Hematological parameters of *Salmonella typhi* and *paratyphi* culture positive patients from Kathmandu Valley, Nepal

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Abstract

Introduction: *Salmonella enterica* causes febrile illnesses leading to changes in hematological parameters. Developing countries like Nepal remain an endemic area for this disease. However, the changes in hematological parameters among Nepalese population have not been well documented in correlation with culture diagnosed cases.

Methods: A total of 204 individuals (102 cases and 102 controls) were included in a cross-sectional, analytical comparative study. The cases and controls were recruited from the tertiary care centers in Kathmandu. The hematological parameters from both culture diagnosed and controls were measured.

Results: Enteric fever (102 culture positive cases) caused a significant decrease in total white blood cell ($p < 0.05$), blood platelet count ($p < 0.05$), lymphocyte ($p < 0.05$), packed cell volume ($p < 0.05$), eosinophils ($p < 0.05$) and haemoglobin ($p < 0.05$) compared to healthy controls. Similarly, enteric fever led to significant increase in neutrophil and monocyte count in enteric fever patients ($p < 0.05$).S. typhi was isolated in 60 (59%) and S. paratyphi was isolated in 42 (41%) of the patients. Anemia was found in 24 (23.52 %).Leucopenia was found in 8 (7.84%) patients, leucocytosis in 3(2.94%), neutrophilia in 29 (28.43%), lymphocytopenia in 19 (18.62%), monocytopenia in 48 (47.05%), absolute eosinopenia in 95 (93.13%),thrombocytopenia in 24(23.52%) and thrombocytosis in 2(1.96%).

Conclusion: Enteric fever was associated with leucopenia, mild neutrophilia, lymphopenia, absolute eosinopenia and decrease in haemoglobin among culture positive patients.*Salmonella enterica* serotype Typhi contributed febrile burden higher 59% (60).

Keywords: Enteric fever, Hematological parameters, Salmonella

Introduction

Febrile illnesses remain a significant burden in the modern world especially among developing countries like Nepal. However, enteric fever is one of the largest contributors in Nepal and it remains an important public health problem throughout developing countries.[1,2] It has been estimated that the highest burden of the disease is in Asia and recent studies show that enteric fever still remains a major public health issue in Nepal as well. Enteric fever has been one of the leading causes of deaths of many patients admitted in the hospitals in Kathmandu in the late 1960s.[3-5] The disease mainly affects young adults and school-going children. Hence, the cost of the disease burden affecting productive age group and younger population is immense.

Main source of the disease is culpable to poor

sanitary hygiene especially drinking water and food contamination.[6-8] It is a bacterial infection in the gut by *Salmonella enterica* (enteric bacteria). The genus of the bacteria is further classified into serotypes of *Typhi* and *Paratyphi*. The source of enteric bacteria is infected individuals and their excreta contain excess amount of bacterial colonies which may contaminate food or water in poor hygiene settings. Hence, direct feco–oral transmission may occur.[9] Shellfish taken from sewage-polluted areas are an important source of infection. The transmission also occurs through eating raw fruits and vegetables (e.g. salad) fertilized by human excreta and through ingestion of contaminated milk and milk products whenever they are not well decontaminated. Flies act as a mechanical transporter of the bacteria to cause human infection through transfer of the infectious agents to foods. Pollution of water sources produces epidemics of enteric fever[10], when large numbers of people use the same source of drinking-water.[6]

The prevalence of culture positivity regarding enteric fever depends upon various factors for example, age, region, season (seasonal variations as winter versus summer) and endemicity. In a study by Ochiai et al from China, India, Indonesia, Pakistan and Vietnam, sample were collected from 21874 febrile patients (fever lasting equal to or more than 3 days) and reports indicated that 475 (2%) had *Salmonella typhi* isolated.[6] But the culture positive prevalence varied widely from country to country. Murdoch et al have reported data from Patan Hospital that there is seasonal variation with *Salmonella typhi* (Winter 10% and Summer 20%) while *Salmonella paratyphi* (Winter 6% and Summer 8%) in their culture reports.[23]Maskey et al have found that the culture positive prevalence of enteric fever in Nepal 14.86% (74.7% *Salmonella typhi* and 29.3% *Salmonella paratyphi*) among total blood culture reports from 82467 febrile patients.[24] However, another large study by Karkey et al reported 3898 (7.15%) culture positive enteric fever patients out of 54536 blood cultures performed at Patan Hospital.[15]

The diagnosis of enteric fever involves scoring clinical signs and symptoms along with assessing laboratory parameters. The clinical features are quite vague and non-specific. The definitive diagnosis of enteric fever requires the isolation of *S.* typhi or *S. paratyphi* from blood, bone marrow, other sterile sites, rose spots, stool, or intestinal secretions.[11] The gold-standard practice is to culture blood from febrile patients especially when they have not taken antibiotics by then. Hence, differentiating enteric fever accurately among the many causes of febrile illnesses is a huge challenge[2] especially in developing countries due to limited laboratory facilities, cost-effectiveness and time constraints. Haematological alterations for example, white blood cell (WBC), platelet, lymphocytes, packed cell volume (PCV), eosinophils and haemoglobin (Hb) may serve as an important diagnostic aid for early diagnosis of enteric fever as well as for monitoring the effectiveness of the therapy in such patients.[11-13]Hence this study aims to assess the haematological parameters in enteric fever and compare it with those in healthy individuals from Kathmandu Valley (endemic area of enteric fever) of Nepal.

Methods

A quantitative cross-sectional comparative study was conducted over a period of one year from 1st November, 2012 to 1st November 2013 in the outpatient department and inpatient department of Tribhuvan University Teaching Hospital (TUTH), Helping Hands Community hospital, Chabahil, Kathmandu and Microbiology Laboratory of TUTH.A purposive judgmental non probability sampling method was used to recruit enteric fever (typhoid/paratyphoid) patients defined as true positive by cultural methods of isolation on culture media. Those reporting prior administration of antibiotics were excluded. The informed written consent was obtained from the study participants and ethical approval was taken from Institutional Review Board of Institute of Medicine. The data were expressed as mean ± standard deviation. The statistical analysis was done using software IBM SPSS statistics 20. Chi-square (X^2) test was done wherever applicable. Independent t-test was applied to determine if there was a significant difference between means of the hematological parameters of the two groups (enteric fever group and healthy individuals). The limit of significance was set at $p=0.05$ i.e. $P<0.05$ was regarded as significant.

Results

A total of 204 subjects were included in the study. The confirmation of the enteric fever was done on the basis of blood culture positivity. The clinically suspected cases but not yielding culture positivity were excluded. 102 positive blood culture cases and 102 healthy controls were selected.

Age and gender distribution of the cases

The cases were categorized into the age ranges of adolescents (below 20 years), adult (20-39, 40-59 years) and elderly (above 60 years). Most of the enteric fever patients were of the age group 20 to 39 years (n = 61, 60%) followed by age group below 20 years (n = 32, 31%) as shown in the **Figure 1**.

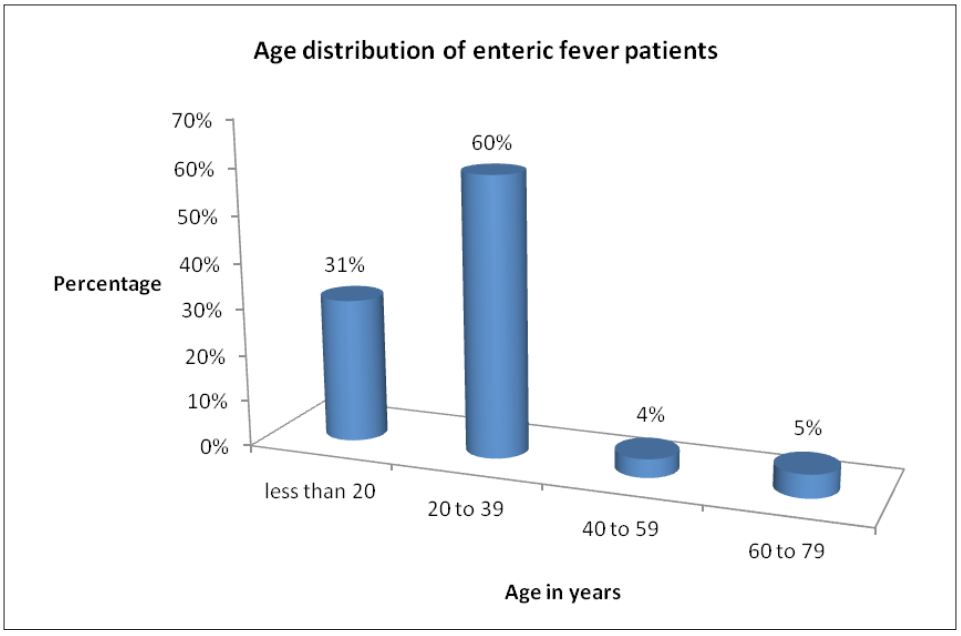

Figure 1. Age distribution of enteric fever patients

The gender distribution of the study population was almost evenly matched with 54.9% males & 45.09% female

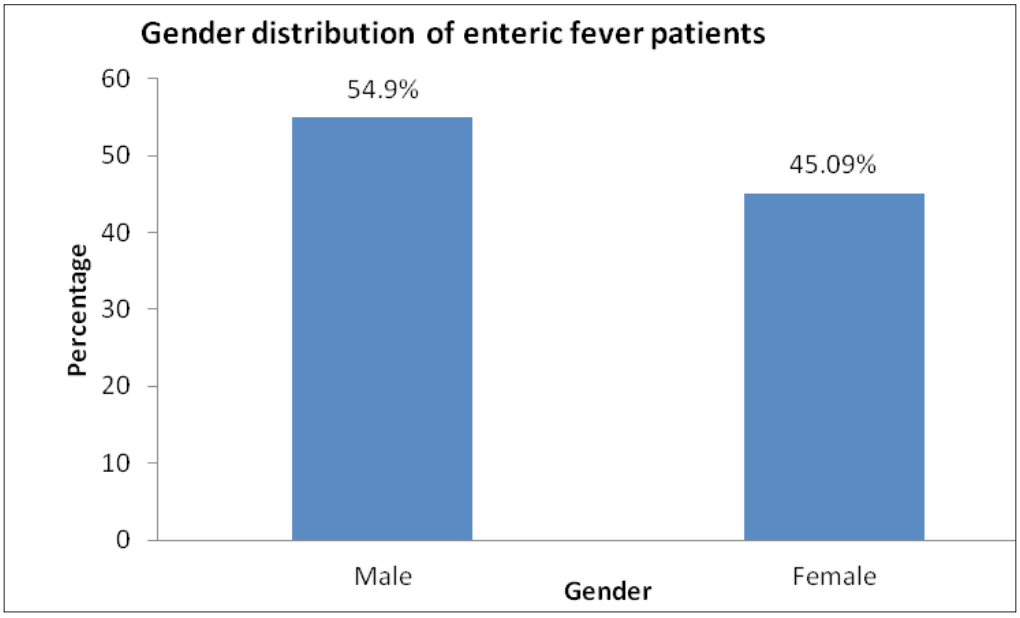

Figure 2. Gender distribution of enteric fever patients

Salmonella serotype distribution

The febrile illnesses caused by culture positive cases were mostly caused by *S. typhi*(n = 60, 59%) and then by *S. paratyphi*(n = 42, 41%) as illustrated in the **Figure 3**.

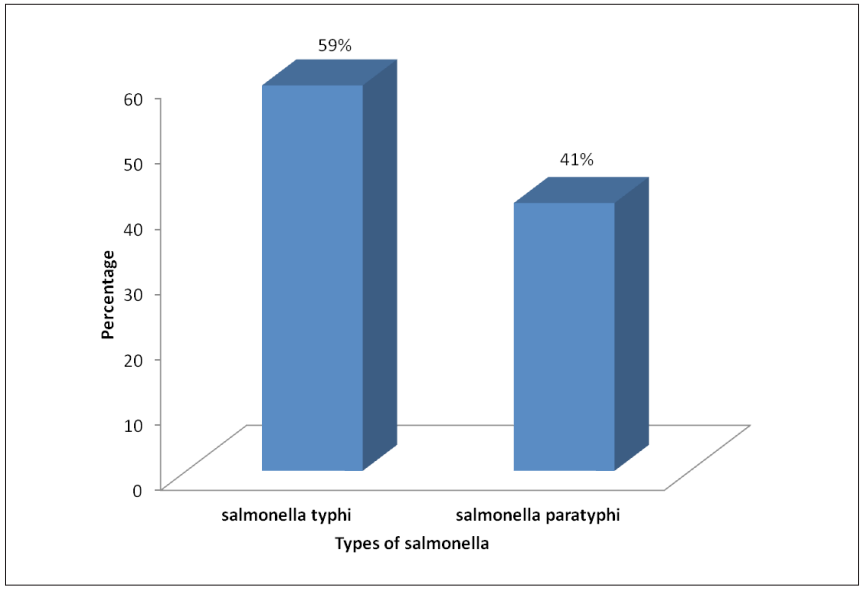

Figure 3. Salmonella distribution of enteric fever patients

Table 1. Hematological parameters on enteric fever patients and healthy individuals

Parameters	Enteric fever patients	HealthyIndividuals	p Value
	Mean±SD	Mean±SD	
Haemoglobin (g/dl)	13.65±1.71	14.63±1.93	p <0.05
Total white blood cell (per mcL)	6210.78±2120.38	7597.49±2048.91	p <0.05
Neutrophils (%)	70.75±8.99	61.56±7.13	p <0.05
Lymphocytes (%)	26.61±8.60	34.54±7.54	p <0.5
Monocytes (%)	2.50±2.84	1.31±1.14	p <0.05
Eosinophils (%)	0.15±0.552	1.96±1.22	p <0.05
Basophils (%)	0±0	0.01±0.099	P > 0.05
Packed cell volume (%)	39.49±4.28	43.93±5.04	p <0.05
Red blood cell count (million/mcL)	4.88±0.57	5.03±0.56	p> 0.05

Enteric fever caused a significant decrease in total white blood cell ($p < 0.05$), blood platelet count ($p < 0.05$), lymphocyte ($p < 0.05$), packed cell volume ($p < 0.05$), eosinophil ($p < 0.05$) and haemoglobin ($p < 0.05$) (Table 1) as compared to the healthy controls. There was significant increase in neutrophil and monocyte count in enteric fever patients in comparison with healthy individuals ($p < 0.05$). The red blood cell count tended to decrease in enteric fever patients although it was not statistically significant.

Table 2. Complete blood count in enteric fever

Parameter	Percentage
Anemia	23.52 %
Leucopenia	7.84 %
Leucocytosis	2.94 %
Neutrophilia	28.43 %
Lymphocytopenia	18.62 %
Monocytopenia	47.05 %
Absolute eosinopenia	93.13 %
Thrombocytopenia	23.42 %
Thrombocytosis	1.96 %
Erythrocytopenia	1.96%

Out of 102 patients, anemia was found in 24 (23.52 %). leucopenia was found in 8 (7.84%) patients, leucocytosis in 3(2.94%); neutrophilia in 29 (28.43%); lymphocytopenia in 19 (18.62%); monocytopenia in 48 (47.05%); absolute eosinopenia in 95 (93.13%); thrombocytopenia in 24(23.52%) and thrombocytosis in 2(1.96%)

Discussion

The major finding of the study are significant decrease in total white blood cell ($p < 0.05$), blood platelet count ($p < 0.05$), lymphocyte ($p < 0.05$), packed cell volume ($p < 0.05$), eosinophils ($p < 0.05$) and haemoglobin ($p < 0.05$) as compared to healthy controls. At the same time, there was a significant increase in neutrophil and monocyte count in enteric fever patients ($p < 0.05$). Most of the enteric fever was caused by serotype *Salmonella enteric* Serotype *typhi*(60, 59%) while *Salmonella enteric* Serotype *paratyphi*contributed (42, 41%).

The enteric fever mainly affects young adults and school-going children.[6,14] Our findings show that the most of the enteric fever patients were of the age group 20 to 39 years i.e. 61 patients (60%) followed by age group below 20 years i.e. 32 patients (31%).Among the study population 54.9% were male whereas 45.09% were female. Similar findings were reported in the previous study from Kathmandu Valley as 57% of the study population (n=30) male and 43 % female.[4]

Here we report *S.typhi* isolated in 60 (59%) andS. *paratyphi*42 (41%) isolated which is consistent with a large study conducted at Patan Hospital where 54,536 blood cultures were done.[15]Karkey A et.al found that 2,672 (68.5%) were *S. typhi* and 1,226 (31.5%) were *S. paratyphi* out of *3*,898 *Salmonella* culture positives.[15]Bhutta et.al estimate that the overall ratio of the enteric fever caused by *S.typhi* to that caused by *S. paratyphi* is about approximately 10:1.[16]Our findings are also consistent with a regional prevalence of the serotype. Jog et.al reported 73 out of 119 (61%) of enteric fever patients were*S.typhi*positive while the remaining of the patients had *S. paratyphi* infection.[17]However, the trend with *S. paratyphi* infections as a cause of febrile illness is an increasing trend in some parts of the world especially in endemic zones.[18-20]

Salmonella infection is expected to suppress the bone marrow during enteric fever period (relatively) in the beginning of infection; but, interestingly, white blood count usually limits itself within normal range. We found that the89% of the study population's WBC count within the normal range while 8% patients had leucopenia which is similar to the study by Jog et al where 85 % of enteric fever patients had normal WBC count and only 11.4 % had leucopenia.[17] Similar results was obtained in the study by Sharma et.al who found 62.5% of the study population (enteric fever) had their WBC count within the normal range and 32 % had leucopenia[3] from Kathmandu valley. Here, we report that a statistically significant decrease in the level of packed cell volume, total white blood cell count, lymphocytes, eosinophils and hemoglobin ($p<0.05$) in enteric fever patients compared to the healthy individuals. In our study 23.52% of enteric fever patients had anemia which is similar to the findings in the study done by Qamar et. al in 2010, in which 61.33% of 150 enteric fever

patients presented with anaemia.[21]The findings are in accordance with the literature regarding hematological changes during typhoid infection[13],which report that significant changes such as anemia, leucopenia, eosinopenia, thrombocytopenia occur; and there may be sub-clinical disseminated intravascular coagulation in severe cases.Interestingly, in our study 93.13 % of enteric fever patients showed absolute eosinopenia (0% eosinophil) which agrees with the findings of Jog et al where 77% of the study population presented with absolute eosinopenia[17] and that of Desmukh et al who reported absolute eosinopenia in 71.4% among paediatric patients diagnosed with enteric fever.[22]

The lower haematological parameters such as leucopenia, eosinopenia, thrombocytopenia and anemia found in enteric fever patients as compared to healthy individuals can be attributed to invasion of haemopoietic organs such as lymph nodes, spleen, tonsils and bone marrow by Salmonella typhi, which radically slows down the rate of haematopoiesis.[13,17] This also explains oligocythaemia, which is usually seen in enteric fever patients. Thrombocytopenia results in causing spontaneous bruises and prolonged bleeding lesions in the intestinal tract with consequent dangers of hemorrhage and intestinal perforation, which are seen in the third week in untreated enteric fever patients.[13,17]*Salmonella typhi* and *paratyphi* remain important enteric pathogens in many parts of the world in association with febrile illnesses;and there a high incidence of mortality and morbidity rate due to salmonella infections in the developing countries of the world.[5,13] Changes in hematological parameters were noticed as early as in 1950s[12]; and these changes still seem to be an important, supporting tool for diagnosis as well as evaluation of the patient's response to treatment especially in peripheral settings where resources are limited.

Conclusions

Enteric fever, which is a major cause of febrile illnesses in Nepal, is associated with leucopenia, mild neutrophilia, lymphopenia, absolute eosinopenia and anaemia which may serve as indicators for the diagnosis of the infection. The clinical features (signs and symptoms) are diverse and the etiology of febrile illnesses is broad leading to the challenges in proper diagnosis, treatment and monitoring of the patients especially in remote and resource limited settings of Nepal. Hence, hematological parameters along with clinical acumen could help clinicians to effectively manage the patients in those settings.

Limitations and Recommendations

Since, Kathmandu is the ultimate referral center of the country, the enteric fever cases may have come from all over the country. Hence, it is not truly the Kathmandu based population. Secondly, it is a cross-sectional study. Probably, it would have been better to have designed a prospective study and followed-up the patients. Third, sample size is not random and smaller considering the disease burden. Therefore, future studies should focus to enroll a larger sample size and follow up the patients with hematological parameters serially monitored.

Conflict of interests: None Declared

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