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Evaluation of clinical profile and response of various antibiotics in typhoid fever

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ABSTRACT:

Typhoid Fever is also known as Enteric fever caused by *Salmonella Enterica Serovar typhi* (*S. typhi*) gram-negative bacteria. In this study sample of 413 patients diagnosed with typhoid fever were taken for evaluation of clinical profile and response of various antibiotics in the treatment of typhoid fever. Our results showed that in 263 normal individuals, C-reactive protein was below 5 mg/dl. In 168 typhoid positive samples the C-reactive protein was found to be higher than 6 mg/dl whereas in 245 typhoid positive samples the C-reactive protein was found to be less than 6 mg/dl. The various biochemical tests that can help identify the bacteria were used. The results showed that catalase positive, oxidase negative, Methyl red positive, nitrate reduction positive, indole negative, urease negative, glucose positive, citrate negative, Voges Proskauer negative gelatin hydrolysis negative and positive for hydrogen sulfide. The antibiotics such as ceftriaxone + salbactum, ciproflaxin and ofloxacin were sensitive to *S. typhi.*

Keywords: *Salmonella enterica*, Typhoid fever, lipopolysaccharide, enterocolitis, diarrhea, bacteremia.

1. Introduction

Gram-negative bacterium *Salmonella typhi* (*S. typhi*) which causing the typhoid fever. The typhoid fever is a preventable, communicable disease and is also a major concern to worldwide public health. In spite of vaccinations, estimates for the year 2000 suggested that there were over 2.16 million episodes of typhoid occurrences global resulting in 216,000 deaths with more than 90% of morbidity and mortality from Asia. [1] Due to the insufficient water supply and indecorous sanitation typhoid fever occurs across the globe. In India, it is endemic with morbidity ranging from 102 to 2219 per 100,000 residents. [2] Due to its changing modes of presentation, as well as the development of multidrug resistance, typhoid fever is fetching increasingly challenge to detect and treat. Advancement in improved in ethics of public health have resulted in a marked decline in the prevalence of typhoid fever in developed countries. [3] In various emerging nations, Widal test is generally used in the diagnosis of Typhoid fever. This is because it is relatively affordable, easy to perform and requires least training and low sophisticated equipment. [4] Till 1948, the gold standard antimicrobial agent for the treatment and management of typhoid was whispered to be chloramphenicol. [5] But, in the last two decades, the resistance of strains of *S. typhi* to chloramphenicol has increased. In endemic areas such as India, book picture of signs and symptoms in enteric fever are not often seen. This may be owing to the widespread and unselective use of antimicrobials and antipyretics, which also contributes to the development of some unusual or atypical presentations of enteric fever and antibiotic resistance in the country. The first line treatment for the typhoid fever is managed and accomplished by using many drugs such as chloramphenicol, ampicillin and co-trimoxazole have become infrequent and quinolones. Since last few there has been substantial increase in the effervescence period in

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patients treated with quinolones. For the treatment of multiple drug resistance case of S.*typhi* the quinolone group of drugs developed as useful drug. But inappropriately, the same factors of indiscriminate antibiotic use and cross resistance within the antibiotic group which led to the appearance of chloramphenicol resistant organisms are still operative. Altered DNA gyrase subunit is accountable for the resistance to quinolone, but it is not plasmid coded. Resistance to ciprofloxacin is now being stated both from the Indian subcontinent and West. [6,7] The aim of this study undertaken is to evaluate the various clinical profile and antibiotic response in typhoid fever.

2. Materials and methods

Hematological profiling

Various hematological parameters in the blood sample were analyzed for parameters like red blood cells (RBC), Hemoglobin concentration (Hb), Total white blood cells (WBC), platelet count (PLT), Lymphocytes, Monocytes etc., values were determined based on the previous study methods. [8,9]

C-Reactive protein (CRP) estimation

The quantification of C-reactive protein (CRP) by latex-enhanced nephelometry based on the latex agglutination was carried (Apsen Laboratories, India). Latex particles complexes human anti-CRP are mixed with a patient's serum containing C reactive proteins, a visible agglutination reaction was observed within 2 minutes. [10]

Identification of S. typhi:

The standard microbiological tests such as Gram's staining followed by Morphological characterization and biochemical characterization tests such as Indole test, Methyl red, Voges Proskauer, Citrate utilization test, Urease test, Catalase test (Slide Test), Oxidase test, Gelatin hydrolysis, Hydrogen sulphide, Glucose test (The Kirby-Bauer Disc Method and The Minimum Inhibitory Concentration (MIC) Method) and Motility test were performed to identify the organism. [11]

3. Results

Hematological profiling

Typhoid fever causes significant hematological complications. Despite the high incidence and serious nature of the hematological changes, these changes are transient and helps in antimicrobial treatment. Out of 413 typhoid positive patients, our results showed that 58 (14.04%) were found to have leukopenia, 47 patients (11.38%) showed anemia (Low Hemoglobin), monocytosis in 63 patients (15.25%), lymphocytosis was found in 42 patients (10.16%), lymphopenia was found in 25 patients (6.05%), neutropenia was found in 49 patients (11.86%), Neutrophilia was found in 27 patients (6.53%), thrombocytopenia was found in 20 patients (4.84%), erythrocytes sedimentation rate was found in 69 patients (16.70%) and pancytopenia was observed in 13 patients (3.14%) (Table.1).

Table 1: Hematological parameters estimation

S. No.	Parameters	Number	Percentage
1	Leukopenia	58	14.04
2	Monocytosis	63	15.25
3	Lymphocytosis	42	10.16
4	Lymphopenia	25	6.05
5	Neutropenia	49	11.86
6	Neutrophilia	27	6.53
7	Anemia	47	11.38
8	Thrombocytopenia	20	4.84
9	Erythrocytes Sedimentation rate (ESR)	69	16.70
10	Pancytopenia	13	3.14

C-Reactive protein (CRP) estimation

The results showed that in 263 normal individuals, C-reactive protein was below 5 mg/dl. In 168 typhoid positive samples the C-reactive protein was found to be higher than 6 mg/dl whereas in 245 typhoid positive samples the C-reactive protein was found to be less than 6 mg/dl (Figure. 1).

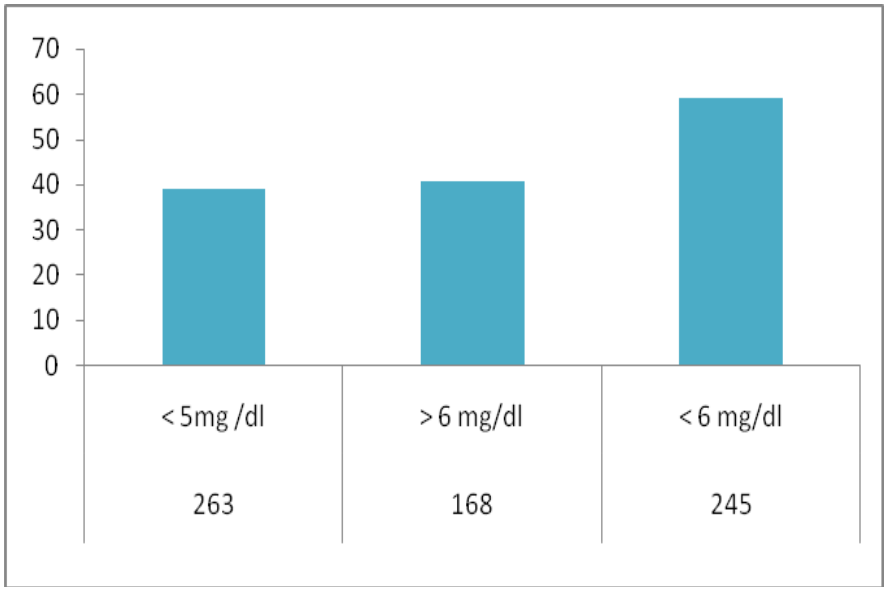

Fig. 1: C-reactive protein

Estimation of Biochemical parameters

The various biochemical tests that can help identify the bacteria were used. The results showed that catalase positive, oxidase negative, Methyl red positive, nitrate reduction positive, indole negative, urease negative, glucose positive, citrate negative, Voges Proskauer negative gelatin hydrolysis negative and positive for hydrogen sulfide (Table. 2).

Table 2: Biochemical tests for *S. typhi*

S. No.	Biochemical test	Result
1	Catalase	Positive
2	Oxidase	Negative
3	Methyl red	Positive
4	Nitrate reduction	Positive
5	Indole	Negative
6	Urease	Negative
7	Citrate	Negative
9	Gelatin hydrolysis	Negative
10	Hydrogen sulphide	Positive
11	Motility	Motile
12	Voges Proskauer	Negative
13	Glucose	Positive
14	Gram staining	Negative

Antibiotic sensitivity

The result of the *in-vitro* antibiotic sensitivity test showed that isolates of *S. typhi* were resistant to antibiotics like ampicillin, amoxicillin, tetracycline, cotrimoxazole, gentamycin nalidixic acid. The intermediate antibiotics were azithromycin and cepodoxime whereas antibiotics such as chloramphenicol, ceftrioxne + salbactum, ciproflaxin and ofloxacin were sensitive to *S. typhi*. In the present study, MDRP shows that ampicillin, cotrimazole, gentamycin, tetracycline, azithromycin was resistant whereas ceftriaxone with salbactum, chloramphenicol, were 100% sensitive and ciproflaxin and cepodoxime were also sensitive against *S. typhi* (Table. 3).

Table 3: Drug sensitivity pattern of *S. typhi* against various antibiotics. (Where, S=Sensitivity, I=Intermediate, R=Resistant)

S. No.	Antibiotics	Zone of inhibition (mm)	Drug Sensitivity	MIC (µg/ml)
1	Ampicillin	0	R	-
2	Chloramphenicol	10.2	R	-
3	Cotrimazole	0	R	-
4	Gentamycin	0	R	-
5	Tetracycline	0	R	-
6	Azithromycin	11.9	I	0.03125
7	Ceftriaxone + Salbactum	17.5	S	0.5
8	Ciproflaxin	14.8	S	0.125
9	Cepodoxime	12.6	I	0.0625
10	Nalidixic acid	0	R	-
11	Amoxicillin	2.5	R	-
12	Ofloxacin	15.3	S	0.25

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) assay was performed for the sensitive and intermediate antibiotics for *S.typhi*. The results showed that azithromycin shows MIC value of 0.03125, ceftriaxone + salbactum with MIC 0.5, ciproflaxin with MIC 0.125, cepodoxime with MIC 0.0625 and ofloxacin with MIC value of 0.25 µg/ml concentration respectively.

4. Discussion

The most common hematological condition detected in typhoid positive patients were leukopenia, monocytosis, lymphocytosis, lymphopenia, neutropenia, neutrophilia, anemia, elevated erythrocytes sedimentation rate (ESR), thrombocytopenia and pancytopenia. The outcomes revealed that hematological parameters such as leukocytes decreases in typhoid positive patients however it increases in typhoid positive patients. The hematological manifestation supports in monitoring the course, diagnosis and treatment of typhoid. leucopenia was found also with anemia or as part of pancytopenia.

Neutropenia in typhoid fever has been recognized to increased margination and defective granulopoeisis. [12] It is distinguishing that tissue invasion by micro-organism is escorted by substantial stimulation of neutrophils granunopoesis which account for increase in neutrophils counts in the blood. [13] Firkin et al., stated that subject with sub-acute or chronic infection which showed that production of neutrophils increases up to 12 times the mean rate in healthy individuals. [13]

For carbohydrate fermentation tests, triple sugar iron agar (TSIA) slant reaction, methyl red-Voges-Proskauer (MR-VP) and Indole reaction tests were carried out for identification of suspected Salmonella according to the methods described by Douglas et al. [14] and OIE [15]. The carbohydrate fermentation test was performed by

inoculating a loop full of NB culture of the organisms into the tubes containing different sugar media. Acid production was indicated by the color change reddish to yellow in the medium and the gas production was noted by the appearance of gas bubbles in the inverted Durham's tube. The TSIA slant was used to identify the lactose fermenters and the saccharose and dextrose fermenters. The medium also helped to regulate the ability of the organisms to produce H_2S. Pinkish slant and yellow butt or black slant and yellow butt were documented as the positive reaction for *Salmonella*.

Since pancytopenia is an alarming finding and occasionally measured synonymous with aplastic bone marrow, typhoid patients might give an aplastic like picture but it is transient. Pancytopenia was found in 13% of the patients in our study, as has been reported by others as severe pancytopenia and bone marrow hemophagocytosis. [16] The outcomes showed that in 263 normal individuals had 38.9% of C-reactive protein below 5 mg/dl. Whereas 168 typhoid positive samples had 40.67% of C-reactive protein higher than 6 mg/dl and in 245 typhoid positive samples had 59.32% of C-reactive protein below 6 mg/dl. C-reactive protein (CRP) is an acute-phase protein with increased concentrations in the blood during infections [17]. Bacterial or inflammatory diseases increase the level of CRP. These inflammatory conditions root the release of interleukin-6 and other cytokines that activate the synthesis of CRP and fibrinogen by the liver. [18-19]

The results of various biochemical tests showed *that S. typhi* were catalase positive, oxidase negative, Methyl red positive, nitrate reduction positive, indole negative, urease negative, glucose positive, citrate negative, Voges Proskauer negative gelatin hydrolysis negative and positive for hydrogen sulfide.

5. Conclusion

The study of 413 patients sample diagnosed with typhoid fever were taken for evaluation of clinical profile and response of various antibiotics in the treatment of typhoid fever revealed that out of 413 typhoid positive patients, our results showed that (14.04%) were found to have leukopenia, patients (11.38%) showed anemia (Low Hemoglobin), monocytosis in patients (15.25%), lymphocytosis was found in patients (10.16%), lymphopenia was found in patients (6.05%), neutropenia was found in patients (11.86%), Neutrophilia was found in patients (6.53%), thrombocytopenia was found in patients (4.84%), erythrocytes sedimentation rate was found in patients (16.70%) and pancytopenia was observed in patients (3.14%). Our results showed that in 263 normal individuals, C-reactive protein was below 5 mg/dl. In 168 typhoid positive samples the C-reactive protein was found to be higher than 6 mg/dl whereas in 245 typhoid positive samples the C-reactive protein was found to be less than 6 mg/dl.

6. Acknowledgment

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