

RESEARCH ARTICLE

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False sero-positivity of *Salmonella typhi* Specific Antibody in Dengue and Corona Virus Infected Patients: An Observational Study

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Abstract

Typhoid fever is a major concern in developing nations. People living in endemic area may frequently get exposed to the typhoid bacilli and can carry some amount of antibody in their circulations which can easily lead to misdiagnosis during other febrile illness. To assess the frequency of false sero-positivity of *Salmonella* specific antibody in Dengue and COVID-19 patients. An observational case control study was conducted in a multispecialty teaching hospital in north India. A total of 110 serum samples which included dengue IgM positive patients (N= 40), COVID-19 RT-PCR positive patients (N=40) and control subjects (N=30) without any febrile illness were studied. All the samples were tested for *Salmonella* specific antibody by Widal test and by immune chromatography strip test (ICT). Patient's detail were documented in a structured Performa. Out of 110 samples 63(57%) were male and 47(43%) were female with age mean age ± 45 years. Among the dengue IgM positive patients 8(20%) patients sample showed significant O and H titre of *Salmonella typhi* (O:80; H:160) in Widal test. Similarly in SARS-CoV-2 RT-PCR positive patients sample 8(20%) patients serum sample showed significant titre of O and H antigen. In control group population significant O antigen along with H antigen of *Salmonella typhi* were observed in 2(6.6%) sample. False positivity in the serological test should be reported with caution and repeated test should be performed until the etiological agents were not confirmed, in this way we can minimize the misdiagnosis and can prevent the morbidity and mortality as well.

Keywords: Widal Test, Dengue, COVID-19, *Salmonella typhi*

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Citation: Singh M, Chakraborty A, Tyagi AK. False sero-positivity of *Salmonella typhi* Specific Antibody in Dengue and Corona Virus Infected Patients: An Observational Study. J Pure Appl Microbiol. 2023;17(1):434-438. doi: 10.22207/JPAM.17.1.35

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INTRODUCTION

Typhoid and Dengue fever are the most concurrent diseases in the tropical country for many years and along with these, coronavirus emerged as one of the most lethal disease in the world.¹ Although these diseases are caused by two separate agents, they have some similar symptoms which can easily end up with misdiagnosis. So, diagnosis of the causative agents is important in the treatment of these diseases which are directly related with population density, urbanization, endemicity, mobility as well as mortality. During COVID-19 pandemic and dengue outbreak it has been found that some patient's sample frequently show sero-positivity of two or more infections leading to pretending challenges in clinical diagnosis and treatment.^{2,3} Baseline titre of *Salmonella* antibodies are not always available in every region hence serological test for typhoid should be reported with caution and requires surveillance and constant follow-up of these patients during COVID-19 pandemic and Dengue viral diseases (DVD) outbreak.^{4,5} In order to overcome these difficulties we undertook this study to assess the frequency of false sero-positivity of *Salmonella typhi* specific antibody test in DVD and COVID-19 patients.

SUBJECTS AND METHODS

Participants and clinical samples

The study was done from August 2021 to November 2021, from patients of the multispecialty teaching hospitals in north India after obtaining permission from the institutional ethical committee (ECR/1432/Inst/UP/2021). A total of 110 subjects which included 40 patients with dengue IgM ELISA positive, 40 RT-PCR positive COVID-19 patients and thirty healthy controls who had no signs and symptoms of an acute illness that integrated with fever and myalgia with retro-orbital pain, headaches, facial flushing, or a petechial rash were included in the study. All the 110 study subjects blood culture report were negative for typhoid and paratyphoid bacilli, there were no history of TAB vaccination and all the individuals were negative for malaria Ag test at the time of this study. Clinical details of patients were collected in a structured proforma.

Laboratory analysis Specimen collection

Using universal precautions, 5-8 ml of venous blood was collected in a plain vacutainer. All the samples were further processed in BSL-3 laboratory, Blood was allowed to clot at room temperature for half an hour, after which the serum was separated by centrifugation at 3000 rpm for 2 minutes. After centrifugation the serum samples were used for Widal test and for detection of *Salmonella typhi* antibodies using immunochromatography strip test.¹

Serodiagnosis of *Salmonella enterica* serotype typhi specific antibody

Widal test, which is a tube agglutination test were performed to detect the antibodies against O as well as H antigens of *Salmonella typhi* and H antigens of *Salmonella paratyphi* A and B. Titre values of $\geq 1:160$ for H and $\geq 1:80$ for O was considered as clinically significant in single acute phase samples which is the base line titre for this region. Furthermore, all the serum samples were tested for *Salmonella typhi* is specific IgM by immune chromatography strip test (Typhoid IgM Rapid Test). All serological assay were done as per manufacturer's instructions.

RESULTS

A total of 110 non-repeated blood samples were included in our study. These included 40 cases of Dengue IgM positive patients serum, 40 cases of COVID RT-PCR confirm patients serum and 30 samples were from healthy individual as a control. Of the 110 samples 63 (57%) were from male and 47 (43%) were from female with mean age group of ± 45 years.

Among the dengue IgM positive patients (n=40) all had symptoms of fever with mean body temperature of 102 ± 1 , followed by headache 39 (97.5%), Myalgia 36 (90%) and retro- orbital pain 36 (90%). On haematological analysis, platelet counts were significantly low in all patients with mean value of $50,000 \pm 20000$ / cu.mm. (Table 1)

Among the 40 Dengue IgM seropositive patients 8 patients sample showed significant anti O antibody titres with the value of 1:80 and anti H titre of 1:160 against *Salmonella typhi* and 18

Table 1. clinical and haematological finding of the study population

	Control N = 30	Dengue positive N = 40	COVID-19 rRT-PCR positive N = 40
Gender (M/F)	(14/16)	(23/17)	(26/14)
Age	35±5	30±5	50±5
Temperature	96±1	102±1	100±1
Headache	NIL	36 (90%)	16 (40%)
Myalgia	NIL	38 (95%)	10 (25%)
Retro-orbital pain	NIL	40(100%)	4 (10%)
Total-WBC Count (cells/cu.mm)	5000+2000	5000+2000	5000+1000
Platelets Count (cells/cu. mm)	15000+25000	50000+2000	12000+10000

Table 2. Widal test finding among the study population

WIDAL TEST result		TYPHOID IgM N=40	DVI N=40	COVID-19 N=30	Control N=40
Anti O titre	Anti H titre				
No agglutination	No agglutination	00	18	16	16
1:20	1:40	01	08	07	06
1:40	1:80	21	06	09	06
1:80	1:160	18	08	08	02
1: 160	1:320	00	00	00	00

patients sample showed no agglutination in Widal test.

Out of 40 dengue positive samples, 20 (50%) of the patients sera were also positive for *Salmonella typhi* specific IgM by immunochromatographic strip test.

Among the 40 COVID RT-PCR positive patients all had symptoms of fever with mean body temperature of 100±1, followed by headache 16 (40%), Myalgia 10 (25%) and retro-orbital pain 04 (10%). On haematological analysis, 5 patients total leukocyte count were significantly high with 12000 cells/cu.mm, Platelet counts were normal in all the patients with mean value of 150000±25000 cu/mm. (Table 1)

Blood samples of 8 patients showed significant titre *S.typhi* Ab (O= 1: 80; H= 1:160), Nine (22.5%) patients serum showed anti O titre of 1:40 and 1:80 in *S.typhi* specific anti HAb. And 16 patients serum samples showed no agglutination at all.

Of the total of 40 COVID-19 RT-PCR positive patients blood sample, 19 (47.5%) patients sera also showed positive band in *S.typhi* specific IgM by immunochromatographic strip test.

In case of control group samples, 2 (6.5%) of the sera showed significant titre of anti O (1:80) and anti H(1:160) of *Salmonella typhi*. Details of the Widal test and ICT result were summarized in Table 2.

DISCUSSION

In recent years, COVID-19 as well as Dengue viral disease along with typhoid fever are the major public health emergency in the developing country,⁶ and physicians often find difficulties for diagnosis and treatment as there are many similarity in clinical presentation in early stage of these diseases. Microbiology laboratory should play a major role to overcome these situations.

The result of our study on 80 patients which included dengue and COVID-19 positive patients and 30 healthy control subjects showed around two in four subjects serum samples antibody titre of *Salmonella* enteric serovar *typhi* O and H antigen were more than 1:20 and 1:40, which is similar to the study done by Ramya and Sunitha where they found 42% of their study

population had anti O titers above 1:20 and 52% had anti H titres above 1:20 against serotype *S.typhi*.¹

In the present study, among the dengue IgM positive patients 20% samples antibody titre of O Ag of *Salmonella* enteric serovar *typhi* were 1:80 and antibody titre for H antigen were 1:160, and they also showed IgM positive bands on ICT test. However, their blood culture showed no growth after 5 days of incubation and clinically no signs and symptoms of typhoid fever. Several other investigator also find the similar findings where they reported about 50% of their study population have dengue IgM with significant Widal test titre.⁷

We also observed that in case of SARS-COV-2 infected patients around 20% subjects showed titre of anti O \geq 80 and titre of anti H were \geq 160 which is considered as a base line titre of the local population. However, around 18% of the patients had anti O titre of 1: 20 and anti H of 1:40. There is very limited data in favour of our finding in the data base.

In control group population we also observed that, around 20% patients whose blood culture were negative with no clinical evidence of typhoid fever had anti O titre of 1:40 and anti H titre of 1:80 and around one in two patients have no agglutination in Widal test. We also observed that none of the control group population were positive with IgM in *Salmonella* antibody specific ICT test. The above findings are similar with the study done by Shukla et al.⁸

In correlation between base line titre of *Salmonella typhi* specific antibody with serum samples from DVI, COVID-19 even in control group population no significant P value were observed and indeed around 50% of the population had some of *Salmonella* specific antibody with low titre value.

Many studies had reported that *Salmonella typhi* somatic and flagella antigen is shared by large number of organism from *Enterobacteriaceae* family^{9,10} and even in several other febrile illness such as Malaria, Tuberculosis, Schistosomiasis and in Dengue virus infection there are reports of false sero positivity of Widal test where there is no signs and symptoms of acute *Salmonella* infection.^{7,11-13} In a recent study by Seema et al.¹⁴ had reported around 20% of

their COVID-19 positive patients were shown false positive result of Widal test which is similar to our study finding where we reported 20% of our COVID-19 patients had a significant titre of Widal test. Yet in another study from India by Adata et al. also find similar finding in their study population.¹⁵ As Dengue and COVID-19 both are febrile illness the false positivity of the Widal reaction may be due to the individual immune responses where there might be activation of memory T cells that causes positive Widal reaction in previously sensitized patients.¹⁶ There might be other factors for Widal test false positivity as it cannot interpret the difference between a person's acute or chronic infection with *S.typhi*, one who has recovered and in a carrier stage or one who simply has been vaccinated against the disease. While this low-cost point-of-care test may be indicative of enteric fever in certain clinical situations however its use is often discouraged due to its low specificity. A false diagnosis of enteric fever through Widal agglutination test may result in the unnecessary use of antimicrobial drugs and provoke the development of drug-resistant bacteria.¹⁷⁻¹⁹

Based on our finding we can conclude that, false positivity in the serological test should be interpreted carefully and base line titre should be monitored frequently to overcome the misdiagnosis. There should be a continuous follow up of the patients and more accurate diagnostic test should be performed to rule out the false positivity. As typhoid is endemic in many part of the world there is also a possibility of both the infections co-existence, so proper preventive measure should be taken to reduce the mortality and morbidity of the diseases.

Limitation of our study

Our study have a limitation because the study was conducted in a small group of population and there is a requirement of studies with large group of population and molecular diagnosis to be performed for the diagnosis of typhoid fever to rule out the co-infectivity with Dengue and COVID-19. In our study typhoid and dengue were diagnosed based on serological test as there were no IgM detection kit for SARS-COV-2, so IgM response of those patients were not evaluated.

ACKNOWLEDGMENTS

The authors would like to thank ICMR Delhi, India and Director General of Medical Education Government of Uttar Pradesh for providing the Kit and Infrastructure to conduct the study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Motilal Nehru Medical College Institutional Ethical Committee (ECR/1432/Inst/UP/2021).

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