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RESEARCH ARTICLE

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Persistence of scrub typhus IgM and IgG antibodies among patients from Karnataka, India

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ABSTRACT

Background: The longevity of scrub typhus IgM and IgG are not clear. A region-specific diagnostic cut-off for enzyme-linked immunosorbent assay (ELISA) is a necessity for improved diagnosis.

Objectives: To determine a region-specific optical density (OD) value cut-off for scrub typhus IgM and IgG ELISA and to study the persistence of these antibodies.

Methods: A prospective cohort study was conducted among patients diagnosed with scrub typhus admitted to Kasturba Hospital, Manipal, Karnataka, India from August 2019 to April 2023. An equal number of scrub typhus patients and healthy volunteers were enrolled to determine region-specific scrub typhus IgM and IgG ELISA OD value cut-off. A receiver operating characteristic (ROC) curve analysis was performed to determine the OD value cut-off with an optimal combination of sensitivity and specificity. The patients were followed up prospectively at varying time points up to 18 months and scrub typhus IgM and IgG ELISA were performed in all the collected samples.

Results: The ROC curve analysis of scrub typhus IgM revealed an optimal OD value cut-off of 1.309 with a sensitivity and specificity of 98.7% (95% CI: 93.1%–100%). The ROC curve analysis of scrub typhus IgG revealed an optimal OD value cut-off of 0.9 with a sensitivity of 71.7% (95% CI: 60.5%–81.4%) and specificity of 93.5% (95% CI: 85.7%–97.9%). At 18 months of follow-up, scrub typhus IgM (OD value > 1.309) and IgG (OD value > 0.9) were above the newly derived diagnostic cut-off in 17 (32%) and 40 (75.4%) patients, respectively.

Conclusion: Scrub typhus IgM and IgG antibodies were persistent above the newly derived regional diagnostic cut-off for up to 18 months.

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Scrub typhus; *Orientia tsutsugamushi*; antibody persistence; IgM; IgG

Introduction

Scrub typhus is a vector-borne infection caused by the organism *Orientia tsutsugamushi*. The infection is transmitted by the larval stage *Leptotrombidium* mite bite [1]. An eschar forms at the site of the chigger bite, which is important in diagnosing scrub typhus [2]. In the absence of an eschar, diagnosis is reliant on enzyme-linked immunosorbent assay (ELISA) in limited resource settings. ELISA is a reliable test for diagnosing infectious diseases in clinical laboratory settings owing to its reproducibility [3]. In ELISA, IgM and IgG antibodies against *O. tsutsugamushi* 56 kDa Type-specific-antigen are detected [4]. However, the antibody kinetics of scrub typhus IgM and IgG remain unclear [5]. Limited studies have explored the longevity of these antibodies in scrub typhus [5–9].

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In India, InBios Scrub typhus IgM ELISA kit is being used extensively for scrub typhus diagnosis [5,10–12]. The kit comes with 96 well plate coated with recombinant 56 kDa type-specific-antigen of *O. tsutsugamushi* Karp, Kato, Gilliam and TA716 strains [4]. Various diagnostic accuracy studies performed using the kit across the country had demonstrated a range of optical density (OD) value cut-offs (0.5–1.0) [5,11–13]. ELISA is influenced by factors such as strain composition and the level of background antibodies [3]. With varying endemicity of scrub typhus in different regions of India, high background antibody levels can lead to false positives [14]. The cut-off value determined for a geographic location cannot be used for another. A lower cut-off may lead to false positives, while a higher cut-off may result in false negatives. Lack of appropriate cut-off has implications for diagnosis, seroepidemiological and clinical studies, and most importantly, the initiation of appropriate antibiotic therapy [15]. Hence, the current study focused on deriving a region-specific scrub typhus IgM and IgG OD value cut-offs and determining the persistence of these antibodies among scrub typhus patients from Karnataka, India.

Methods

A prospective cohort study was conducted during August 2019 and April 2023 on adult scrub typhus patients admitted to Kasturba Hospital, Manipal, Karnataka, India. The patients willing for follow up, were followed up at varying time points (day 7, 14, 28 and 3, 6, 9, 12 months) up to 18 months post infection. Blood sample of 2 ml was collected in a plain tube from each patient at different time points. Serum was separated from the plain tube and was subjected to scrub typhus IgM and IgG ELISA using Scrub typhus Direct™ IgM ELISA (InBios International, Inc., Seattle, WA, USA) kit and Scrub typhus Direct™ IgG ELISA (InBios International, Inc., Seattle, WA, USA) kit.

Determining scrub typhus IgM & IgG OD value cut-off

The scrub typhus IgM and IgG optical density (OD) value cut-off was determined by collecting samples from healthy volunteers and confirmed scrub typhus patients. Healthy volunteers in our study were enrolled from Kasturba Hospital Blood Center, Manipal, Karnataka, India. Volunteers fulfilling the criteria for blood donation were enrolled in the study after obtaining written informed consent. A case of confirmed scrub typhus was defined as samples positive for either *O. tsutsugamushi* nested polymerase chain reaction (nPCR) or Realtime PCR. Serum samples of cases and control group patients were subjected to scrub typhus IgM and IgG ELISA.

Confirmation of scrub typhus cases by PCR

A 4 ml of blood was drawn in to an ethylenediaminetetraacetic acid (EDTA) tube. The EDTA tube was centrifuged at 1000 × g for 15 min. The blood was separated into plasma and buffy coat. Approximately 300 µL of buffy coat was separated. The 300 µL of buffy coat was transferred to a 1.5 ml micro centrifuge tube and 900 µL of RBC lysis buffer and 100 µL of 1x Triton X were added. The tube was gently vortexed and centrifuged at 6000 × g for 5 min. After centrifugation the supernatant was discarded. The same step was repeated two more times till a clear white pellet was obtained. To the white pellet 260 µL of WBC lysis buffer and 40 µL of sodium dodecyl sulphate (SDS) reagent were added. The tube was gently vortexed till the pellet is dissolved and was placed for incubation at room temperature for 20 min. After 20 min, 120 µL of 6 M sodium chloride was added. The tube was gently mixed and centrifuged at 6000 × g for 5 min. The supernatant was transferred to ice cold isopropanol and the centrifugation step was repeated. After which a 70% ethanol wash was performed and the DNA pellet was kept for drying. The DNA pellet was re-suspended in 50 µL of Tris-EDTA buffer. The extracted DNA was subjected to a nested polymerase chain reaction (nPCR) targeting the 483 bp segment of *Orientia tsutsugamushi* 56-kDa type-specific-antigen (TSA) gene [16]. Nested PCR was performed in a total reaction volume of 25 µL. Each reaction contained 12.5 µL of 2× Takara Emerald Master mix (Takara Bio, Shiga, Japan) 0.5 µL (10 µM) each of forward and reverse primers, 10.5 µL of molecular-grade water and one µL of DNA template. The cycling conditions were an initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 30 s and extension at 72 °C for 1 min 30 s. The final extension step was performed at 72 °C for 5 min. For the second round of nested PCR reaction, one µL of PCR product of first round was used a template. The PCR master mix components and the cycling conditions were similar for the second round of nested PCR reaction. The amplicons were resolved on a 1.5% agarose gel and were visualized in a UV Gel documentation system.

Scrub typhus IgM and IgG ELISA

A 2 ml of blood was drawn into a plain tube. The plain tube was centrifuged at 1000 × g for 5 min. The serum was separated to a separate tube. The Scrub typhus Direct™ IgM ELISA (InBios International, Inc., Seattle, WA, USA) kit and Scrub typhus Direct™ IgG ELISA (InBios International, Inc., Seattle, WA, USA) kit contents were brought to room temperature. The 1× wash buffer was prepared by adding 120 ml of 10× wash buffer provided by the manufacturer to 1080 ml of distilled water. The samples and controls were prepared to a 1/100 dilution by adding 4 µL of serum to 396 µL of sample diluent buffer. The prepared samples and controls were thoroughly mixed and a 100 µL of sample and controls were added to the antigen coated 96 well ELISA plate. The plate was incubated at 37 °C for 30 min in an incubator. After the incubation, the contents were discarded and the plate was washed manually for six times using the freshly prepared 1× wash buffer. The plate was gently tapped to remove any residual 1× wash buffer. To the plates 100 µL of ready to use enzyme horseradish peroxidase (HRP)-conjugate was added and the plates were incubated a at 37 °C for 30 min in an incubator. After the incubation, the plates were washed manually as mentioned in earlier step. A 150 µL of EnWash was added to all the wells and the plate was placed for incubation at room temperature for 5 min. After which, the plate was manually washed. To the wells, 100 µL liquid 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate was added and the plate was incubated at room temperature for 10 min. After the incubation, 50 µL of stop solution was added to all the wells. After a couple of minutes, readings were taken in an ELISA plate reader at a wavelength of 450 nm. The protocol followed was same for both scrub typhus IgM and IgG ELISA kits.

Sample size calculation for determining the optimal OD value cut-off for scrub typhus antibodies

The sample size was calculated using estimation of area under the Receiver-operating characteristic (ROC) curve for establishing diagnostic ability. Assuming the area under curve to be at least 80%, with precision of 10% and confidence interval of 95%, the sample size required was 78 participants' each in scrub typhus group and healthy volunteers' group.

Sample size calculation for determining the longevity of scrub typhus antibodies

To detect a difference of at least 35% in IgM from baseline to 18 months with intra class correlation 0.15, power 0.90 and with a dropout rate of 30%, the sample size required was 60.

Analysis

Scrub typhus IgM and IgG ELISA OD values obtained were recorded. ROC curve analysis was performed using MedCalc software Version 22.001 (MedCalc Software Ltd, Ostend, Belgium) to determine the OD value cut-off with an optimal combination of sensitivity and specificity. Once an optimal OD value was determined, IgM and IgG ELISA were performed in all scrub typhus patients and their follow up samples to determine longevity of these antibodies.

Results

Determining the IgM and IgG ELISA OD value cut-offs

A total of 78 *O. tsutsugamushi* PCR confirmed cases were enrolled. Simultaneously, 78 healthy volunteers were enrolled. Among the healthy volunteers 62 (79.5%) were males and the mean age of the healthy volunteer group was 30.88 ± 9.2 years. The healthy volunteers belonged to Udupi (29, 37.2%), Shivamogga (16, 20.5%), Davangere (7, 9%), Chikkamagaluru (5, 6.4%), Uttara Kannada (5, 6.4%), Bangalore (3, 3.8%), Chitradurga (3, 3.8%), Haveri (3, 3.8%), Dakshina Kannada (2, 2.6%), Vijayanagara (2, 2.6%) and Gadag (1, 1.3%) districts of Karnataka and 2 (2.6%) patients belonged to Kannur district, Kerala. Scrub typhus IgM and IgG ELISA were performed in all the 156 samples. The median IgM and IgG OD values of healthy volunteers were 0.46 (0.36, 0.60) and 0.31 (0.26, 0.45) respectively. Among the healthy volunteers' scrub typhus IgM and IgG OD values were elevated in 3 and 5 samples respectively (Figure 1). The scrub typhus IgM cut-off calculated from healthy volunteer was mean OD (0.531) + 3 standard deviation (0.275) = 1.35. The scrub typhus IgG cut-off calculated from healthy volunteers was mean OD (0.51) + 3 standard deviation (0.17) = 0.88.

An ROC curve was plotted using all 156 OD values for both scrub typhus IgM and IgG individually (Figure 2). The ROC curve analysis of scrub typhus IgM revealed an optimal OD value cut-off of 1.309 with a sensitivity and specificity of 98.7% (95% CI: 93.1%–100%) and an area under curve of 0.998. The ROC curve analysis of scrub typhus IgG revealed an optimal OD value cut-off of 0.9 with a sensitivity of 71.7% (95% CI: 60.5%–81.4%) and specificity of 93.5% (95% CI: 85.7%–97.9%) and an area under curve of 0.855 (Figure 2).

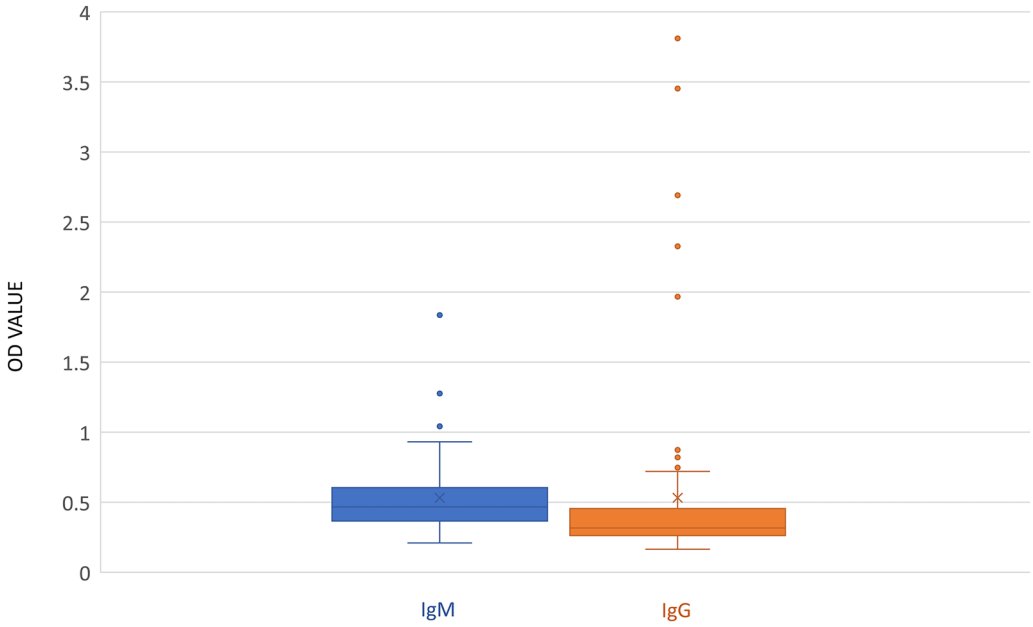

Figure 1. Scrub typhus IgM and IgG OD values among healthy volunteers.

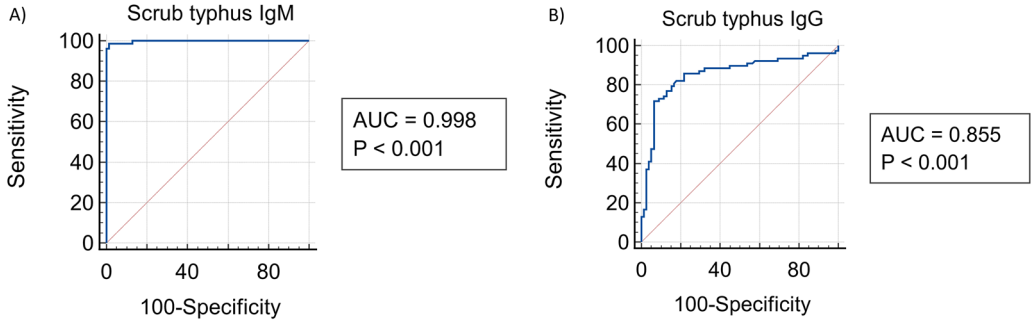

Figure 2. Receiver operating characteristic (ROC) curve analysis of (A) scrub typhus IgM and (B) scrub typhus IgG ELISA.

Longevity of scrub typhus IgM and IgG antibodies

During the four-year study period, a total of 162 patients were enrolled, and 329 serial blood samples were collected from patients willing for follow up. At the time of admission 67 (41.4%) and 69 (42.6%) patient samples were positive for *O. tsutsugamushi* nested PCR and Real-time PCR respectively. The mean age of the cohort was 50.35 ± 15 years and males were (105, 64.8%) were predominant. The median duration of fever among the cohort was 7 (5, 10) days. The patients enrolled were from 9 districts of Karnataka viz. Davangere (31, 19.1%), Shivamogga (26, 16%), Chikkamagaluru (22, 13.6%), Chitradurga (22, 13.6%), Udupi (21, 13%), Haveri (16, 9.9%), Uttara Kannada (15, 9.3%), Vijayanagara (5, 3.1%) and Bellary (3, 1.9%). One (0.6%) patient was from Anantapur district, Andhra Pradesh.

Scrub typhus IgM was elevated up to three months post infection among the cohort and dropped gradually there after till nine months post infection (Figure 3). IgM slowly picked up again exactly after one year among the cohort and gradually dropped by 18 months (Figure 3). Real time PCR performed at one year follow up in samples that had elevated IgM were negative for *O. tsutsugamushi* DNA. Scrub typhus IgG gradually rose from first week of infection and reached peak at nine months post infection. There after IgG declined by 12 months and persisted at the same level till 18th month (Figure 3).

Scrub typhus IgM and IgG were persistent above the diagnostic cut-off at varying time points among our cohort up to 18 months (Table 1). At 12 months of follow up, scrub typhus IgM (OD value > 1.309) and IgG (OD value > 0.9) were above the newly derived regional diagnostic cut-off in 7 (50%) and 13 (92.8%) patients respectively. At 18 months of follow up, scrub typhus IgM (OD value > 1.309) and IgG (OD value > 0.9) were above the regional diagnostic cut-off in 17 (32%) and 40 (75.4%) patients respectively (Table 1).

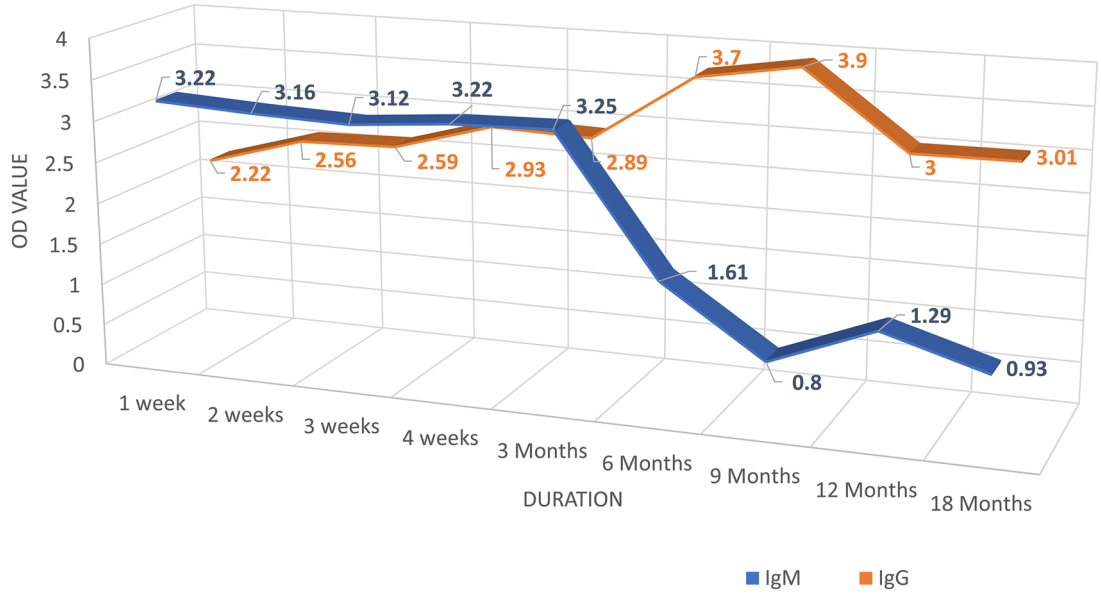

Figure 3. Scrub typhus IgM & IgG median OD values of the cohort over 18 months period.

Table 1. Scrub typhus IgM and IgG antibody positivity among scrub typhus patients at varying time points stratified by different cut-offs.

S. no.	Duration of illness	No. of patient samples collected	IgM positive (≥0.5 OD value) n (n%) DHR-ICMR cut-off [17]	IgM positive (≥1.3 OD value) n (n%) Current study cut-off	IgG positive (≥1.8 OD value) n (n%) Varghese et al. cut-off [5]	IgG positive (≥0.9 OD value) n (n%) Current study cut-off
1	Day 1–7	81	81 (100)	77 (95)	44 (54.3)	57 (70.3)
2	Day 8–14	81	81 (100)	78 (96.3)	52 (64.2)	63 (77.7)
3	Day 15–21	44	44 (100)	44 (100)	29 (65.9)	35 (79.5)
4	Day 22–28	20	20 (100)	19 (95)	17 (85)	17 (85)
5	1–3 Months	12	12 (100)	12 (100)	9 (75)	10 (83.3)
6	3–6 Months	15	13 (86.6)	9 (60)	14 (93.3)	15 (100)
7	6–9 Months	9	7 (77.7)	3 (33.3)	8 (88.8)	8 (88.8)
8	9–12 Months	14	13 (92.8)	7 (50)	13 (92.8)	13 (92.8)
9	12–18 Months	53	38 (71.6)	17 (32)	35 (66)	40 (75.4)

Comparison of OD value cut-offs

The scrub typhus IgM OD value data was stratified according to probable case definition outlined in 'DHR-ICMR guidelines for diagnosis & management of rickettsial diseases in India' [17]. When this cut off (OD value > 0.5) was applied, from first week of illness till three months post infection all the samples were positive for scrub typhus IgM (Table 1). At 12 months post infection, 92.8% samples were positive for scrub typhus IgM according to DHR-ICMR cut-off and only 50% were positive according to the newly derived regional cut-off (OD value > 1.309). Similarly, at 18 months post infection, as per DHR-ICMR cut-off, 71.6% samples were positive for IgM and 32% were positive according to newly derived regional cut-off (Table 1). In a similar manner, scrub typhus IgG cut-off was compared with the cut-off determined by Varghese et al. in Vellore, Tamil Nadu, India [5]. A comparison between various cut-offs is portrayed in Figure 4.

Scrub typhus IgM and IgG among severe and non-severe patients

The 162 enrolled patients were stratified to severe and non-severe groups. Severity was defined as patient's requirement for intensive care admission. Among the cohort 55 (33.9%) patients required intensive care admission. The median IgM OD value of the severe group was 3.15 (2.9, 3.88) and non-severe group was 3.18 (2.94, 3.84) at the time of admission. The median IgG OD value of the severe group was 1.89 (0.68, 3.01) and non-severe group was 2.62 (0.84, 3.3) at the time of admission. There was no significant difference in IgM and IgG OD values between severe and non-severe groups at the time of admission. The detailed clinical data of the patients has been published separately [18,19].

Among the cohort, there was one patient whom we could follow up at 9 time points starting from Day 8 of illness till day 510 post infection (Figure 5).

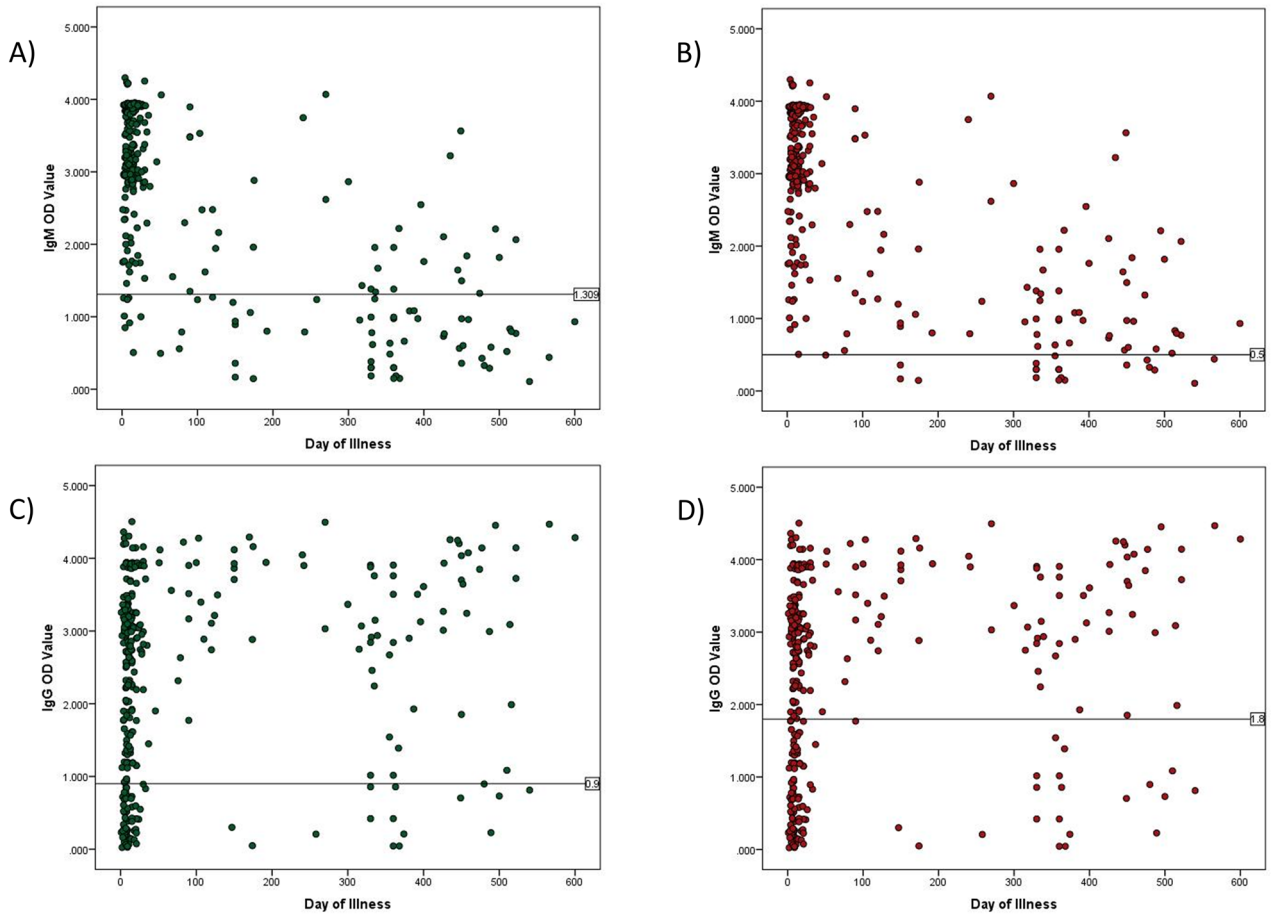

Figure 4. A comparison of various OD value cutoffs for scrub typhus IgM and IgG ELISA. (A) Scrub typhus IgM current study cut-off (OD value > 1.309), (B) DHR-ICMR scrub typhus IgM cut-off (OD value > 0.5), (C) scrub typhus IgG current study cut-off (OD value > 0.9) and (D) scrub typhus IgG cut-off determined by Varghese et al. (OD value > 1.8).

The patient had a high initial scrub typhus IgM and IgG OD value, which maintained till 28 days post infection. While, IgM dropped rapidly by 106th day, IgG gradually decreased by day 270 post infection. Later, IgM and IgG gradually peaked at the same time by 300th day of illness. After which, IgM gradually declined by day 510 post infection, and IgG reached its peak (Figure 5).

Discussion

In the current study, we have determined the region-specific scrub typhus IgM and IgG antibody ELISA OD value cut-offs and have prospectively followed up scrub typhus patients up to 18th month post infection to determine the persistence of IgM and IgG antibodies. In our study, scrub typhus IgM ELISA OD value cut-off of 1.309 was optimal with a sensitivity and specificity of 98.7% (93.1%–100%) each and scrub typhus IgG ELISA OD value cut-off of 0.9 was optimal with a sensitivity and specificity of 71.7% (95% CI: 60.5%–81.4%) and 93.5% (95% CI: 85.7%–97.9%) respectively. Diagnostic accuracy studies carried out on scrub typhus IgM in various geographic locations globally had OD value cut-off from 0.2 to 2.0 [14]. The cut-offs used in various regions of India ranged from 0.5 to 1.0 [5,11–13]. 'It is not ideal to implement the diagnostic cut-off generated in a different geographic location owing to the variation in background antibody levels' [15].

The *O. tsutsugamushi* 56 kDa type-specific-antigen is the major immunodominant protein. There are very few studies conducted that studied the IgM and IgG kinetics of antibodies produced against *O. tsutsugamushi* 56 kDa type-specific-antigen [5–9]. Among Korean population, a rapid increase in IgG antibody titre than the IgM antibody titre during first one to two weeks after infection was observed [7]. Similarly, another group of researchers have observed that IgM and IgG reached their peak level by 15 to 25 days after fever onset [6]. These findings contrast with our observations. In our study IgM was elevated from first week of illness, and IgG reached its peak by 9 months post infection (Figure 3), and both IgM (32%) and IgG (75.4%) remained

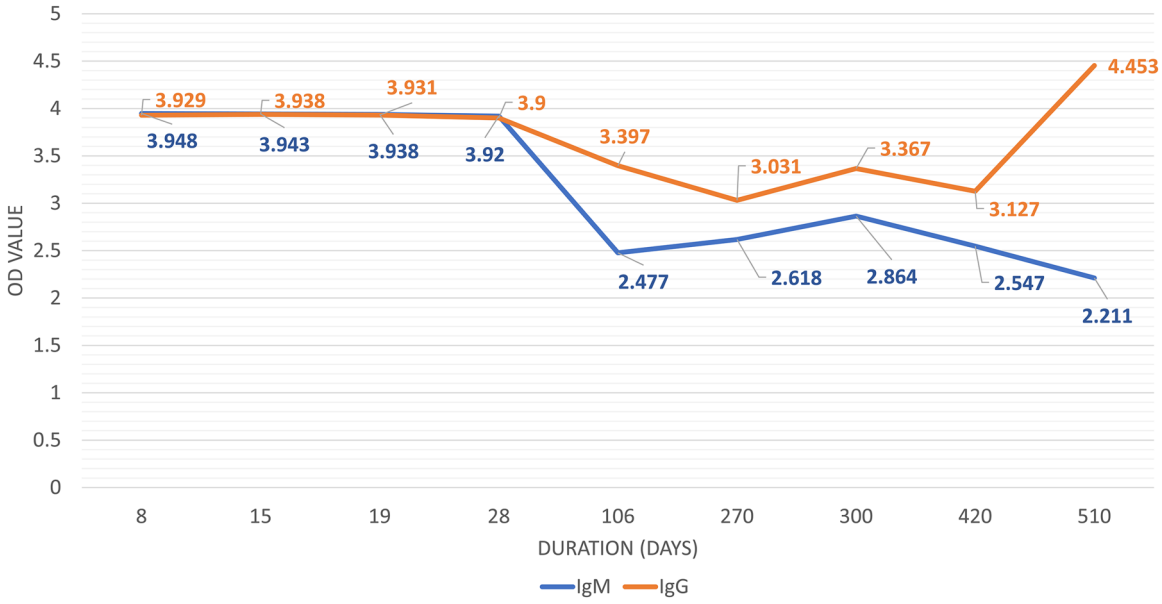

Figure 5. Scrub typhus IgM & IgG OD values of a patient over 18 months period.

above the newly derived regional diagnostic cut-off up to 18 months (Table 1). A similar finding was reported among patients from Vellore, India. Among these patients, IgG reached its peak at 10 months post infection and was above the diagnostic cut-off up to 36 months post infection [5]. Long-lasting immunity is seen with the antibodies that are produced against the 56 kDa protein, when compared with 47 kDa and ScaA [6,9]. While the antibody response against 47 kDa was delayed and less pronounced, anti-ScaA antibodies declined rapidly [6,9]. Owing to the persistence of anti-56 kDa IgM for up to 18 months, clinicians need to exercise caution while interpreting single serum sample IgM ELISA result. Furthermore, performing PCR can improve the diagnosis. When initial IgM and IgG OD values of the severe and non-severe patients were compared, there was no significant differences in our study. However, a previous study has reported that initial high IgG is associated with higher risk of complications [8]. Further studies with a larger sample size can be carried out to determine the utility of IgG as prognostic marker for severe scrub typhus.

We have compared our newly derived regional diagnostic cut-off with DHR-ICMR cut-off for scrub typhus IgM and the cut-off derived in Vellore with scrub typhus IgG. As per DHR-ICMR cut-off, 71.6% samples were positive for IgM and 32% were positive according to regional cut-off at 18 months follow-up. Implementing DHR-ICMR cut-off to this geographical region would lead to scrub typhus IgM false positivity in 39.6% of samples. As per cut-off determined in Vellore, 66% samples were positive for IgG and 75.4% were positive according to regional cut-off at 18 months follow-up (Table 1). Implementing cut-off determined in Vellore population to this geographical region would lead to scrub typhus IgM false negativity in 9.4% of samples. This highlights the importance of regional assessment of antibodies for determining optimal ELISA OD value cut-offs [3,20].

Immunity against *O. tsutsugamushi* is complicated due to great antigenic diversity, weak cross protection among various strains and the waning homologous immunity. Macrophages, neutrophils and anti-*O. tsutsugamushi* antibodies play a major role in clearing the organism. These antibodies inhibit cell invasion of the organism by binding to *O. tsutsugamushi* 56-kDa TSA protein [21]. Over a period of time the homologous immunity wanes off and reaches baseline values two years post recovery in humans [9].

Initially killed *O. tsutsugamushi* vaccine was successful in animals. However, in the studies conducted in Japan and Burma, the killed vaccine failed to stimulate long-lasting heterologous protection in humans [22]. Later the vaccine efforts were shifted on to the 56-kDa TSA protein [22–24]. Multiple studies were performed on recombinant 56-kDa TSA protein for its immunogenicity in mice and non-human primates (NHP). However, only partial protection against homologous strains was achieved [22]. Later it was discovered that *O. tsutsugamushi* ScaA along with 56-kDa TSA to be more effective when mice were challenged with heterologous strains [23]. The same group of researchers developed a novel recombinant antigen derived from the conserved regions of 56-kDa TSA (cTSA56). The cTSA56 conferred immunity to both homologous and

heterologous strains when mice were challenged with lethal doses [24]. Further studies are ongoing to develop an effective vaccine to prevent the illness that causes one million cases annually.

To the best of our knowledge this is the first study to prospectively follow up scrub typhus patients from 9 districts of Karnataka, for 18 months to study the persistence of scrub typhus IgM and IgG antibodies. However, our study has certain limitations. Due to the long duration of the follow-up period, many patients were unable to adhere to the follow-up schedule. Also, the COVID-19 pandemic and travel restrictions hindered the patient follow-up timelines. In the current study, we could determine longevity of antibodies by performing only ELISA and could not perform Immunofluorescence assay (IFA) owing to funding constraints. Though the antibodies are persistent above the diagnostic cut-off for a longer duration, we could not determine the underlying mechanism. Furthermore, animal studies can be useful in determining the factors that contribute to the persistence of these antibodies for longer durations. Also, studies can be performed to explore the protective effect of these persistent antibodies against reinfection with both homologous and heterologous strains in animal models.

The method of detecting the antibodies in previous studies and the current study were different. While IFA was used to detect antibodies in most of the previous studies [6,7,9], ELISA was used in the current study. Factors such as, previous infection to scrub typhus, host immune status, strain composition, method of detection of antibodies, time of sample collection and storage could be some of the contributing factors for such differences in antibody responses among the studies.

In Karnataka, scrub typhus IgM ELISA OD value cut-off of 1.309 can be utilized for a probable diagnosis of scrub typhus. In limited resource settings, ELISA can be used as a standalone test to diagnose scrub typhus after assessing background antibodies among the population [3]. ELISA can be instrumental in improving the detection rate, timely initiation of antibiotics, thus by reducing morbidity and mortality associated with scrub typhus. To increase ELISA sensitivity, region-specific antigenic preparations based on circulating *O. tsutsugamushi* strains can be used.

Conclusion

Scrub typhus IgM was elevated up to 3 months post infection among the cohort and dropped gradually till 9 months post infection and stayed above the newly derived regional diagnostic cut-off in 32% of the cohort up to 18 months. Scrub typhus IgG gradually rose from first week of infection and reached peak at nine months post infection. IgG levels declined by 12 months and remained stable until 18 months, with 75.4% of the cohort having IgG levels above the newly derived regional diagnostic cut-off.

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Ethics statement

The study was reviewed by Kasturba Medical College and Kasturba Hospital Institutional Ethics Committee and received approval with number IEC:412/2019. Written informed consent was obtained from all the patients. This study was performed in line with the principles of the Declaration of Helsinki.

Author contributions

Kiran Chunduru: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization. Manoj A.R.: Software, Validation, Formal analysis, Investigation, Data Curation, Writing – Review & Editing, Visualization. Subhadra Poornima: Methodology, Software, Validation, Formal analysis, Investigation, Writing – Review & Editing. Nagalakshmi Narasimhaswamy: Investigation, Writing – Review & Editing. Indira Bairy: Conceptualization, Resources, Writing – Review & Editing, Funding acquisition. Mridula M: Investigation, Writing – Review & Editing. Manjunatha Hande H: Writing – Review & Editing, Supervision. Shamee Shastry: Investigation, Writing – Review & Editing. Ramakrishna Devaki: Methodology, Validation, Formal analysis, Investigation, Resources, Writing – Review & Editing, Supervision. George M Varghese: Conceptualization, Methodology, Writing – Review & Editing. Kavitha Saravu: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. All authors have read and approved the final version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy/ethical restrictions which contains information that could compromise the privacy of research participants.

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