Prevalence of Co-Infection with Salmonella Typhi and Malaria Parasites in Tertiary Care Centre Kanpur

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Abstract:

Background: Malaria and typhoid fever often present with mimicking symptoms especially in the early stages of typhoid On the other hand, typhoid fever is also a major public health problem in India. It is an acute systemic infection caused by the bacterium Salmonella Typhi exaggerates the situation. Thus the aim of this work was to investigate the rate of co-infection with respect to the use of widal test and blood culture methods for diagnosing typhoid fever in Kanpur.

Methods: This study was conducted in Rama Medical College, Hospital & Research Centre, and Kanpur. It is a retrospective study conducted from July 2021 to December 2021 A total of 654 blood samples were collected (5ml of blood drawn by vene puncture) from each febrile patients, both OPD and IPD, who were tested for widal test and Malaria card test for malaria parasite detection. Patients were explained about the study and their consent was taken.

Results: The results of this study are based on bacteriological and serological tests for the diagnosis of typhoid fever parasitological examination for malaria parasites and in 126 patients.

The total 654 sample where 126 samples were positive for typhoid by the widal test and 27 (21.4%) (Fig:-2) samples were positive for malaria parasites by card, only 6 by the culture method. The rate of co-infection was significantly high when typhoid was diagnosed by widal (19.26%) than by blood culture method (4.7%). The patients comprised 78 (61.9%) females and 48 (38%) males (fig:-1) aged between 14 to 65 years (mean = 41 years) Malaria parasites were found in 27 (21.4%) samples henceforth known as malaria patients.

Conclusion: The incidence of typhoid and malaria co-infection will greatly reduce if the diagnosis of typhoid fevers in malaria endemic area.

Key words: Typhoid fever, Malaria, co-infection.

Introduction

The treatment of malaria and typhoid co-infection is a common phenomenon in many parts of Africa.[1] Malaria and typhoid remain a treat to many people in Sub Saharan Africa for several reasons: the increasing poverty, deterioration in public health services, compounded by HIV / AIDS and increasing resistance of malaria parasites to chloro quine [2] the lack of portable water and widespread misuse of the Widal agglutination test for diagnosing typhoid fever, [3.4] increased requests for Widal tests as a means of making quick money by private laboratories are other factors[5]. Malaria and typhoid fever often present with mimicking symptoms especially in the early stages of typhoid [6]

On the other hand, typhoid fever is also a major public health problem in India. It is an acute systemic infection caused by the bacterium Salmonella Typhi. Although the two infections are caused by very different agents and transmitted via different mechanisms, both diseases share rather similar symptoms like fever, headache and spleenomegaly. The severity of the two diseases is Compounded by increasing drug resistance of the two a etiological agents [7].An association between malaria and typhoid fever was first described in the medical literature in the middle of the 19th century, and was named typho-malarial fever by the United States Army [8]. Within the last few decades an unusually high number of illnesses have been diagnosed as malaria co-existing with typhoid fever. Both typhoid and malaria share social circumstances which are imperative to their transmission. Therefore, a person living in such an environment is at risk of contracting both these diseases, either concurrently or an acute infection superimposed on a chronic one.

Malaria and typhoid fever are among the most endemic diseases in the tropics. Both diseases have been associated with increasing poverty, deterioration in sanitation, poor public health services, compounded by increasing drug resistance of the two a etiological agents. Although the two infections are caused by very different agents and transmitted via different mechanisms, both diseases share rather similar symptoms [9-14]. This presents a challenge of diagnostic error. Definitive laboratory-based diagnosis is, thus, required to differentiate the two infections as well as detect co-infections.

A reliable diagnosis of typhoid is based on culture of blood, stool and bone marrow. [15] Bone marrow aspiration has technical difficulties and stool cultures are positive in most patients only in the third week of

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infection. This leaves blood culture as the best method for diagnosing early Salmonella typhi infections in the absence of other alternatives. The aim of this study was to determine the rate of co-infection with malaria parasites and S. typhi in patients with respect to the use of a single Widal test and blood culture methods for the diagnosis of typhoid fever in tertiary care centre Kanpur

Material and Methods

This study was conducted in Rama Medical College, Hospital & Reasearch Centre, Kanpur. It is a retrospective study conducted from July 2021 to December 2021 A total of 654 blood samples were collected (5ml of blood drawn by venepuncture) from each febrile patients, both OPD and IPD, who were tested for Widal test and Malaria card test for malaria parasite detection. Patients were explained about the study and their consent was taken.

Widal test- The widal agglutination test was performed on all blood samples by rapid slide agglutination method using commercial antigen suspension (Span diagnostic kit) for the somatic O and flagellar H antigen. Titres with TH>1:320; TO>1:320 were considered significant in widal test.

Malaria card test- Malaria card test was performed and both antibody (by J Mitra Antibody detection card test) and antigen (SD bioline Antigen detecting card test) were detected for each sample. These tests were performed according to manufacturer's instructions. The antigen detection card detects HRP-II (Histidine-rich protein II) specific to P. falciparum and pLDH (Plasmodium lactate dehydrogenate) pan specific to P. species in human blood sample [7]. Malaria antibody detecting card test detected all isotopes of antibody against the same antigens.

Patients found to be positive by any of the tests i.e., widal or typhidot or any malaria card test positive (antigen or antibody) tests were considered suffering from co-infection and were further tested for isolation of S. Typhi or Paratyphi A and B by bacteriological culture of blood and stool specimen and for confirmation of malaria a peripheral blood smear stained by Leishman's stain was prepared.

Bacteriological blood culture- A minimum of 10 ml of blood was ascetically introduced into Hi media blood culture bottle containing 70 ml of glucose broth from individuals found to be suffering from both malaria and typhoid fever by above rapid diagnostic tests. All blood culture bottles were incubated at 37°C for an initial period of 24 hrs and sub-cultured on MacConkey agar after 24 hrs,72 hrs and finally at 7th day. S.Typhi / S. Paratyphi A and B organisms were identified on the basis of standard cultural, microscopic and biochemical characterization. Inoculated blood culture media was discarded as negative if there was no growth after 7 days.

Selection of RT-PCR Kit

For the present study, TruPCR SARS-CoV-2 RT-qPCR Kit (Kilpest India Ltd., India) were selected on the basis of multiple SARS-CoV-2 specific gene targets in a single tube with simultaneous detection of each target on different detection channel. This kit was targets Envelope gene (E) and RNA dependent RNA polymerase (RdRp) and Nucleocapsid (N) genes of SARS-CoV-2, was used for SARS-CoV-2 RNA detection according to the manufacturer's instructions. A Cycle threshold (Ct) value of less than 35 was reported as positive The CFX-96 real-time thermal cycler (Bio-Rad Laboratories, USA) was used for amplification.

Sources of Sample

Respiratory samples, mainly nasopharyngeal and throat swabs were collected from 100 suspected COVID-19 cases, at Rama Medical College Hospital, & University, U.P, India, from January to December 2021. Samples were mixed in 2 ml of viral transport media (VTM), consisting of Hanks' balanced salt, 0.4% fetal bovine serum, HEPES, antibiotic and antifungal agents. Samples were transported at 2–8 °C to the Microbiology laboratory, Rama Hospital, for processing within a few hours. All specimens were processed in Bio safety level-3 (BSL-3) and Bio safety level-2 enhanced (BSL-2 +) facilities with full personal protective equipment.

Statistical Analysis

The numbers of positive samples were compared using two by two contingency table. The agreement between the antigen test and rRT-PCR techniques was evaluated using the Cohen's weighted kappa index (K value) [18]. Socio-demographic and clinical profile was described using percentages and mean. Sensitivity, Specificity, PPV and NPV of RAT was calculated using relevant formulas by keeping qRT-PCR as a gold standard. Cohen's Kappa was calculated. [19].

Result

Rapid antigen test sensitivity and specificity were evaluated by institutions using total 100 number of respiratory samples. Certain rapid tests may be used at the point-of-care and thus offer benefits for the detection and management of infectious diseases. In order to assess the potential of the rapid antigen test in this context, 100 respiratory samples collected from individuals living in a shared housing were analyzed head to head by Rapid antigen test and RT-PCR using CFX-96 real-time thermal cycler. Out of 100, 50 negative samples by RAT, 21 (10.5%) of the samples were found positive by SARS-CoV-2 by rRT-PCR with cT values ranging between 17.32–32.91 and 50 positive samples by RAT, they were all 50 (100%) samples found positive by SARS-CoV-2 by rRT-PCR with cT values ranging between 16.62–33.91 The antigen test

diagnosed the infection status with a sensitivity of 79.0 % (79/100) and a specificity of 100 %. Cohen's weighted kappa value of 0.511 indicated moderate agreement between rRT-PCR and the rapid antigen test. The overall concordance between the rRT-PCR and the antigen test was 21.0 % (79/100).

[Table/Fig-1]: Comparison of RT-PCR and Rapid Antigen Test (RAT).

Rapid Antigen Test (Rat) Result	RT-PCR Test		Total
	Positive	Negative	
Positive	50	0	50
Negative	21	29	50
Total	71	29	100

[Table/Fig-2]: Socio-demographic and clinical profile of study subjects

Variables	N - %
Gender	
Male	64%
Female	36%
Age (in years)	
18 to 40	41%
41-60	45%
≥61	14%
Residence	
Urban	38%
Rural	42%
Symptomatic at testing	
Yes	100%
No	0
Type of symptoms in those symptomatic cases	
Fever	68%
Cough	71%
Sore throat	69%
Myalgia	33%
Diarrhea	11%
Anosmia	5%
Primary reason for testing	
Severe Acute Respiratory Infection (SARI)	33%
Symptomatic Influenza-Like Illness (ILI)	24%
High risk contact	22%
Low-risk contact	16%
Voluntary testing	3%
Surgical clearance	2%
Past history of COVID-19	
No	100%
Yes	0
Pre-existing medical conditions	
Hypertension	56%
Diabetes	39%
Chronic lung diseases	21%
Chronic Kidney diseases	9%
Malignancies	7%
Others*	8%

[Table/Fig-3]: Statistics for Rapid Antigen Test (RAT) in comparison with RT-PCR.

Statistic	Value
Sensitivity	77.00%
Specificity	100%
Positive Predictive Value (PPV) (*)	100%
Negative Predictive Value (NPV) (*)	58%

Discussion

S.No.	Study	Year	Results
1	**E. Albert et al[20]**	2021	Between 2nd September and 7th October 2020 this prospective study enrolled 412 patients with clinical suspicion of COVID-19 of whom 327 were and 85 children, attending primary care centers of the Clínico-Malvarrosa Health Department in Valencia (Spain).
2	**Seema Aleem et al[21]**	2022	A cross-sectional study was conducted by Government Medical College, Srinagar; The sample size was estimated at 359. A total of 473 were included in the study.
3	**In the present study**	2022	This study was be conducted in the Department of Microbiology Rama Medical College Hospital and Research Centre Kanpur. Total 100 known samples, Out from 50 positive by RAT patient and 50 negative patients already tested by RAT kit for Covid -19 test.
4	**Seema Aleem et al[21]**	2022	A total of 473 subjects were included in the final analysis. The selection of study subjects in depicted in [Table/Fig-2]. The subjects comprised of 277 (58.6%) males and 196 (41.4) females. The mean age of subjects was 38.4±12.2 years and 57.29% of subjects belonged to urban areas. A total of 124 subjects (26.2%) had any symptom at the time of testing. The most common presenting symptom was fever reported by 71 subjects (15.01%). Loss of smell was reported by seven (1.5%) subjects. A 13% of subjects had a previous history of COVID-19. The primary reason for testing included a positive contact history 221(47%) subjects, symptoms 124(26%) and voluntary testing 116 (24.5%). A total of 1/5th of subjects had any concomitant co-morbidity.
5	**In the present study**	2022	A total of 100 subjects were included in the final analysis. The subjects comprised of 64 males and 36 females. The mean age of subjects was 38 years and 38 of subjects belonged to urban areas. A total 100 subjects had any symptom at the time of testing. The most common presenting symptom was cough reported by 71 subjects and fever was reported by 68 subjects. None of any subjects had a previous history of COVID-19. The primary reason for testing included a Severe Acute Respiratory Infection (SARI) 33% subjects and Symptomatic Influenza-Like Illness (ILI) 24%.
6	**Chutikarn Chaimayoet al22**	2020	The results were interpreted as positive when both control (C) and SARS-CoV-2 antigen (T) lines appeared within 30 min, as shown in Fig. 1. Comparing SARSCoV-2 antigen detection to RNA detection by RT-PCR assay, the sensitivity and specificity of rapid SARS-CoV-2 antigen detection to identify COVID-19 were 98.33% (59/60; 95%CI, 91.06–99.96%) and 98.73% (389/394; 95%CI, 97.06–99.59%), respectively,
7	**Seema Aleem et al[21]**	2022	The present study estimated the sensitivity and specificity of RAT to be 54.43% (42.83% to 65.69%) and 99.24 (97.79% to99.84%), respectively. The overall accuracy was estimated at 91.75%..
8	**In the present study**	2022	The results were interpreted as positive when both control (C) and SARS-CoV-2 antigen (T) lines appeared within 30 min. Comparing SARSCoV- 2 antigen detection to RNA detection by RT-PCR assay, the sensitivity, specificity PPV and NPV of rapid SARS-CoV-2 antigen detection to identify COVID-19 were 77.%, 100%,100% and 58% respectively,

Conclusion

In view of this significant difference and in order to role out any case of malaria with mimicking Symptoms, or the influence of anamnesis response the practical use of cultural methods for the diagnosis of typhoid fever should be emphasized in our clinical laboratories. This will also improve patient management by cutting down cost of treatment and eliminate other risks associated with misuse of antibiotics.

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