

Endemic titres to Salmonella Typhi as determined by the Widal titres in a tertiary care centre in south Kerala

Dr. Sneha Mariea Sebastian¹, Dr. Mercy John Idikula²

¹Post Graduate-MD Microbiology Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla, Kerala.

²Professor and Head of Department, Department of Microbiology, Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla, Kerala.



Abstract:

Background: In developing nations endemic to typhoid, laboratory diagnosis by blood cultures is still not considered an affordable option. In this context serological tests like the Widal test, are widely used and preferred.¹ In order to interpret Widal test on a single sample in endemic countries for diagnosis of typhoid fever, baseline titers need to be determined. These endemic titers change with time and require periodic revision.

Methods: This is a cross-sectional observational study done in Department Microbiology, in a tertiary care center in south India. The sample size was 206 and comprised sera of consenting patients with no recent history of fever in past 3 weeks to represent apparently healthy population of a community. Widal tube agglutination test was done and endemic and significant cutoffs were determined.

Results: Among the 206 non-febrile individuals included in the study, the majority (41.7%) were in the 41–60-year age group and males formed a higher proportion (57.3%) compared to females (42.7%). 95th percentile of O and H Antibody titres was 1:40, which can be considered the endemic baseline and titres, hence above the endemic titres (more than or equal to 80) can be considered as significant titres.

Keywords: *Salmonella; S. Typhi ;Enteric fever; Typhoid ;Widal test; Agglutinins; Baseline titre; Endemic titres Significant Cut off; Typhiwell IgM ELISA; India; Kerala; Pathanamthitta; Thiruvalla , Endemic countries.*

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Corresponding Author:

Dr. Sneha Mariea
Sebastian

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Introduction

Enteric fever includes typhoid fever caused by *Salmonella enterica* subsp. *enterica* serovar Typhi (S. Typhi) and paratyphoid fevers caused by *Salmonella Paratyphi* A, B, and C. It is a common, life-threatening bacterial infection in low to middle-income countries of Asia, Africa and South America. Route of transmission is ingestion of contaminated food and water and prevalence is high in regions with poor access to adequate sanitation and safe drinking water supply.^{1, 2, 3}

The World Health Organization (WHO) estimates as of 2019 up to 9 million cases of enteric fever and 110,000 deaths each year worldwide, reported from both rural and urban settings

.However, the actual burden of the disease is unknown since major disease burden lies in developing nations with the limited resources and available data.

India holds a major portion in the global burden of typhoid and a study done to survey national disease burden of typhoid, reported an incidence of 360 cases of typhoid fever per 100 000 person-

years, with 4.5 million cases estimated annually and 8930 deaths with higher incidence being reported in southwestern states and urban settings in the north India.^{5, 6}

In most endemic areas including India, typhoid fever is more prevalent than paratyphoid fever. Typhoid and paratyphoid fevers are often indistinguishable from each other and other acute febrile illnesses. Clinical presentations are often nonspecific and atypical. Thus, differentiating typhoid fever from viral, parasitic and other bacterial infections, that are also common in endemic areas becomes difficult,² and is more likely to manifest as Fever of Unknown Origin later¹¹

As per WHO recommendations, the definitive diagnosis of typhoid fever is by isolation of *Salmonella enterica* serotype Typhi from bone marrow or blood cultures. Blood cultures are optimally sensitive only in the first week of illness after which the sensitivity declines. Conventional blood cultures can be tedious and erroneous and automated techniques are expensive in most regions in developing countries with a relatively long turnaround time (TAT),

thus making it an impractical solution to diagnose Typhoid especially in resource limited settings.¹

In this context, the Widal Test is a simpler, rapid, and cost-effective serological test which can easily be done performed even in small laboratories.¹ However, interpretation of Widal test for diagnosis of typhoid fever requires demonstration of a fourfold rise of antibody titre in paired sera from suspected patients taken 10-14 days apart. However, such a rise is not always demonstrable, even in blood culture-confirmed cases. In most developing countries endemic to typhoid, obtaining a second serum sample from patients is often not feasible owing to poor patient compliance, financial constraints and late patient presentation during the course of illness. Hence, interpretation of a single titre value compared to a cut-off value determined from the baseline titre in the community, is the norm and perhaps the only means to diagnose typhoid in developing countries.

The O and H agglutinin titres in the population are referred to as the 'baseline / endemic titres' and vary regionally and also in the same region with respect to time in the same population. In endemic areas these anti-salmonella antibodies are present invariably in the community in varying titres due to the constant exposure and re-exposure resulting in clinical and subclinical infections.

Thus, interpretation of a single Widal test demands a knowledge of the prevalent baseline titres in the community. Based on this, a cut-off titre would be assigned for the community. In a febrile patient, the finding of a titre higher than the cut-off titre in a single Widal test would serve as presumptive evidence of infection. Thus, in different geographical areas the baseline and significant titres need to be determined.⁵

In India, antibody titres in Widal test considered generally significant for presumptive diagnosis of typhoid is >100 for O Ag and >200 for H Ag.⁴

However a study conducted in Kannur, Kerala in 2012 showed Widal titre of significance reduced to >80 for O and H antigens because the baseline titres were determined as <40 for O and H antigens.¹² The current cut off titres, for the serodiagnosis of typhoid fever followed in our institution since 2015 is >80 for O antigen and >160 for H antigen.

A note of caution is that a rise in Widal titres could be non-specific to typhoid, occurring due to anamnestic response to cross reacting antigens.¹²

ELISA and other point of care tests for the rapid diagnosis of febrile illnesses, such as malaria and dengue, have been studied extensively. Research and standardized evaluations of these have provided evidences to the World Health Organization (WHO) to formulate diagnostic algorithms in endemic regions. However, diagnostic tests for acute enteric fever have yet to be evaluated in this rigorous manner.¹¹

Until such time that more sensitive and specific tests are available for antigen or antibody detection, the use of Widal test cannot be phased out in most areas where enteric fever is likely to be endemic. The newer tests, if available may be used to complement the Widal test.

Hence a study was warranted to determine the prevalent endemic titres to *Salmonella Typhi* in this geographical region and thereby review the cut off value to determine the significant titre for the presumptive diagnosis of typhoid

Although determination of endemic antibody titers to *S. Typhi* is done as a field/ community-based study, we opted for a hospital-based study setting for better patient compliance, accessibility to laboratory facilities, easy transport and storage of samples as has been done at other centers.^{12, 15}

Materials and Methods

This was a cross-sectional observational study done at Department of Microbiology, Pushpagiri Institute of Medical Sciences and Research Centre,

Sera of 206 consenting patients with no recent history of fever in past 3 weeks were recruited to represent healthy population for determination of endemic titres to *S. Typhi* by Widal tube agglutination test to fulfil primary objective of the study.

Calculation of Endemic titre

Calculation of Endemic/Baseline Widal titre to *S. Typhi*

Highest titre among healthy individuals in an area is referred to as endemic titres.

Baseline titers or endemic titres are the normal antibody levels found in healthy people in a specific geographic area, which can be high due to past exposure to Salmonella or vaccination.

The reported methods of calculation of endemic titres are:

- Determining modal titre and 2 standard deviations above it denotes endemic or baseline titre⁵⁷
- Highest titre seen in >10% of population.⁵⁸
- Highest titre corresponding to 95th percentile⁵⁶

The endemic titre in this study, was calculated by the third method ie analyzing distribution of antibody titres to Salmonella Typhi 'O' and 'H' by Widal test at a starting titre of 10 and taking 95th percentile as baseline titre (Collard et al) above which the titre is considered significant⁵⁶

For calculating the 95th percentile, the data was first sorted in ascending order.

The total number of sera in afebrile group was multiplied by 0.95 to find the position.

$$N = K \times 0.95.$$

$$N = 206 \times 0.95$$

$$N = 195.7$$

The obtained value for N, was rounded to the nearest whole number ie 196th position

The antibody titres corresponding to 196th position, when all the titres are arranged in ascending order, corresponds to 95th percentile and the titre, was taken as baseline/endemic titres to O& H antibodies.

Calculation of titres of significance or cutoff values Widal titre to *S. Typhi*

Significance titers, or cutoff values, are titres above the normal baseline titres and are used to interpret a single Widal test result, indicating a high likelihood of an active or recent infection with *S. Typhi*. The titer of significance is determined from the local baseline titer and a single reactive Widal titre above this value suggestive of Typhoid fever

- Determining the **modal titre**⁵⁷, then multiplying with 4

- Determining endemic titres, multiplying 2, denotes significant titres.⁵⁸
- Adding one double dilution titre more than 95th percentile, above or equal to which represents titre of significance⁵⁶

The locally derived **cutoff** values can be used to interpret the Widal test results of febrile patients, to assess its diagnostic reliability for enteric fever in this region of South Kerala.

Patient Data and results were properly coded and entered in Microsoft Excel and analysed using SPSS Version 25 statistical software. Fisher's exact test was applied to determine statistically significant differences.

Results

Table 1: Distribution of Salmonella Typhi O and H antibody titres according age groups

Age group (Years)	O & H titres and frequency in %								Frequency	Percentage
	10		20		40		80			
	O	H	O	H	O	H	O	H		
≤20	50	16.7	50	33.3	-	33.3	-	16.7	6	2.9
21-40	33.8	1.5	63.2	58.5	1.5	20	1.5	20	65	31.6
41-60	54.6	23.2	32.6	64	12.8	2.8	-	-	86	41.7
>60	44.9	18.4	51	67.3	4.1	14.3	-	-	49	23.8
Total									206	100.0

Determination of endemic titres and significant cutoffs by 95th percentile method

The 95th percentile of O Antibody titres was 1:40, and that of H Antibody titre was 1:40

Hence the endemic titre for our population was determined as less or equal to 40 for both O and H antibody titres Therefore, titres more than or equal to 80 can be taken as titres of significance.

Determining endemic titre by alternate methods

- Modal titres for O antibody were 10 and for H antibody, 20. Multiplying by 2 (dilution factor) to determine endemic titres ie Endemic titres Antibody- 20, H Ab-40. Significant cutoff titre by this method is modal titre multiplied by 4(2x SD) ie O-40, H-80.
- Highest Titre shown by >10%-Endemic titre, above which its significant ie 40 for both O and H antibodies above this titre ie greater than or equal to 80 is significant titres for both O and H antibodies.

Discussion

Need for determination of Endemic/Baseline titres

Interpretation of Widal test is complicated by background antibodies in people living in endemic areas due to prior exposure and re-exposure.

Current Study

Among the 206 non-febrile individuals, males formed a higher proportion (57.3%) compared to females (42.7%), showing a slight male predominance in the study population.

Majority (41.7%) were in the 41–60-year age group, followed by 31.6% in the 21–40-year group. Participants aged above 60 years constituted 23.8%, Paediatric population was not well represented in this study (2.9% of study population).

Overall, the distribution of both O and H antibody titres was similar between males and females. Fisher's exact test showed no statistically significant difference in the proportion of participants with different O antibody titres between genders and across age groups.

- Only 2.9% of the study population were constituted by ≤20 years age group. Since criterion for selection also included sufficient serum volume, probably this group of participants got eliminated because they did not have adequate volume or afebrile patients were less frequently investigated with blood examination. Further studies are warranted for whether or not this age group requires a different cutoff to diagnose typhoid as literature warns us that although incidences maybe less than adults, disease severity of typhoid fever is more in children.^{39,42,44}
- Over 60 years predominantly had O Antibody titres less than or equal to 20 (1:10 ie 44.9%) and 1:20 ie 51.0%) (Results-Tables 11)
- The distribution of Salmonella Typhi H Antibody titres among non-febrile participants showed maximum variation in the ≤20-year age group (16.7% had a titre of 1:10 33.3% had 1:20, 33.3% had 1:40, and 16.7% had 1:80) Among participants aged 21–40 years majority (58.5%) had 1:20 as titre, 20% had 1:40, and 1.5% had 1:80. In the 41–60-year group and participants over 60 years, majority patients had a predominant titre had 1:20 (ie 64% and 67.3% respectively) (Results-Tables 12).
- Our results were in concordance to a study done in North Kerala in 2012, where endemic titres to O and H antibodies were determined as <40¹². Our results showed lower titres compared to two studies done in Kerala by

Table 2- Results of four studies done in Kerala to determine endemic antibody titres and significant cutoff to S. Typhi

Author (Year)	District in Kerala	Population Studied	Endemic Titre	Significant Titre	Reference No.
Maulingkar S et al. (2015)	Wayanad	Apparently Healthy adult population visiting hospital	Anti-TO 1:40 Anti-TH 1:80	≥ 1:160 (TO) ≥ 1:320 (TH)	(15)
Sreenath K et al. (2013)	Kollam	Healthy blood donors	Anti-TO 1:80 Anti-TH 1:160	≥ 1:80 (TO) ≥ 1:160 (TH)	(55)
Aruni I S et al. (2014)	Thiruvananthapuram	Healthy coastal population	Anti-TO 1:80 Anti-TH 1:160	> 1:80 (O) > 1:160 (H)	b
Bijapur S et al. (2014)	North Malabar, Kerala region	Healthy local individuals	Anti-TO 1:40 Anti-TH 1:40	≥ 1:80 (TO, TH)	(12)

Summary

Recommendations based on the study findings

- The study findings of endemic titres for Widal test and significant cutoff is less than with the titres followed in our institution since 2015(80-O , 160-H), Both O and H antibody titres have reduced over the past decade, this shows that endemic titres must be periodically assessed in order to diagnose and treat typhoid cases of a region , as well as to prevent antibiotic misuse when interpreting the Widal test in patients from regions with high baseline Widal titres.
- Increasing awareness of preventive measures such as proper hand hygiene, access to safe clean drinking water, improved sanitation screening of workers handling food especially migrant labourers from areas of high endemicity must be implemented.
- To prevent the incidence of typhoid and reduce antimicrobial resistance, World health organization (WHO) recommends use of typhoid conjugate vaccine. However, in India, the typhoid vaccine is not yet a part of the national immunization schedule. If implemented this could bring down the high burden of typhoid in India.

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