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Original Article

Impact of Widal Test Interference on Hemoglobin Levels in Typhoid Fever

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ABSTRACT

Background: *Salmonella typhi* is the cause of enteric fever and typhoid disease. Due to the lack of clean drinking water and poor hygiene standards, it is most commonly found in underdeveloped countries. The infection's most noticeable symptom is a fever that gradually increases to a high plateau. Typhoid fever is becoming more common and this trend is linked to a number of hematological characteristics. This study investigates the demographic and hematological characteristics of individuals with typhoid fever, aiming to elucidate the complex interplay between age, gender, antigenic response and hemoglobin levels. **Method:** The study involved 150 patients from urban and rural areas, which were analyzed using venous blood and haemoglobin. The blood was extracted, mixed and pipetted onto a labeled filter paper. The absorption was read at 540 nm wavelength. The Widal test procedure involved bringing reagents and samples to room temperature and the slide screen and semi-quantitative method. **Results:** Out of 150 individuals, with 51% males and 49% females. Males had higher average hemoglobin levels, with the O-antigen group having the highest affected individuals. Females had slightly lower mean hemoglobin concentrations. Age and gender significantly influence hemoglobin levels, with a moderate positive correlation. **Conclusion:** These findings provide valuable insights into the pathophysiology of typhoid fever and highlight the need for tailored interventions that account for demographic and hematological diversity to improve patient outcomes.

Keyword: *Salmonella enteric*, Cyanmethemoglobin method, Widal test, Typhoid Fever.

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INTRODUCTION

Typhoid fever, caused by *Salmonella enterica* serotype *Typhi*, remains a significant global health concern, particularly in regions with poor sanitation and limited access to clean water.[1,2] The clinical manifestations of typhoid fever are diverse, ranging from mild symptoms to life-threatening complications, making accurate diagnosis crucial for timely intervention.[3] One of the commonly used diagnostic tests for typhoid fever is the Widal test, which detects antibodies against *Salmonella* antigens.[4] However, the reliability of the Widal test has been a subject of debate due to its susceptibility to interference from various factors, potentially leading to false-positive or false-negative results.[3] In addition to its diagnostic limitations, emerging evidence suggests that the Widal test may interfere with hematological parameters, particularly hemoglobin levels, thereby complicating the interpretation of test results.[5,6] Hemoglobin, a vital component of red blood cells responsible for oxygen transport, is often used as a marker of anemia, a common complication of infectious diseases such as typhoid fever.[4] Several studies have reported discrepancies between Widal test results and hemoglobin levels in patients with typhoid fever, raising concerns about the accuracy of diagnosis and clinical management.[7]

Therefore, this paper aims to explore the potential interference of the Widal test with hemoglobin levels in patients with typhoid fever. By synthesizing existing literature and examining relevant studies 2-5, we seek to elucidate the mechanisms underlying this interference and its implications for diagnostic accuracy and patient care using cyanmethemoglobin method. This method offers three key advantages compared to alternative methods including quantifies all hemoglobin forms except sulfhemoglobin, which typically isn't found in blood, standardization is straightforward and the cyanmethemoglobin reagent exhibits exceptional stability.

Understanding the relationship between the Widal test and hemoglobin levels is essential for optimizing diagnostic strategies and improving clinical outcomes in individuals affected by typhoid fever.

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MATERIALS AND METHODS

Ethics: Informed consent was taken before using biological samples and study is approved by Institutional human ethics committee (IHEC).

Study Design and population: A cross sectional observational study includes patients who visited in outpatient department of AGMC, tertiary care hospital Tripura.

Sample collection criteria: For this study, A total of 150 patients who had suffered from fever in 6 days duration or more than 6 days of fever and Widal test were done to confirm typhoid fever were enrolled in the study. Hemoglobin tests were performed by the spectrophotometric based cyanmethemoglobin method.

Sample: The study was carried out with the sample size of 150 patients residing in urban and rural area. Venous blood was drawn and haemoglobin was estimated by direct cyanmethaemoglobin methods.

Biochemical analysis by direct cyanmethemoglobin method: Biochemical study was done as described by Bansal *et al*., 2016.[8] Briefly, 5 mL of venous blood were extracted and placed in a vial containing Ethylene Diamine Tetra Acetic acid (EDTA). The blood in the EDTA vial was mixed well and then 20 µL of blood was pipette onto a labeled Whatman's Filter paper, number I. After allowing the filter paper to dry, it was sealed in a ziplock bag and taken to the lab for examination. Blank, standard and test were prepared as per protocol and the absorption was read at 540 nm wavelength.

Concentration of hemoglobin in the specimen was calculated using the following formula:

$$\text{Conc. of hemoglobin in the specimen (g/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times \text{Conc. of Standard}$$

Concentration of the standard is 15 g/dL, Alternatively, a calibration graph can be prepared using multiple standards of varying concentration and the result can be obtained quickly by checking hemoglobin concentration which corresponds to obtained absorbance. This is markedly accep- when a large number of specimens are daily processed on the same instrument.[7]

Widal test procedure: Bring reagents and samples to room temperature before testing

Test was performed as described in Tydal® widal antigen set/ antigens kit.[9]

Slide Screen and semi-quantitative method: Test was performed as described in Tydal® widal antigen set/antigens kit.

RESULTS

Total sample size of 150 individuals, with males comprising 51% (*n*=76) and females 49% (*n*=74). Among males, 47% (*n*=36) reside in urban areas, while 53% (*n*=40) are in rural settings. On the other hand, among females, 43% (*n*=32) reside in urban areas, whereas 57% (*n*=42) are situated in rural regions. The mean age of males in the sample is 42.58 years with a standard deviation of 17.2, while the mean age of females is 40.11 years with a standard deviation of 17.1. This suggests that, on average, males tend to be slightly older than females in the dataset, with both groups exhibiting similar variability in age. The mean hemoglobin level for males is 12.06 g/dL with a standard deviation of 2.44, whereas for females, the mean hemoglobin level is slightly lower at 11.06 g/dL with a standard deviation of 2.30. This indicates a slight difference in hemoglobin levels between males and females, with males exhibiting a higher average hemoglobin level compared to females.

The number of individuals affected at different dilution

At a dilution of 1:80, the O-antigen group exhibited the highest number of affected individuals (57), followed by the H-antigen group (53) and the AH-antigen group (46). Similarly, at dilution 1:160, the O-antigen group continued to have the highest number of affected individuals (58), followed by the H-antigen group (51) and the AH-antigen group (22). Notably, at the highest dilution of 1:320, the number of affected individuals drastically decreased across all groups, with the AH-antigen group showing no individuals affected. Regarding hemoglobin levels, the mean hemoglobin concentration (±SD) was highest in the O-antigen group across all dilutions, with values of 11.28±2.27 g/dL (Table 1).

Across all age groups, females consistently exhibited slightly lower mean hemoglobin concentrations compared to males. In the age group 0-10, females accounted for 27% of the population, with a mean hemoglobin concentration of 11.09 g/dL, while males constituted 73% with unspecified mean hemoglobin concentration. During adolescence (11-20 years), a more balanced gender distribution was observed, with females comprising 48% and males 52% of the population. The mean hemoglobin concentration increased to 12.2 g/dL in this age group. Subsequently, in the age groups 21-30, 31-40 and 41-50, the gender distribution remained relatively balanced, with females representing 49% and males 51% of the population in each group. Mean hemoglobin concentrations ranged from 11.33 to 11.4 g/dL across these age groups. Notably, in the older age groups, a shift towards a higher percentage of females was observed. In the age groups 51-60, 61-70 and 71-80, females constituted 44-54% of the population, with males accounting for 46-56%. Mean hemoglobin concentrations ranged from 11.51 to 12.6 g/dL, with a slight increase observed in the 51-60 and

Table 1: Number of individuals affected at different dilutions (1:80, 1:160 and 1:320) for O-antigen, H-antigen and AH-antigen, along with the mean hemoglobin levels.

Parameter	Dilution 1:80	Dilution 1:160	Dilution 1:320	g/dL
O-antigen	57	58	8	11.28±2.27
H-antigen	53	51	3	
AH-antigen	46	22	0	

Table 2: Distribution of Hemoglobin Levels by Age Group and Gender.

Age (Years)	Male	Female	g/dL (Mean±SD)
0-10	73%	27%	11.09±1.62
11-20	52%	48%	12.2±2.76
21-30	51%	49%	11.4±1.82
31-40	55%	45%	11.33±1.75
41-50	55%	45%	11.33±2.33
51-60	56%	44%	12.6±1.65
61-70	46%	54%	11.51±2.19
71-80	56%	44%	12.22±2.22
>80	57%	43%	11.92±1.41

Table 3: Linear r (pearson) correlation test.

	Male	Female	Hemoglobin (HB)
Male	1	0.49909	-0.2601
Female	0.49909	1	0.2601
HB	-0.2601	0.2601	1

71-80 age groups. In individuals above 80 years of age, females constituted 43% of the population, with males comprising 57%. The mean hemoglobin concentration in this age group was 11.92 g/dL. These findings underscore the influence of age and gender on hemoglobin levels, with variations observed across different age cohorts. Table 2 represents the distribution of Hemoglobin levels (HB) among different age groups, categorized by gender, along with the corresponding mean hemoglobin concentrations (±SD).

A Pearson correlation test was conducted to explore the relationship between hemoglobin levels in widal positive cases and gender. The correlation coefficients revealed the strength and direction of these relationships. The correlation coefficient between "Male" and "Female" was found to be approximately 0.49909, indicating a moderate positive correlation between gender categories. Regarding the relationship between gender and Hemoglobin levels (HB), the correlation coefficient between "Male" and "HB" was approximately -0.2601, while the correlation coefficient between "Female" and "HB" was approximately 0.2601 (Table 3). These coefficients suggest a weak, yet statistically significant, association between gender and hemoglobin levels. Specifically, males exhibited a slight negative correlation with hemoglobin levels, while females displayed a slight positive correlation. The p-values associated with these correlations were extremely low ($p<0.001$), indicating that these findings are statistically significant at conventional levels of significance. The results indicate a moderate positive correlation between male and female genders and weak but statistically significant correlations between gender and hemoglobin levels among widal positive cases.

DISCUSSION

The study examined demographic and hematological characteristics of 150 typhoid fever-sample participants, finding a balanced gender distribution and a slightly higher proportion of males, suggesting a higher incidence of the disease in certain geographic regions.[1] Regarding residential status, our findings revealed differences in urban-rural distribution between males and females. While a larger percentage of males resided in rural areas, the reverse was observed among females.[10,11] This disparity in residential distribution may reflect underlying socio-economic factors influencing exposure to typhoid fever, such as access to clean water and sanitation facilities. Furthermore, our study highlighted age-related differences between genders, with males exhibiting a slightly higher mean age compared to females. This finding is in line with previous research indicating that typhoid fever incidence tends to peak in adolescence and early adulthood, with a gradual decline thereafter.[1] Similar variability in age between genders suggests that both groups may be equally susceptible to typhoid fever across different age

ranges.[12] Importantly, we observed variations in hemoglobin levels between males and females, with males exhibiting a higher mean hemoglobin level compared to females. This finding is consistent with studies reporting gender-based differences in hemoglobin levels and the prevalence of anemia.[5] However, our study did not directly investigate the etiology of anemia in this population, existing literature suggests that infectious diseases such as typhoid fever can contribute to the development of anemia through hemolysis and bone marrow suppression.[13] Overall, our findings underscore the complex interplay between demographic factors, hematological parameters and infectious diseases like typhoid fever. Future research should aim to further elucidate the underlying mechanisms driving gender disparities in disease incidence and hematological profiles, with implications for targeted interventions and public health strategies.[1,5,12] By integrating these results into the discussion section, we provide valuable insights into the broader context of typhoid fever epidemiology and its relationship with demographic and hematological factors.

Our study investigated the number of individuals affected at different dilutions, shedding light on the antigenic response in typhoid fever patients. Here, we discuss the implications of these findings in the context of existing literature. At a dilution of 1:80, O-antigen group exhibited the highest number of affected individuals (57), followed by H-antigen group (53) and AH-antigen group (46).[3,11] Similarly, at dilution 1:160, the O-antigen group continued to have the highest number of affected individuals (58), followed by the H-antigen group (51) and the AH-antigen group (22).[12] Notably, at the highest dilution of 1:320, the number of affected individuals drastically decreased across all groups, with the AH-antigen group showing no individuals affected.[3] The study indicates that typhoid fever patients have distinct immune responses to different antigens, with the O-antigen exhibiting the strongest response at lower dilutions and the mean hemoglobin concentration was highest in the O-antigen group.[12] Research indicates a possible link between antigenic response and hemoglobin levels in typhoid fever patients, with further investigation needed to understand the underlying mechanisms and clinical implications.[5] The study offers valuable insights into the antigenic response and hematological profiles of typhoid fever patients, aiding in understanding disease pathogenesis and potential therapeutic targets. The study found that females had slightly lower mean hemoglobin concentrations compared to males across all age groups during typhoid fever.[4] In the age group 0-10, females accounted for 27% of the population, with a mean hemoglobin concentration of 11.09 g/dL, while males constituted 73% with unspecified mean hemoglobin concentration.[14] Adolescence showed a balanced gender distribution with females comprising 48% and males 52%. Mean hemoglobin concentration increased to 12.2 g/dL. In age groups 21-30, 31-40 and 41-50, females represented 49% and males 51%, respectively. In older age groups, females constituted 44-54%, with males accounting for 46-56%.[14] In individuals above 80 years of age, females constituted 43% of the population, with males comprising 57%. Age and gender significantly influence hemoglobin levels in typhoid fever patients. Further research is needed to understand the underlying factors and their implications for disease susceptibility and management. Our study employed a Pearson correlation test to investigate the relationship between hemoglobin levels in Widal positive cases and gender, revealing significant associations. Correlation coefficients demonstrated a moderate positive correlation between male and female genders, with a coefficient of approximately 0.49909. Furthermore, weak but statistically significant correlations were observed between gender and Hemoglobin Levels (HB), with males displaying a slight negative correlation and females showing a slight positive correlation. These findings underscore the importance of considering gender differences in the assessment and management of hemoglobin levels in individuals with typhoid fever. The results contribute to our understanding of disease pathophysiology and patient care, emphasizing the need for tailored approaches to address gender-specific factors influencing hemoglobin concentrations in typhoid fever patients.[4,14]

CONCLUSION

In conclusion, our comprehensive investigation into the demographic and hematological characteristics of individuals with typhoid fever has provided valuable insights into the complex interplay between age, gender, antigenic response and hemoglobin levels. The observed variations in gender distribution across age cohorts, coupled with differential immune responses to specific antigens, highlight the multifactorial nature of typhoid fever pathogenesis. Furthermore, the correlation between gender and hemoglobin levels underscores the importance of considering gender-specific factors in disease assessment and management. These findings have significant implications for the development of targeted interventions aimed at improving outcomes for individuals affected by typhoid fever, emphasizing the need for tailored approaches that account for demographic and hematological diversity.

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AUTHOR CONTRIBUTION

JD: Data collection; writing-original draft, SG: Supervision; project administration; review and editing, TM: concept making and methodology; formal analysis; review and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

g/dL: Grams Per Deciliter; **SD:** Standard Deviation; **HB:** Hemoglobin level; ***p*-value:** Probability value; **EDTA:** Ethylene diamine tetra acetic acid; ***S. typii:*** *Salmonella typhi;* **IHEC:** Institutional Human Ethics Committee.

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