

Original Article

Widal test interpretation in COVID-19 patients: a study from a dedicated COVID center

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Abstract

Background: There have been several outbreaks of coronaviruses including the present SARS-CoV-2 outbreak. The clinical presentation of COVID-19 infection may mimic febrile phase of dengue fever, typhoid fever, malaria, and several other diseases. During the COVID-19 pandemic, more number of typhoid cases were reported. The goal of this study was to determine the seropositivity of the widal test amongst RT-PCR (Real Time Polymerase Chain Reaction) positive COVID-19 patients.

Methods: A cross-sectional study was done on RT-PCR positive COVID-19 patients at the Department of Microbiology, Guru Teg Bahadur Hospital, a designated COVID-19 hospital in Delhi, India between March 2020 to January 2021 and April 2021 to July 2021.

Results: Out of 136 samples received for widal testing, 58 patients showed evidence of recent infection (significant TO titre ≥ 128) and 32 patients (significant titre TH ≥ 256) may have been in the convalescent stage of enteric fever. An increased number of female patients showed widal seropositivity as compared to males. In our study, 50 (36.76%) and 26 (19.11%) patients with significant titre (TO ≥ 128 ; TH ≥ 256) were less than 40 years of age.

Conclusion: Documenting the prevalence of typhoid fever, especially during the COVID-19 pandemic, is very difficult. Serological results like widal tests can be misleading, sometimes; due to the cross reacting antigens and the endemicity. Cases of co-infections or false positivity amidst an ongoing pandemic are likely to get unreported unless the clinicians are mindful of the rare occurrence of co-infections by various pathogens. Our study concludes that careful surveillance and interpretation of widal seropositivity in COVID-19 patients is crucial, especially in endemic settings.

Keywords: COVID-19; False Positive; Pandemic; Widal.

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Introduction

Over the past twenty years, various outbreaks of coronaviruses notably severe acute respiratory syndrome (SARS) and Middle East respiratory syndromes (MERS) have been reported from Central Asia and Middle East Asia in the years 2002 and 2012 respectively. The

present coronavirus, initially called novel coronavirus (nCoV), emerged from the Wuhan district of Hubei Province of China in late December of 2019; and then spread all over the World within a span of few months (1,2). On 31st January 2020, WHO declared this as a Public Health Emergency

of International Concern. On 11th February 2020, the International Committee on Taxonomy of Viruses (ICTV) named the virus as SARS-CoV-2 and on the same day WHO named the disease as “COVID-19”. Subsequently, on 11th March 2020, a pandemic situation was declared (3).

The SARS-CoV-2 affects the parenchyma of lung leading to severe interstitial inflammation expressive of acute respiratory distress syndrome (4). The clinical presentation of COVID-19 infection may vary from an asymptomatic or a paucisymptomatic form to a severe clinical disease characterized by multisystem involvement, ultimately resulting in a multiple organ dysfunction syndrome (4). In humans, CoVs mostly cause respiratory and gastric manifestations. The clinical presentation during a COVID-19 infection may mimic various other diseases especially prevalent in tropical countries. Dengue fever, typhoid fever, paratyphoid fever, malaria, and several other diseases may mimic the coronavirus infection in the febrile phase; that may often lead to difficulty in reaching a definitive clinical diagnosis. It is worth mentioning that these symptoms may not be present every time; hence, a high level of clinical conjecture for SARS-CoV-2 infection is needed. With the current toll of COVID-19 cases, the numbers are increasing and the conditions are getting worse; co-infections will continue to occur and patients will concurrently be infected with other ubiquitous or endemic pathogens.

Recent studies have reported the burden of co-epidemics/co-infections of COVID-19 and various other diseases on already compromised healthcare facilities. Co-infections are not uncommon in the light of acute illnesses being caused by bacteria, viruses, or parasites. During the COVID-19 pandemic, more cases of enteric fever were reported than COVID-19, as per a report that mentions nearly 20000 typhoid patients in June 2020 (5). Illnesses that are convalescing, recent, non-responding to

conventional diagnosis and treatment, deteriorating or atypically presenting; may be associated with coronavirus infection. Misleading serological testing due to cross reactions could further complicate the problem. Since COVID-19 is still evolving; a lot remains to be still known about the immunopathogenesis, that may affect such co existing or co prevalent diseases. WHO reports 11 to 21 million infected from enteric fever worldwide leading to 128 000 to 161 000 deaths every year (6). Enteric fever is a major health issue worldwide, with endemicity in the developing world due to their poor level of sanitation and unhygienic surroundings; the annual incidence being up to more than 100 cases per 100,000 population in endemic settings. An Indian estimate of annual enteric fever incidence rate has been reported at 493.5 cases per 100,000 person-year (7). Furthermore, the newer strains of typhoid are reported resistant, making the treatment more challenging and expensive. 90% of the reported morbidity and mortality among all the cases worldwide are from Asia. The COVID-19 pandemic has had a negative effect on the control of infectious diseases, mainly in the exhausting and limited health-care delivery systems. With a high influx of patients in hospitals during the ongoing COVID-19 pandemic, difficulties in diagnosis and differentiating diseases with almost identical clinical presentation becomes a matter of concern, keeping in mind the possibility of a co-infection or a false positive titre. Due to a lack of literature on interpretation of Widal titers in COVID-19 patients from India, the present study was done and examination of Widal titer among suspected cases during the period when the hospital was a dedicated COVID center was done.

Methods

A cross-sectional study was done to determine the seropositivity by Widal test among COVID-19 patients admitted to a designated COVID-19 treatment facility between March 2020 to January 2021 and

April 2021 to July 2021. The samples were received at the Microbiology Laboratory for suspicion of enteric fever in these patients. Blood samples of all these patients were positive for COVID-19 by RT-PCR.

Inclusion Criteria:

All COVID-19 RT-PCR test positive patients with clinical features suggestive of enteric fever.

Exclusion criteria:

- Patients with dengue, malaria or any other known cause of fever.
- Patients positive for HBsAg or Anti HCV antibody.
- Patients with autoimmune hepatitis, alcoholic hepatitis and drug induced hepatitis.

Blood samples received for Widal testing were allowed to stand at ambient temperature until serum could be separated by centrifuging the samples at 1200 rpm for 15 minutes. Widal was performed by Tube agglutination method using Typhocheck kit (manufactured by Tulip diagnostics pvt. Ltd) on equal volume of serial dilutions of patient serum (1:64 to 1:512).

For any serum sample an antibody titer of $\geq 1:128$ for O and $\geq 1:256$ for H, AH and BH antigens of Salmonella Typhi was considered significant.

Data from all those COVID-19 RT-PCR positive patients admitted in clinical wards and suspected of having enteric fever and prescribed Widal testing by clinicians was collected. On this basis, a sample size 136 patients was taken.

The data thus collected using the study tools was converted into a computer based Excel sheet and analyzed. For ethical considerations, the principles of Declaration of Helsinki were followed at the time of study.

Results

A total of 136 samples from confirmed RT-PCR positive COVID-19 patients were received for widal testing during the study period. Out of these, 87 samples were from females and 49 were from male patients. The male to female ratio was 0.56:1.

The majority of the patients (84 out of 136; 61.76%) in our study were between 11-40 years of age.

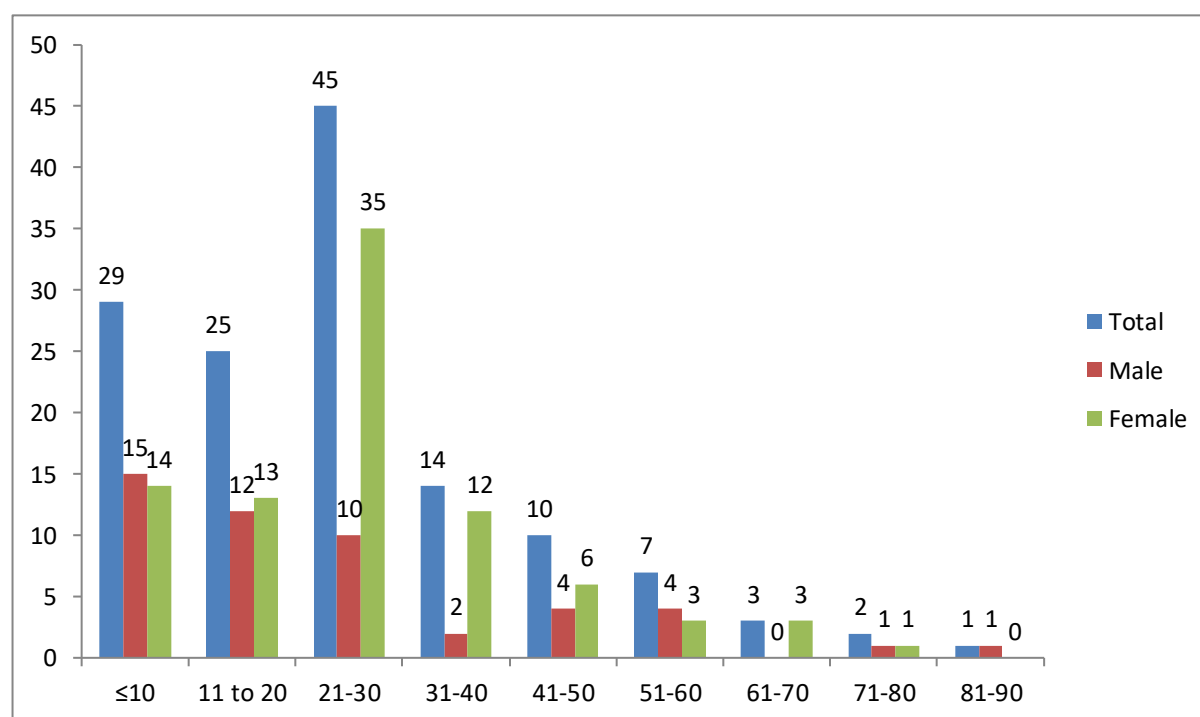


Figure 1. Age and sex distribution of the suspected typhoid cases during the COVID period (n=136)

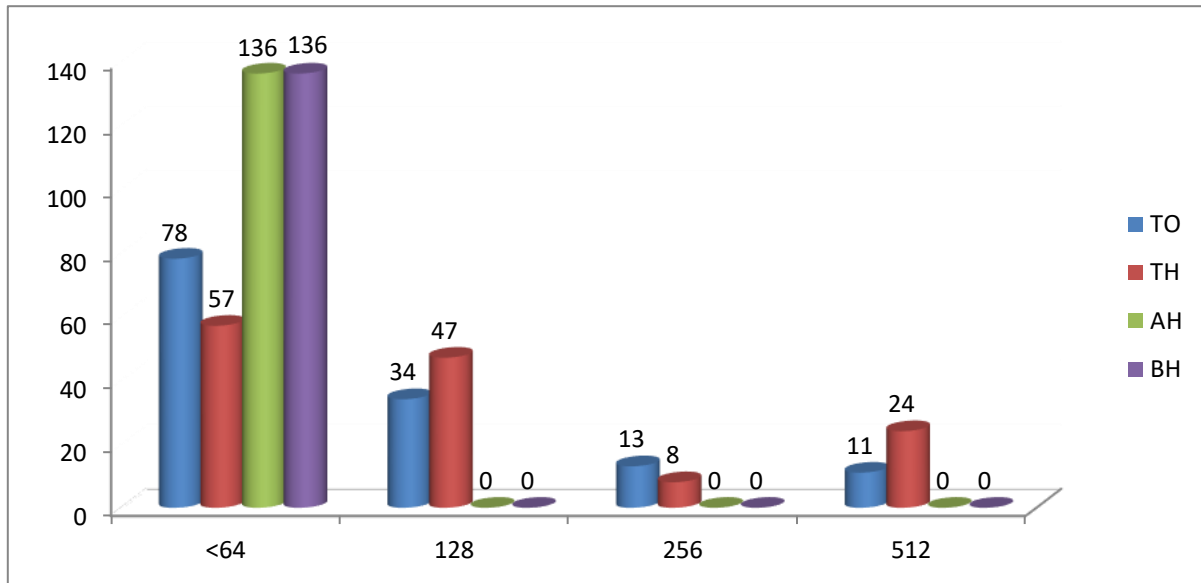


Figure 2. Widal titres among the suspected typhoid cases during the COVID period (n=136)

The age and gender wise distribution of the suspected cases of enteric fever is shown in Figure 1.

The TO and TH titres of 128, 256 & 512 were seen in 34, 13 & 11 and 47, 8 and 24 patients respectively. The titres of AH and BH were less than 64 in all the cases. (Figure 2) Widal test was considered positive for patients with antibody titre $\geq 1:128$ to O antigen and $\geq 1:256$ for H, AH and BH antigens of *Salmonella Typhi*. It

can be deduced from these observations that 58 patients showed evidence of recent infection (TO titre ≥ 128) and 32 patients showed may have been in convalescent stage of the disease at the time of presentation.

The significant titre of more than 128 in case of TO was seen in 34 females and 24 males. Whereas significant titres of more than 256 in case of TH was seen in 15 females and 17 males. (Figures 3 & 4).

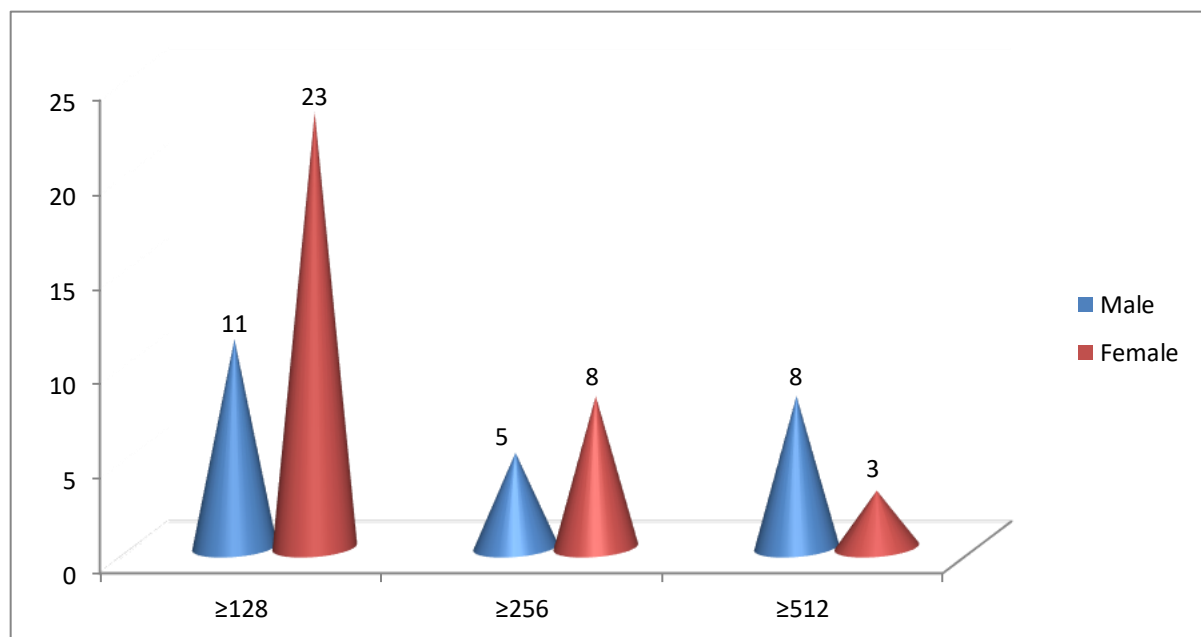


Figure 3. Gender-wise distribution of the TO titre among suspected typhoid cases during the COVID period

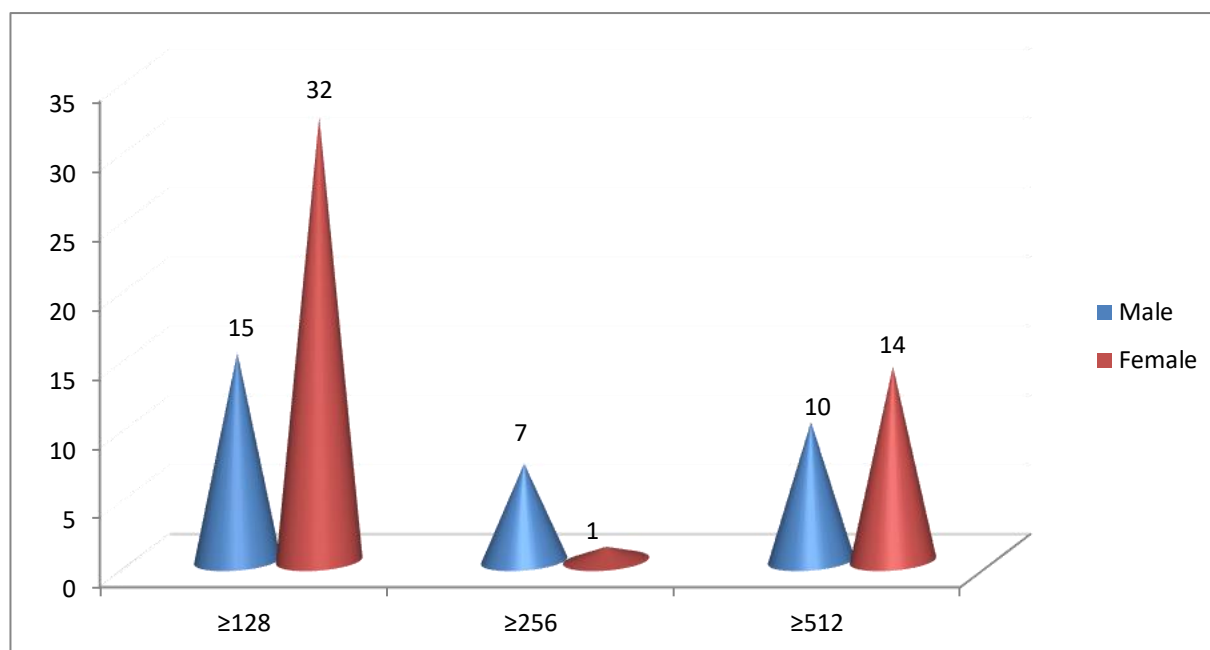


Figure 4. Gender-wise distribution of the TH titre among suspected typhoid cases during the COVID period

Our study points towards the finding that an increased number of female patients showed Widalseropositivity as compared to male patients.

Table 1 shows age wise titres of TO & TH. As can be seen from the table; in case of TO titre, 50 out of 136 patients (36.76%) with significant titre (≥ 128) were less than 40 years of age while 26 out of 136 (19.11%) patients with significant titre (≥ 256) were less than 40 years of age in case of TH titre.

Table 1. TO & TH titres among various age groups of the study population

Age Group (Yrs)	TO titre			TH titre		
	≥ 128	≥ 256	≥ 512	≥ 128	≥ 256	≥ 512
<20	15	9	6	20	3	14
21-40	16	1	3	19	3	6
41-60	2	0	0	5	2	1
>60	1	3	2	5	0	3

We tried to collect baseline widal data during the immediate previous non covid period. Figure 5 shows TO, TH, AH and BH titres of 632 Widal seropositive patients during the non-COVID period from March to December 2019. The TO, TH, AH, BH

titres of 128, 256 and 512 were seen in 265, 239, 128 patients; in 177, 289, 166 patients; in 7, 9, 4 patients and in 3, 1 patients. Widal test was considered positive for patients with antibody titre $\geq 1:128$ for O antigen and $\geq 1:256$ for H, AH and BH antigens of *S. Typhi*. There was a high widalseropositivity during non-COVID interval and this positivity was mostly seasonal in nature with cases declining towards the end of the year.

Figure. 6 shows a comparison between the antibody titres to TO, TH, AH, BH antigens during non-COVID and COVID periods. As can be seen from the figure, 632 (26.74%) patients had titres more than 128 in case of TO antigen, 455 (19.25%) patients had titres more than 256 in case of TH antigen, 13 (0.55%) patients had titres more than 256 in case of AH antigen and only 01 (0.04%) patient had titres more than 256 in case of BH antigen during the non-COVID interval out of a total 2363 samples that were tested (March-December 2019). When comparing with titres during COVID interval (between March 2020 to January 2021 and April 2021 to July 2021), a total of 136 samples were tested, out of which 58 (42.64%) patients had titres more than 256 in case of

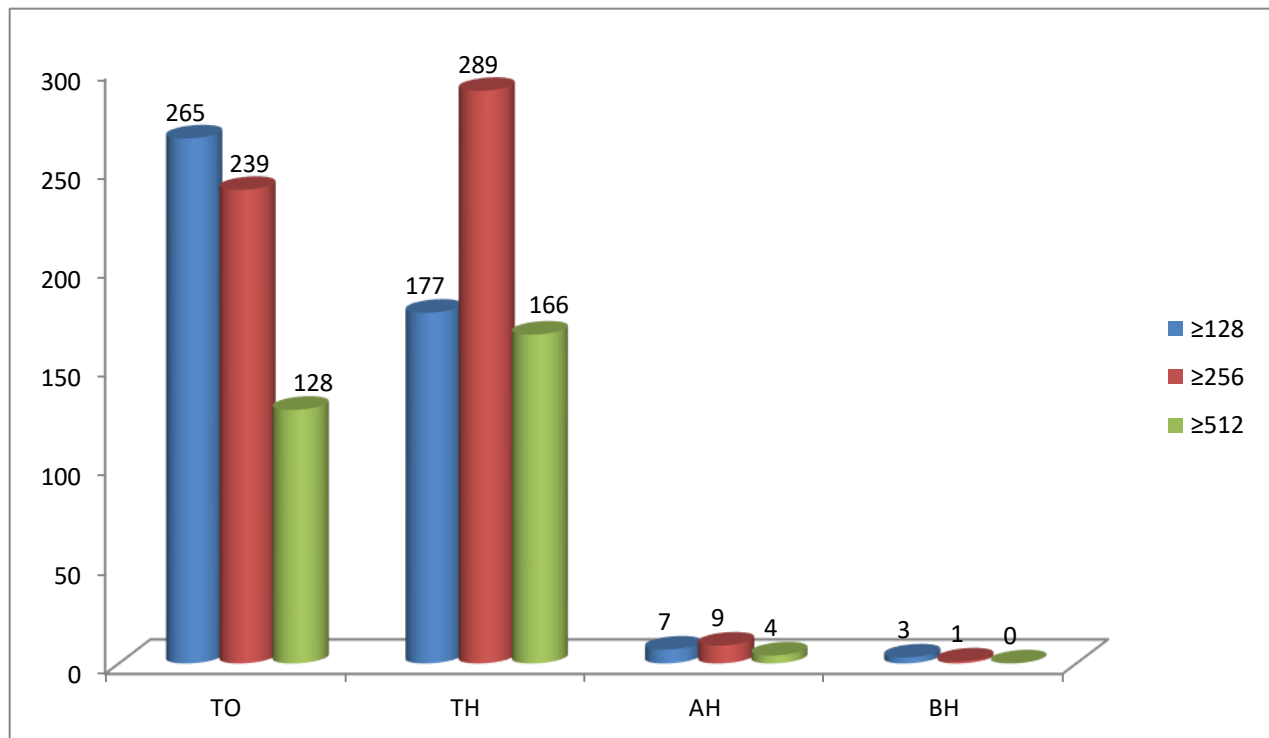


Figure 5. TO, TH, AH & BH titres of Widal positive patients during March to December 2019 (non-COVID period) (n= 632)

TH antigen; though no significant rise in antibody titre to AH and BH antigens was observed. It can be deduced from the figure

6 that there was increased Widal positivity during COVID interval as compared to non-COVID interval.

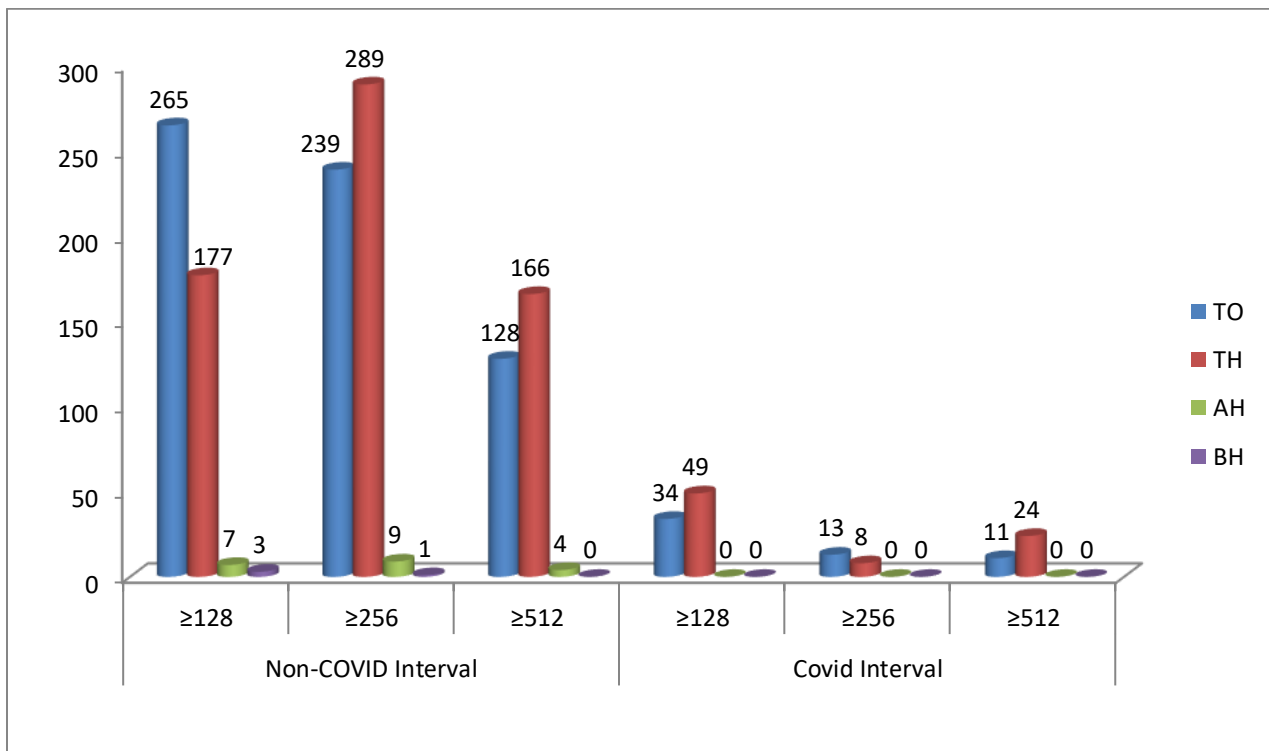


Figure 6. Comparison between antibody titres to TO, TH, AH , BH antigen during non-COVID and COVID period

Discussion

Documenting the prevalence of typhoid fever, especially during the pandemic, has been difficult due to the absence of a nation-wide surveillance system and limited infrastructure. Underreporting of the same may be possible due to several other factors including administration of antibiotics before confirmation of the diagnosis, or lower frequency of patients seeking health care or using lab services for the same. Moreover, there has been negligence in our response against several pre-existing prevalent infections due to the diversion of the already limited infrastructure and efforts towards our fight against COVID-19. The clinical mimicry of typhoid with the current pandemic further challenges the situation. The resulting delay in the diagnosis and management has been reported to be worsening the outcomes in both the diseases (8). Antimicrobial resistance to anti typhoidal drugs, which was already on a rise, has further increased due to the use of few such antibiotics in the treatment of COVID-19. The COVID-19 pandemic has provided us an opportunity to learn to adequately respond to both the diseases and improve the prevention of such a situation by understanding the epidemiology, increasing surveillance, detecting high risk areas or factors and bringing about awareness.

A recent study from Bangladesh on various co-infections in COVID-19 among children, reported 12 of 30 co-infections to be positive for typhoid that amounted to around 40% rate of COVID-19 and typhoid co-infection; which was reported predominant among various other infections (9). Pakistan has also recorded higher rates of co-infections of typhoid with COVID-19, reporting around 48% positivity for typhoid from a high burden COVID province. These were increasingly reported in the 21 to 40 years age group and among males (5).

A total of 136 samples from confirmed COVID-19 patients were sent for widal

testing during the period for which our hospital was a designated COVID center. Significant titers of more than 128 and more than 256 in case of TO and TH were reported in 58 and 32 cases respectively. Unless a detailed continuous follow up is done for such patients, it will be very difficult to conclude if all of these were actual co-infections or were cases of false positive titers in the context of typhoid. A recent Indian retrospective analysis emphasizes on the importance of recognizing false positivity serology titres in COVID-19 patients for typhoid. The study, which retrospectively analyzed 104 COVID-19 patients confirmed by RT-PCR and classical CT scan changes, reported a series of 6 positive cases of typhoid that were later confirmed to have COVID-19. All cases were negative on blood culture, though. Serological results can be misleading, sometimes; due to the cross reacting antigens and the endemicity for such infections in our setting (10). The same study concludes that a careful surveillance and interpretation with caution along with a diligent follow up is crucial, especially in endemic settings.

Such cases of co-infections or false positivity amidst an ongoing pandemic are likely to get unreported unless the clinicians are mindful of the rare occurrence of co-infections by serious pathogens and maintain a broad differential, when the epidemiology too is suggestive. A recent report of Enteric fever and COVID-19 co-infection from a teenager does suggest that such concurrent infections had more biochemical abnormalities than typical with either of enteric fever or COVID-19 infection individually (11).

Though India has been navigating the waves of COVID-19, one matter of concern appears to be the fact that the potential overlap of several endemic infections (including typhoid) has not been widely addressed to. Also adds to the complexity around this, is the continued evolution of

COVID-19 disease, due to which a lot remains still unknown of the pathogenesis and its interaction with other diseases. Due to the rapid transmission of this ongoing global pandemic, more than millions of human infections have been lab confirmed with few hundred thousand deaths. This unprecedented crisis has resulted into development of several immune based rapid tests targeting a diverse range of antigens for the virus. There should be an equal and parallel emphasis on the development of interventions that can adequately address the infections that are co- prevalent in different settings, so that the patients can be managed in the best possible way. This manuscript is an attempt to apprise the readers of the noticeable existence of co-infections during these times, that tend to be usually ignored and falsely reported.

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Authors' contribution

Dr Bineeta Kashyap Developed the study concept, design and revision of the manuscript. Dr Nadeem Ahmad participated in Drafting, writing of article and data interpretation. Dr Rajat Jhamb participated in Data analysis and revision. Dr Kapil Singh participated in the Collection of data.

Ethical considerations

For ethical considerations, the principles of the Declaration of Helsinki were followed at the time of study.

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Conflicts of interest

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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