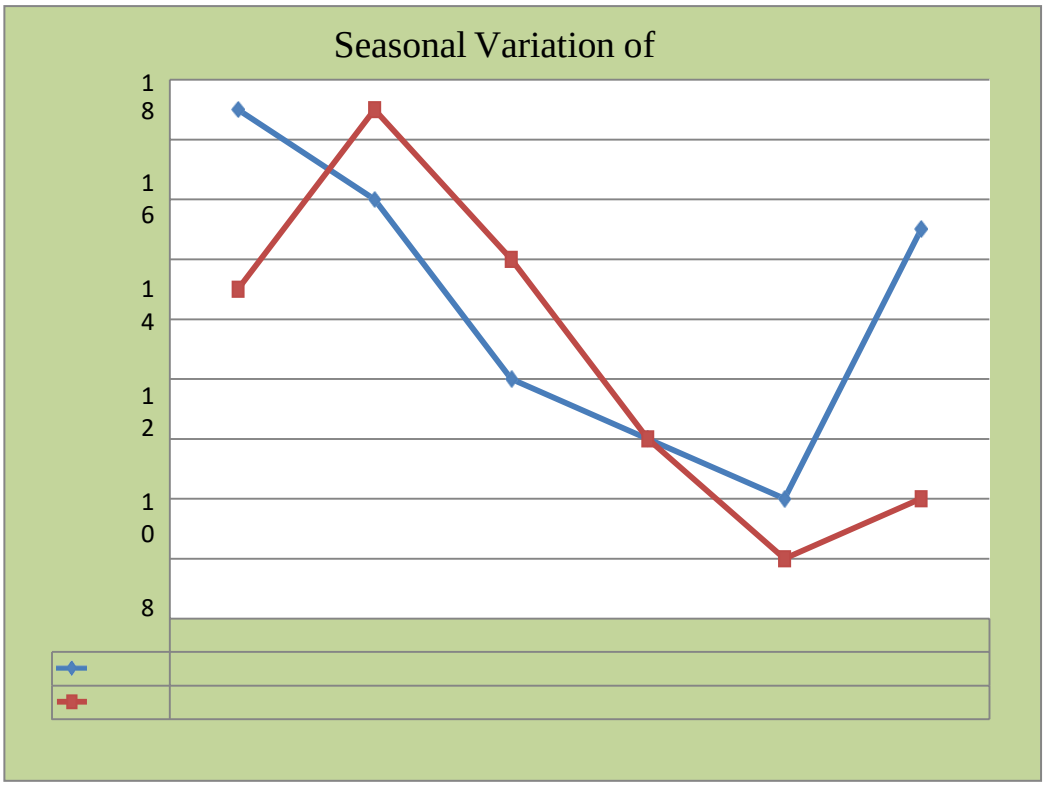
Geographical mapping of the positive cases was also done. Coimbatore had 38 cases, Erode 17, Niligiris nine, Dindigul three, salem one, Namakal two and trichy one case.

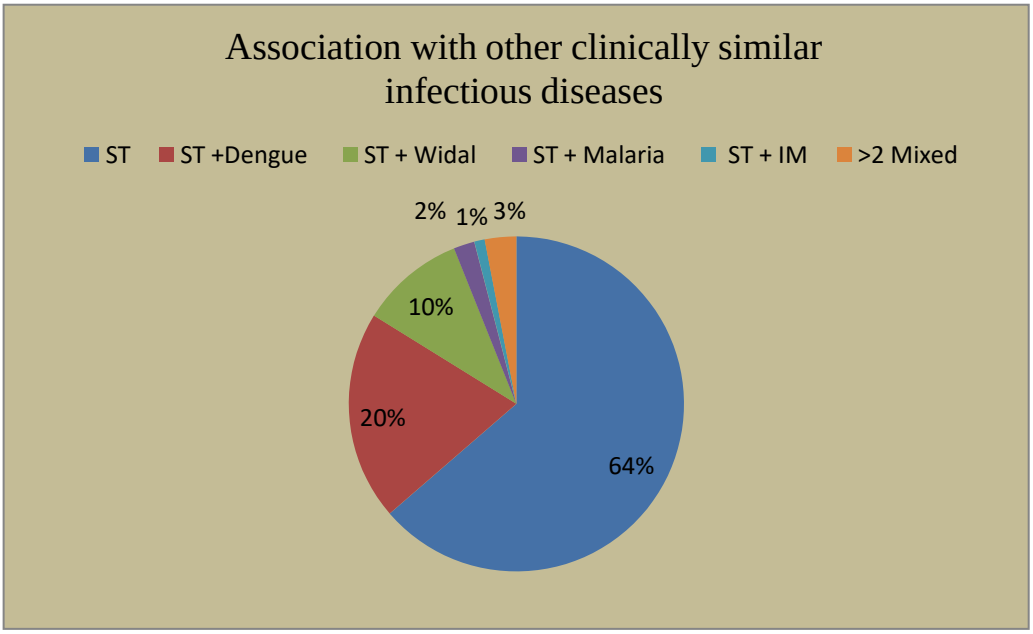


Picture of eschar seen over the lower right rib




Age group distribution of scrub typhus
Number of cases
Number of cases
3
2
2
1
1
5
0
2 - 10 yrs
11 - 20 yrs
21 -30 yrs
31 - 40 yrs
41 - 50 yrs
51 - 60 yrs
71 - 80 yrs
81 - 90 yrs
numb
1
2
1
9


Seasonal Variation of
1
8
1
6
1
4
1
2
1
0
8



	[BER OFCASES	PLATELET <1.5 X 10^3	NORMAL PLATELET 1.5 – 4 X 10^3
SCRUB TYPHUS	**63**	**30**	**33**
T+ DENGUE	**20**	**17**	**3**

Platelet counts in Scrub typhus alone and Scrub typhuswith Dengue.

SCREENING TEST EVALUTION

Indirect Fluorescent Antibody assay which is the serological gold standard for Scrub typhus was done on serum samples of patients with suspected Scrub typhus. A total of 50 IFA negative and 50 IFA positive samples were selected. These samples were subjected to Weil Felix and ELISA (for detection of IgM antibody against Orientia tsutsugamushi) for comparison of results against IFA.

Of the 50 IFA positive cases, 20 were Weil Felix positive (at a titer of 1:160) and 48 ELISA positive, and 20 were both Weil Felix (at a titer of 1:160) and ELISA positive. Of the 50 IFA negative cases, 47 were Weil Felix (titer less than 1:160) negative and 44 were ELISA negative and 40 were both together negative.

The sensitivity of Weil Felix at a titer of 1:160 was found to be 40% and specificity 94%, Positive predictive value was 86.9% and negative predictive value 61.0%. The sensitivity of Weil Felix at a titer of 1:80 was found to be 54% and specificity 92%, Positive predictive value was 87% and negative predictive value 61%. The sensitivity of ELISA was found to be 96% and specificity 88%, Positive predictive value was 88.8% and negative predictive value 95.6 %.

Discussion

Scrub typhus disease is endemic to 13,000,000 km^2 area of the Asia – Pacific rim, extending from Afghanistan to China, Korea, the islands of the south western Pacific and northern Australia [5]. Scrub typhus is areemerging disease due to the increasing movement of people from

rural to urban areas and deforestation activities. [9]

Scrub typhus is one of the causes of acute undifferentiated fever. Our study showed that of the 633 suspected cases of Scrub typhus from October 2012 to Aug 2014, 95 cases were positive by Weil Felix or ELISA constituting 15 % of the cases. In a study from Thailand, Scrub typhus accounted for 19.9% of the acute undifferentiated fevers [87].A study in Secunderabad showed a similar finding.[86]

Our study showed that the predominant clinical feature was fever with an average of 10 days followed by headache, vomiting, cough, myalgia, breathlessness, loose stools, reduced urine output, abdominal pain and nausea. The other features which we observed in our study were joint pain and constipation. These findings correlated with the other studies from Goa and Pondicherry. Eschar was observed in 6 out of the 95 patients (6.3%) in our study. The percentages of patients with scrub typhus having eschars arevariable with different studies citing different results. The rate of the eschar depends on the investigators. A study from Korea showed that as much as 92.04% of the patients presented with eschar.[18] In Goa,one study showed that eschar was seen in 13.3% [78], from Pondicherry in 46%[79] .

It has been reported that the eschars were more frequently detectable in the fair skinned Japanese children than the dark skinned Thai children .It's also reported that in dark skinned patients the early eschar lesions were atypical and could be easily overlooked. A retrospective study on dark skinned Thai pediatric patients showed thatonly in 7% was the eschar detectable.[18] Another reason for variable reports could be because the presence of an eschar could be easily missed on routine physical examination and the vector bite is painless so the patients wouldn't notice it either. In our study we observed an almost equal preponderance of scrub typhus in both genders. Out of the 95 cases, 48(50.5%) were males and 47(49.4%) were females. Other studies showed variable reports with some showing male predominance and others showing female predominance. In a study from Andhra Pradesh, the malesconstituted 59.3% and females 40.7%[81] From Pondicherry the study showed out 50 patients 28 were females and 22 were males.[79]

Regarding the age distribution of Scrub typhus our study gaveresults with similar pattern to another study from KMC, Manipal.[83] Our study showed maximal incidence in the age group 41 – 50 yrs withdecreasing incidence as one goes further from this peak to either side whereas the study from Manipal showed maximal peak in the age group 31 – 40 yrs.

About 50% of the patients were agricultural workers showing an increased risk among those involved in this occupation. Literature also reports Scrub typhus is generally seen among those whose occupation or recreational activities bring them in contact with the scrub vegetation. [71]

We observed for the seasonal variation from Sept 2012 to August 2014 and noticed an increased number of cases from September to February, which coincided with the cooler months of the year. It has been reported that outbreaks of scrub typhus in some areas

are seen more often in the cooler months [82]This could be due to the growth of secondary scrub vegetation (mite islands commonly seen in the post monsoon season from September to early months of next year)which is the habitat of the trombiculid mites.[16]. Some studies have shown the incidence of scrub typhus to be more in the rainy season[81].

Scrub Typhus presents as one of the causes of acute undifferentiated fever and this is made more complex by the presence of dual infections. Dual infections should be suspected when the patient presents with atypical clinical features of either disease or whenpatient responds poorly to treatment. [41]

In this study it was observed that of the 95 cases, 63 of them suffered from Scrub typhus alone and the remaining 32 cases suffered from mixed infections. Literature shows case reports of patients with Scrub typhus along with other infections but prospective studies for theincidence of the same were not found.[88,89]

Our study showed that the level of platelets less than 1,50,000/mm^3 was seen in 47.6% of those suffering from scrub typhusalone and in patient with scrub typhus and dengue, thrombocytopenia was seen in 85% of the patients. Thus correlating with study stating that the platelet levels (<1, 40,000/mm^3) were found to be much lower in those suffering from Dengue as compared to scrub typhus infection. [84] Suputtamongkol et al from Thailand showed that thrombocytopenia was associated in 20.9% of the patients suffering from scrub typhus. [85] In our study, the results of Weil Felix and ELISA werecompared to the serological gold standard IFA. The sensitivity of Weil Felix OX K at a break point of 1:160 was found to be 40% and specificity 94%, positive predictive value was 86.9% and negative predictive value 61.0%.These results correlated well with other studies from Thailand and South India where both show Weil Felix OX K having a sensitivity of 30 – 40 % and specificity of 93.3%. The ELISA containing r56 recombinant – antigen showed sensitivity to be 96% and specificity 88%, positive predictive value was 88.8% and negative predictive value 95.6 %. This was similar to the results stated in another study using the same assay and titer which showed sensitivity to be 97.5% and specificity to be 82.5%. For the treatment of scrub typhus 67.3% of the cases were administered doxycycline, 9.4% was administered azithromycin and for the remaining cases the data was unavailable. One patient did not respond to doxycycline and combination therapy of azithromycin and rifampicin was given. The patient had responded better to the combination therapy but unfortunately succumbed to other complications.

Conclusions:-

Many of our finding have correlated well with other studies. Theconclusions we have drawn based on this study are that, there is an occupational risk with those involved in agriculture, more in the age group of 41 – 50 yrs with an increasing incidence during the cooler months of the year.

Contrary to some studies we have observed good specificity with Weil Felix but it has a low sensitivity. Therefore if the results by Weil Felix are negative it does not rule out Scrub typhus and may require further testing. The good specificity we have observed could also be because of a low endemic titer in the local population. ELISA showed good sensitivity thus it is a preferable screening test though it is not very specific and may require confirmation with IFA whenfeasible.

Scrub typhus is a significant disease in this part of the country therefore it should be kept in mind as a possible diagnosis in undifferentiated fevers even though an eschar is not found. Scrub typhus was found to constitute 15 % of the acute undifferentiatedfevers. This being a treatable disease further emphasizes the need forits timely and accurate diagnosis.

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