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The "Baseline Widal Titre" Amongst Apparently Healthy Adults Working at a Tertiary Care Medical Institution, a Pilot Study, Uttarakhand, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: As typhoid fever is known for its endemicity all over the India, healthy people may contain antibodies against salmonella *typhi* and *paratyphi*. These antibodies may react up-to a variable titre either due to past exposure, vaccination or/and cross -reacting antigen. Therefore,

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antibody titre in widal test varies widely from place to place and is referred as baseline titre of that particular area. Two properly staged tests (2-4 weeks interval) show about a four -fold rise in antibody levels leads to a reliable widal test. Single widal test values are not considered significant for diagnosis but many places still use this as diagnostic marker which further depends upon baseline titre prevalent in that particular area.

Aims: This study was undertaken in jhajra, Uttarakhand to determine the baseline titre in this geographical region for calculating significant widal titres.

Material and Methods: Blood samples were collected from apparently healthy individuals which included medical students, nursing staff, doctors, and other health care workers (aged 19-45) with proper consent. All the samples after being screened by slide agglutination test were further confirmed by Quantitative tube agglutination test, to find out the baseline titre. The tube agglutination test was carried out by taking 0.5 ml of two-fold serially diluted sera (dilutions from 1:20 to 1:320) in 0.9% normal saline with an equal amount of antigen.

Results: Out of total 110 serum samples,36 (32.7%) sera were positive (titre ≥ 1:20) and 74 (67.3%) were negative(titre<1:20). A total of 36 samples were positive for agglutinins of “O” antigen,32 for agglutinins of “H” antigen,16 for *paratyphi A* and 10 for *paratyphi B*. 23 out of 36 (63.8 %) sera were positive for agglutinins of 'O' antigen of *Salmonella typhi* (TO) at a titre of 1:40 followed by 6 sera positive at 1:20 (16.6%) and 1:80 (16.6%) and 1 serum sample positive at 1:160 (2.77%) 59.3 % (19) sera were positive for 'H' antigen of *Salmonella typhi* (TH) at a titre of 1:80 and 10 % sera (11) were positive for 'H' antigen at a titre of 1:40 and 2(1.8%) were positive at 1:20.16(44.4%) sera samples were positive for of *Salmonella paratyphi A* (AH) and *Salmonella paratyphi B* (BH) respectively, both at a titre of 1:20 and 8.3% sera were positive at 1:40 respectively.

Conclusion: The most frequently recorded titre of the reactive sera was 1:40 and 1:80 for anti-O antibodies and anti-H antibodies and 1:20 for AH and BH. Thus, the significant cut-off level for TO and TH was ≥ 1:80 and ≥ 1:160 that for AH and BH was ≥ 1:40.

Keywords: Baseline titre; widal test; slide and tube agglutination test; typhoid/enteric fever; Salmonella typhi.

1. INTRODUCTION

“Enteric fever is endemic in developing countries like India since ages and it is still a cause of major health issue” [1]. “This systemic infection is caused by the bacterium, *Salmonella enterica serotype typhi*, *paratyphi A and B*. Due to its high adaptivity and immune escape mechanisms in its host (mainly humans) *salmonella* ensures its survival and transmission” [2,3,4,5]. “Salmonella nearly comprises more than 2000 serotypes or species and majority of them are pathogenic. Non-typhoidal species of *Salmonella* are also there which can cause gastroenteritis, septicaemia or localized infections in humans. Enteric fever affects the local community as well as travellers to the endemic areas. The prevalence peaks during the rainy season due to water clogging and its contamination with sewage” [2,6]. “Other contributing factors includes poor personal hygiene, habits, open air defecation, urination which leads to spoiled food and water. Typhoid fever is almost eliminated in majority of the developed countries due to improvement in sanitation whereas its endemicity is prevalent and of great concern in developing countries like India. Bacteriological diagnosis of enteric fever can be done by isolation of bacteria from the various clinical specimens and demonstration of antibodies in patient’s serum” [6,7]. Definitive diagnosis of enteric fever although depends on isolation of *Salmonella* from blood, stool/urine, bone marrow, bile or other body fluids. However, due to irrational use of antibiotics and lack of adequate diagnostic facilities as well as financial and time constraints, isolation of organism (culture positivity) is being seen in limited number of cases only. For these reasons, we have to be dependent upon serological tests like widal for diagnosing enteric fever.

“Now, as per historical records this serological test was developed by Georges Fernand Isidore Widal in 1896. This was used as a diagnostic test for enteric fever as an alternative to the microbial culture since its introduction which was almost 10 decades ago” [8]. “Reliability of this test depends on minimum two properly staged tests showing four -fold rise in antibody levels” [7,8]. “In many places, single widal is also used for diagnosis

and management of enteric fever for which we need the baseline titre prevalent amongst healthy individuals in that particular geographical area. The interpretation of the widal test is done by comparing test results with baseline titre prevalent amongst the healthy individuals in that particular geographical area. These titres vary among the healthy populations of different areas. Variation of baseline titre depends upon the endemicity of typhoid in each area, which keeps on changing during a period of time" [9,10]. "Thus, continuous updating and monitoring of baseline titre is essential for the proper interpretation of the test" [10-12]. "In our current health care settings, most of the patients present late to the hospital and due to requirement of immediate diagnosis and a specific treatment, a single sample cut-off value has to be relied upon, instead of paired serum samples" [13-15]. Interpretation of a single widal test result needs to be based on the average baseline titre which is seen among healthy individuals therefore, the present study was undertaken to establish the normal baseline titre in apparently healthy health care workers.

2. MATERIALS AND METHODS

This prospective epidemiological cross-sectional study was conducted in the Department of Microbiology, Gautam Budha Chikitsa Mahavidyalaya, Dehradun, India from July 2022 to December 2022 after taking ethical clearance from the Institute's Ethical committee. The IEC number provided is GBCM/IEC/2024/05-01. The blood samples were collected from 110 healthy adults (>18 years old) either male or female with no gender bias who were apparently free from any disease and not vaccinated for typhoid and not had fever for past six months. In order to calculate baseline antibody titres against the O, H, AH and BH antigens of *Salmonella*, slide and tube agglutination method was adopted. The antigens used were from Tulip Diagnostics kit, ISO certified (13485), Goa, India. Kit literature was strictly followed. The tube agglutination test was carried out by taking 0.5 ml of two-fold serially diluted sera (dilutions from 1:20 to 1:320) in 0.9% normal saline with an equal amount of antigen.

3. RESULTS

A total of 110 serum samples were collected and screened for slide and tube agglutinins against *salmonella typhi and paratyphi A and B*. Females outnumbered males in our study with Male: Female ratio being 1:0.71. Maximum volunteer involved were of age group 18-40 years (89.09%) followed by >40 years being10.9% [Table 1]. Sera positivity rate for widal test in our study showed 32.7 % (36/110) samples were positive for the agglutinins (≥1in 20) whereas 74 (67.20%) samples did not show agglutinins (≤ 1in 20). The agglutinins to S. typhi were the most prevalent among the sera of various dilutions (32.7% for the O antigen and 29.09% for the H antigen) which were tested. The levels of the agglutinins for *Salmonella paratyphi AH* and *paratyphi BH* were comparatively low (only 14.5% and 9.09% for the AH and the BH antigens respectively) [Table 2].

Table 1. Age/Gender wise distribution of samples (n=110)

	Age wise distribution		
	18-40	**>40**	**Total**
Male	42	4	46
Female	56	8	64
Total	98	12	110

Table 2. Number & percentage of sera with cut-off titres in healthy volunteers (n=110)

Antigen	Dilution (1:20)	Dilution (1:40)	Dilution (1:80)	Dilution (1:160)	No. of positive samples
Salmonella typhi O	6(5.4%)	23(20.9%)	6(5.4%)	1(0.9%)	36(32.7%)
Salmonella typhi H	3(2.7%)	10(9.09%)	19(17.2%)	0	32(29.09%)
Salmonella paratyphi AH	14(12.7%)	2(1.8%)	0	0	16(14.5%)
Salmonella paratyphi BH	7(6.3%)	3(2.7%)	0	0	10(9.09%)

Table 3. Comparative analysis of Baseline titre of O and H agglutinins in different regions of India till date

Author	Place	Year	Baseline titre			
			TO	TH	AH	BH
Shukla S et al	Central India	1997	1:80	1:80	0	0
Patil Anand M et al	Karnataka	2007	1:80	1:80	1:40	1:40
Punia JM et al	Chandigarh	2003	1:80	1:160	1:20	1:20
Shekhar Pal et al	Srinagar (Garhwal)	2011	1:40	1:80	1:20	1:20
Prashant Peshattiwar et al	Andhra Pradesh	2011	1:40	1:80	1:20	1:20
Saxena et al	Hadoti (Rajasthan)	2012	1:40	1:40	1:20	1:20
Sreenat et al	Kollam	2014	1:40	1:40	1:20	1:20
Vadsmiya et al	Ahemdabad	2014	1:40	1:40	1:20	1:20
Maulingkar et al	Wayanad	2015	1:40	1:80	1:40	1:40
Shethwala et al	Himmatnagar (Gujarat)	2017	1:40	1:40	1:40	1:20
Present study	Jhajra (Dehradun)	2022	1:40	1:80	1:20	1:20

Table 3 shows- 63.8 % **(**23/36) sera were positive for agglutinins of 'O' antigen of Salmonella typhi (TO) at a titre of 1:40 (commonest) followed by 6 sera positive at 1:20 (16.6%) and 1:80 (16.6%) respectively. Only one serum sample was positive at 1:160 (2.77%). 19 out of 32 (59.3%) sera were positive for 'H' antigen of *Salmonella typhi* (TH) at a titre of 1:80 whereas, 10 % (11 out of 32) sera samples were positive for 'H' antigen at a titre of 1:40. Only 2 (1.8%) samples were positive at a titre of 1:20.A total of 16 sera samples (44.4%) were positive for *Salmonella paratyphi A* (AH) and 10 (9.09%) were positive for *Salmonella paratyphi B* (BH) at a titre of 1:20.Only 2 and 3 samples for paratyphi A and paratyphi B were positive at a titre of 1:40 respectively. The commonest baseline titre in our study was found to be 1:40 for “O”,1:80 for “H” and 1:20 for “AH” and “BH”. Thus, significant titre for widal test is considered as ≥ 1:80 for TO and ≥ 1:160 for TH and for AH and BH was ≥ 1:40 in this particular area.

4. DISCUSSION

“The culture isolation of *Salmonella enterica subspecies enterica* from blood and other body fluids remains the gold standard for the diagnosis of enteric/typhoid fever. But the majority of the normal healthy individual in endemic region also carry detectable range of typhoid antibodies due to the repeated prior exposures or prior vaccination. Also, in an acute febrile illness especially so in endemic region, non-specific clinical picture and use of broad-spectrum antibiotics, a rapid yet accurate, specific and sensitive test should be used to differentiate typhoid fever from other non-typhoidal illness” [8,16-19]. In our country, we definitely need an alternative and reliable laboratory test for typhoid fever. Reasons may be poor cultural isolation yield, empirical use of broad-spectrum antibiotics, self-medication and specimen collection after intake of antibiotics, lack of adequate blood culture facilities and various other financial and time constraints. “Thus, widal agglutination test has become the most common alternative laboratory procedure for diagnosis of enteric fever. The O antigen is the somatic antigen and antibodies against the O antigen are predominantly IgM which rise early in the illness and disappear early. The H antigens are flagellar antigens of *Salmonella typhi, paratyphi A and paratyphi B.* Antibodies to H antigens are both IgM and IgG which rise late in the illness and persist for a longer time” [8-10]. In endemic areas, baseline anti O and anti H antibodies are present due to repeated exposure, vaccination and cross- reacting antigens. [8-10,20,21]. “The specific purpose of this study was to develop local recommendations for the interpretation of widal test. There should be four- fold rise in paired samples 10-14 days apart for reliability but such a rise is not always demonstrable, even in the blood culture confirmed cases. This can occur due to late receiving acute phase sample and high levels of the background antibody in a region of endemicity” [22]. “Furthermore, the patient treatment cannot wait for so long, thus in view of practicality treatment decision are dependent upon single acute phase sample titre” [23,16,17,18,19]. The variation depends on the endemicity it may change over time [16,24,25]. “Thus, single titre many a times is used solely for diagnosis and management of enteric fever [11]. So, every country or region should have a baseline titre of their healthy population, which should also be updated with time” [23,16,17]. The results of this present study showed that the sera of a significant proportion of healthy

individuals in this area contained antibodies which were capable of reacting to the variable titres in the Widal test.

"Among the 110 samples of the healthy volunteers, 32.7 % were positive for the agglutinins against various serotypes of Salmonella enterica. The agglutinins to S. typhi were the most prevalent among the sera which were tested at various dilutions. The most frequently recorded titre of the reactive samples was found to be 1:40 for "O" and 1:80 for "H" and this was considered as the cut off titre. As all the samples individual had a titre of ≤1:40 for the *Salmonella enterica serovar paratyphi A* and *B*, anything above this can be taken as diagnostic titre in this area" [23]. The present study suggests that the current baseline titre for the diagnosis of typhoid fever in the jhajra region, Dehradun city is 1:40 for the anti-O agglutinins and that it is 1:80 for the anti-H agglutinins. Based on this, we have set our own laboratory guidelines of the O and H agglutinin Widal titres of 1:80 and 1:160 as being of diagnostic significance in single acute phase samples. Many other Indian studies from different regions showed the baseline titre of 1:80 for O antigen and same with anti-H agglutination [1,23,16] whereas Pal et al., has documented significant titre of 1:40 for O and 1:80 for H antigen which is similar to our findings in this region of Dehradun city [7]. Similary, Prashant et al and Maulingkar et al are in concordance with our study [26,27]. For the anti-TH antibodies, an agglutinin titre of up to 1:80 was commonly found in many studies whereas only Punia et al showed high titre of 1:160 for "H" agglutinin however only 1 sample showed such a high titre in our study. The titre for "AH" and "BH" was recorded 1:20 which is common in almost all the studies mentioned except few like Maulingkar and Patil et al.

The comparatively lower antibody titre against AH and BH antigens highlighted the lower endemicity of *Paratyphi* infection in our country as compared with typhi infection and/or low antibody response against *Paratyphi* infection. The variation depends on the degree to which typhoid is endemic in each area, a fact which may change over time [10]. So, each country or region should have a baseline titre of their healthy population, which should be regularly updated with time. This variation may be the result of difference in safe water supply and sanitary conditions along with standard of living and available medical facilities and education play a major role [28].

5. CONCLUSION

Due to constant exposure, in endemic areas population is "immunologically sensitized", the response to infection is more rapid and reaches higher levels [17]. Several studies have highlighted the limitations of using the widal serological test in the laboratory diagnosis of Salmonella, the worst being its non-specificity. Still considering the low cost and easy availability the widal tube agglutination test is likely to remain the test of choice in many developing countries, as of ours, provided a baseline antibody titre of healthy individual in the population. *Salmonella* antibodies are common in healthy individuals and subject to variability in due course of time, we may thus conclude that, knowledge of baseline titre of a region is essential for calculating cutoff titres of widal test in a particular geographical area and more such studies in fiture should be done.We may thus conclude that, knowledge of baseline titre of a region is essential for knowing the significant cutoff titre of a particular geographical area and may help in the interpretation for diagnosis of typhoid fever. A single widal can be significant in this endemic region when higher titre of (1:80) for "O" and (1:160) for "H" is obtained.

6. LIMITATION

Due to limited number of subjects, we might suggest to do more such kind to studies in near future.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

As per international standards or university standards, Participants' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

After taking ethical clearance from the Institute's Ethical committee. The IEC number provided is GBCM/IEC/2024/05-01.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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