Concurrent Infections and Louse-Flea Borne Typhus Fever in Acute Febrile Patients Diagnosed Serologically In International Clinical Laboratories, Addis Ababa, Ethiopia

Jelkeba Bali Weyesa[1]

Master's of Sciences in Tropical and Infectious Diseases, Department of Biomedical Sciences, College of Health Sciences, Mizan-Tepi University

Abstract: *Background: Typhus fever, which is one of acute febrile illnesses, remains known enemy of human populations globally, particularly considerable in tropical and subtropical developing countries. Besides, concurrent infections have been reported from many regions of Africa where febrile illnesses are predominantly endemic. Of these, co-infection of typhoid fever and typhus fever is commonly observed in different parts of Ethiopia. But, limited study were conducted previously to measure serological and bacteriological estimations of co-infection of these febrile illnesses. Therefore, this study was designed to determine serological estimates of concurrent infections and Typhus fever from acute febrile patients diagnosed in ICL. Methods: A retrospective study was conducted with electronic data of acute febrile patients who made serological testing from January 2007 to 2011 in the International Clinical Laboratories. Data collection was performed from Dec 29, 2011 to Feb 10; 2012.Data were electronically stored into EpiData 3.1 and exported to SPSS 20 and STATA 11 for statistical analysis. Continuous measures were summarized by mean calculation and standard deviation at 95% CI. For most variables, frequency analysis was performed to descriptively observe them. The relationship of exposure and outcome variables was assessed by Spearson calculation. Ethical clearance was given from Institutional Review Board of ALIBP and additionally approved by Research Committee of ICL. Result: Over five years, a total of 5,029 patients with acute febrile illness were serologically diagnosed for typhoid fever and typhus fever at same time in ICL, Addis Ababa, Ethiopia. The age of patients distributed from less than a year to eight seven years old and mean age was located at 33.39±14.72 years [95% Conf. Interval].Of those patients, smaller numbers (43%) were females with 22% Weil Felix positive and greater numbers (57%) were male with 15 % Weil Felix positive. Around 6% of febrile patients were co-infected with typhoid fever and Typhus fever or 42% of Weil Felix positive. Approximately 18% of patients had significant titer equal to or greater than 1in 80µl that considered national cut value to exclude presence of multiple infections. Conclusion: Multiple infections are commonly known to result in severe disability and death. At early onset, serological diagnosis can exclude absence of Typhoid fever or typhus fever as IgM sero conversion takes time.*

Keywords: Weil Felix, Typhoid fever, Typhus fever, Concurrent infection, seroprevalence, AF

1. Introduction

Typhus fever and its concurrent infections are commonly diagnosed in tropical and subtropical developing countries. The disease is caused by typhus group rickettsia and characterized clinically by presenting features of high fever(40 to 41°C), severe headache, malaise, prostration, and 2 to 4 days after onset of illness maculopapular rash appears.[1]The diseases commonly occur among people living in unhygienic conditions and in overcrowded manner that may result in epidemics. Arthropod vectors infected with blood meal of rikketsemic patients are responsible for transmission of the diseases, particularly ectoparasite body louse and rat flea. The case fatality rate of typhus fever ranges from 10 to 40% and generally known to cause high morbidity and mortality in worldwide.[2,3]

Multiple infections, typhus fever with mainly typhoid fever and Malaria parasites,greatly affects presenting clinical features in all age categories but the degree of severity varies. All patients with multiple infections are highly prone to come down when compared with single infection and the consequences of these infections greatly result in severe outcomes. Most often reported that concurrent infections highly affected by occurrence of diseases in defined population and geographic area. Multiple infections may be more harmful than the most aggressive of the component infections; they can also be as harmful as the most aggressive strain. Most models predict the evolution of higher virulence in populations with frequent multiple infections, than in populations with less multiple infections.[4]

Clinical misdiagnosis of acute febrile illness in tropical countries, especially typhoid fever and typhus fever occurs in most health settings. Typhus fever, both epidemic and endemic types, mostly misdiagnosed as typhoid fever in tropical countries. Sustained high febrile condition that accompanied with symptoms, such as malaise, headache, and myalgia, are usually diagnosed in both typhoid and typhus fever. Also some cases of both typhoid and typhus fevers may also be presented with Jaundice and malena. The typhus group infections generally share this group of symptomatic presentation with an array of infectious diseases that increases likelihood of overlooking of them. However, sub-conjunctival hemorrhage is characteristically identified from patients with typhus fever. High incidence of co-infections typhoid fever and typhus fever usually reported from tropical African countries where there the diseases endemically affecting populations.[1, 3, 5, 6]

In Ethiopia, the diseases are known to have been present in the country for many years and cases continue to be reported from all regions. Rickettsial diseases are among serious causes of morbidity and mortality, mainly in under-five children in all highland of Ethiopia.[7] It constitutes the large endemic focal in Eastern Africa and prone to large geographic area outbreak from several years back. The risk increases during the colder months when human activities encourage the spread of human body lice. Typhoid fever and typhus fever are major health problems that result in co-infections and mistreatment at early stage. The illnesses are predominantly endemic diseases of highland regions of Ethiopia where unhygienic conditions and crowding of population occurs but typhoid fever is estimated to be higher in prevalence.[8, 9]

2. Methods and Materials

2.1. Study Design and Setting

The electronic data analyses were retrospectively conducted at Headquarter of the International Clinical Laboratories, Addis Ababa, Ethiopia. These laboratories are internationally known non-governmental institutions that established in 2004.Now it has four branches that independently function in regional town of Ethiopia. It gives more than 200 tests for clients in Addis Ababa and its neighboring. It fully engaged in 24 hours services each day with 90 professionals working in three shifts. Since establishment, only two big partners, namely: Bioscientia-Germany and LabCorp-USA have been collaboratively working. It is the only known accredited by the Joint Commission International in East Africa.
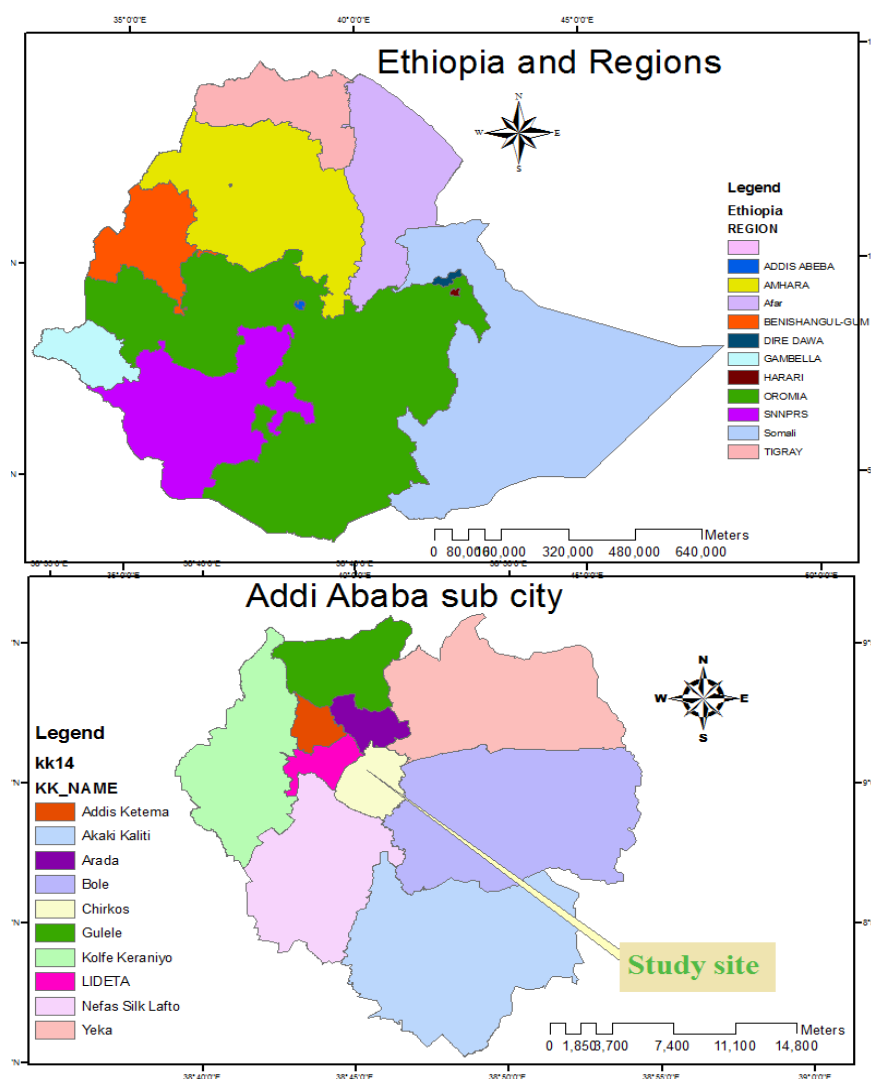

Figure 1: Geographical location of Study site in Addis Ababa

2.2. Blood Sample and Sero Diagnostic Test

The blood sample was drawn mainly in Head branch but a few proportion transported from regional sites. The blood specimens always keep in sterilized sample separator tube (SST) and waited for clotting. Then after, the serum component were separated fully from whole blood by centrifuging at 1000 rpm for 10 minutes at room temperature and remained at 2-8ºC temperature away from direct sunlight until time of titration. Serum from regional sites always kept under favorable conditions (2-8 ºC or frozen at -20 ºC temperature away from direct

Paper ID: 02014630

290

sunlight).These serum was made to re-attain room temperature before test. The sera from regional sites always rejected if there was hemolytic reaction, clotting, lipemic (after high speed centrifugation), Icteric and so on. It should also be sufficient for test and loaded with proportional sample to anticoagulant ratio.

One drop (50 μl) of Serum sample was placed into individual cells of slide with 6 reaction cells. Proteou Ox-19 antigen for rickettsia diseases were pipetted by micropipette into respective cells of slide and homogenized well with the serum by separate disposable applicator sticks. Finally, the glass slide placed on automated rotator with 80-100 rpm to rock antigen-serum complex solution. After approximately 1 minute of rotation, the agglutination pattern was observed under bright artificial light with naked eye.

The semi-quantitative slide test performed after observation of significant reactive antigens. Serum dilution in physiological saline (9g Nacl/l) was performed to have serial dilution of 0.08ml, 0.04ml, 0.02ml, 0.01ml and 0.005ml .Against serial dilutions a drop of antigens was pipetted into six reaction cells slide and quantification process repeated for every reactive antigens. The reagents and serum were well homogenized with separate disposable applicator sticks. Finally, the glass slide placed on automated rotator with 80-100 rpm to rock kit reagent-serum complex solution. After approximately 1 minute of rotation, the agglutination pattern was read under bright artificial light with naked eye. The result was reported in tube equivalent dilution as 1 in 20, 1 in 40,1in 80, 1 in 160, and 1 in 320.

2.3. Data collection and Instruments

The data retrieval performance was conducted consecutively from Dec 29, 2011 to Feb 10, 2012.Data from secondary sources that were electronically entered into Commercial Computer Software from January 2007 to December 2011 were collected for this study. Serological tests of patients with acute febrile illness over those five years were stored electronically in Polytech Technology Commercial Softwares. Patient data regarding Weil Felix tests, certain epidemiologic characteristics and suggested diagnosis were extensively employed in the statistical analysis. Overall, serum activity with agglutinin titer greater or equal to 1 in 80μl was significantly suggestive of typhus fever diagnosis but all data from 1 in 20μl were statistically analyzed. In Weil Felix test, only slide agglutination method was performed that extrapolated into equivalent tube confirmatory tests.

2.4. Data analysis

A vast majority of data was subjected to descriptive analysis primarily but some were inferentially assessed. The data were electronically stored into EpiData 3.1.windows version and exported to latest SPSS 20 and STATA 11 statistical packages for analysis. Mean calculation of independent continuous measures was performed at corresponding 95% confidence interval. The relationships of explanatory and outcome variables were statistically assessed by Spearson correlation. Frequency analysis was appropriate statistics for most study variables that exhaustively performed.

2.5. Ethical statement

The study was ethically approved by the Institutional Review Board of ALIPB before undertaking and additionally by the ICL Review Committee. During data processing, only patient ID number was confidentially taken by excluding personal identifications. All forms of consent weren't possibly requested because secondary data was retrieved manually but data were handled confidentially throughout study processes. Also raw data never shared with third party and handled only by principal investigator.

3. Result

3.1. Epidemiology of Populations

Over five years period from January 2007 to December 2011, a total of 5,029 patients were serologically examined for endemic febrile illness in the International clinical Laboratories. Almost all patients were residents of Addis Ababa and surrounding areas where the services possible given for. Of these patients, 4,781(95%) were tested for typhus fever with rapid slide agglutination method. All patients were made serodiagnostic test for typhoid fever and typhus fever (95%), but few proportion microscopically diagnosed for malaria parasites. The age pattern showed that it ranged from less than year to eight seven years. A greater proportion of patients constituted young adult with mean age of 33.89±14.72 years [95% Conf. Interval]. The analysis also showed that of all febrile patients, adolescent and adult of age category between 20 to 30 years (32.08%) were largest of all in number.

Next, approximately 25% of patients were in age category of 30 to 40 years. The risk of attack by episodes of acute febrile illness was steadily rising in patients older than fifty years but significantly falling towards lower extreme age category. In general, data from this study showed that predominantly patients with age category of 20 to 30 years highly affected by one or mixed febrile infections, unlike school aged children and older (**Figure 1& 3**).
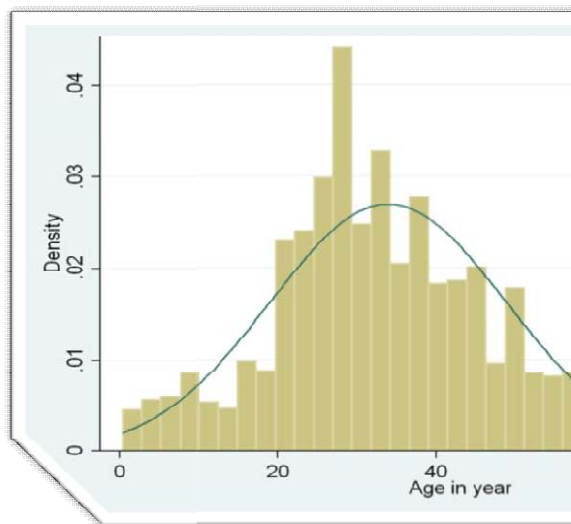
Figure 2: Age distribution of febrile patients from 2007 to 2011

Over five year, the estimate of typhus fever showed increasing trend from 2007 and declined in 2011.The distribution of typhus fever in season greatly varied and showed higher incidence rate during warm months (Figure 2& 3).
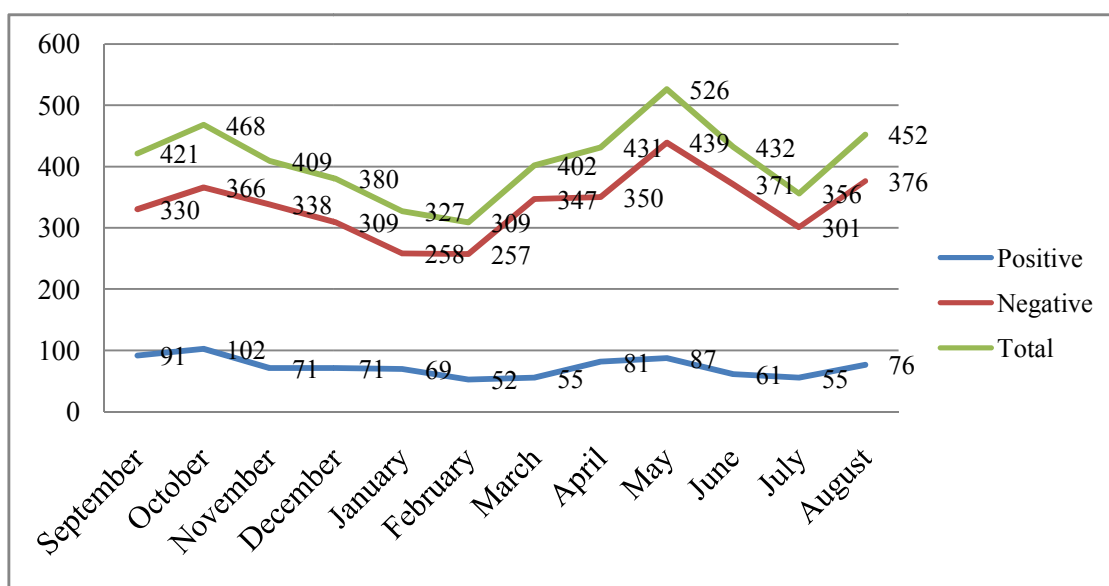
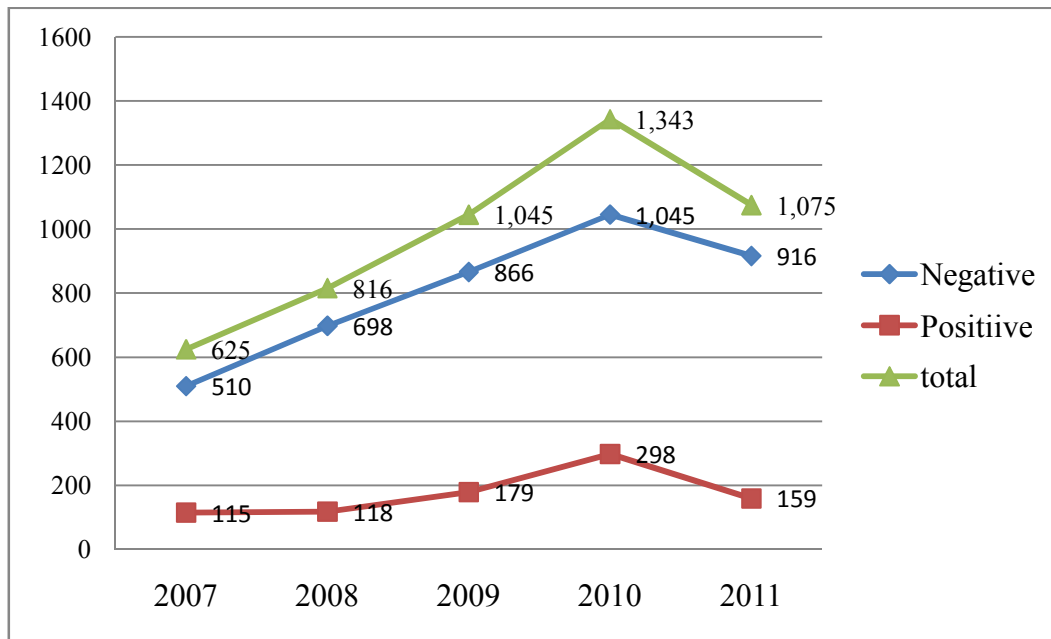**Figure 3:** Seroprevalence of typhus fever by month from 2007 to 2011

Figure 4: Seroprevalence Typhus fever from 2007 to 2011

3.2. Weil Felix (ox19) test

Most sera of patients were tested for more than two febrile illnesses because of the fact that co-infections commonly diagnosed in tropical countries. All patients with acute fever onset but a few were serologically tested for typhus fever and that accounted for a third of patients were co-infected with typhoid fever. The sera of those patients sufficiently reacted to antigen suspension producing co-positive results. Approximately 3,665(75.4%) of patient sera serologically tested for typhus fever responded negative, where nearly 1,121(23%) of patient sera responded positive for typhus fever. By far the largest proportion of sera sero-positivity rate for typhus fever that accounted for 409(37.6%) was observed among population with age category between 21-30 years only. Next, higher positive sera 264(23.3%) were observed in age category between 31-40 years populations. A few positive sera 23(0.3%) were detected in age category 80 years and higher. Nearly equal ratio of male to female sera (Ratio= 0.99) responded positive with varying distribution over all age categories. The febrile patients were slightly found more positive for Typhus fever than Typhoid fever (Table 1 & Figure 3).The epidemiological pattern analysis showed that both typhoid fever and typhus fevers were closely similar in over those years. In this analyses, typhus fever significantly correlated with sex and seasonal variation (Pearson chi2 (28) = 71.7179 & P-value $\leq$ 0.001 for sex and Pearson chi2 (33) =50.1575 & P-value = 0.028 for season).

The agglutinin titers for typhus fever in decreasing order were 1 in 320 in 6(0.28%) females and 6(0.21%) males, 1 in 180 in 2(0.09%) females and 2(0.07%) males, 1 in 160 in 165(7.72%) females and 99(3.46%) males, 1 in 100 in 1(0.05%) females, 1 in 80 in 280(13%) females and 306(10.7%) males, 1 in 60 in 5(0.23%) females and 3(0.1%) males, 1 in 40 in 92(4.31%) females and 113(3.95%) males, and 1 in 20 in 27(1.26%) females and 44(1.54%) males. Approximately 280(13.10%) of female patients and 306(10.70%) of male patients' sera produced 1 in 80 which was the most frequent value. Of all patients, 455(52.3%) of females and 415(47.7%) of male patients' serodiagnosed were positive for typhus fever (Pearson chi2 (4) = 36.9612 & P- value $\leq$ 0.001). Among patients sera positive that accounted for 280(32.2%) were co-infected with typhoid fever but 589(67.7%) were typhoid fever negative. The level of agglutinin titer measured in the serological estimation was almost identical both against Typhoid fever and Typhus fever except for a few patients **(Table 1)**.
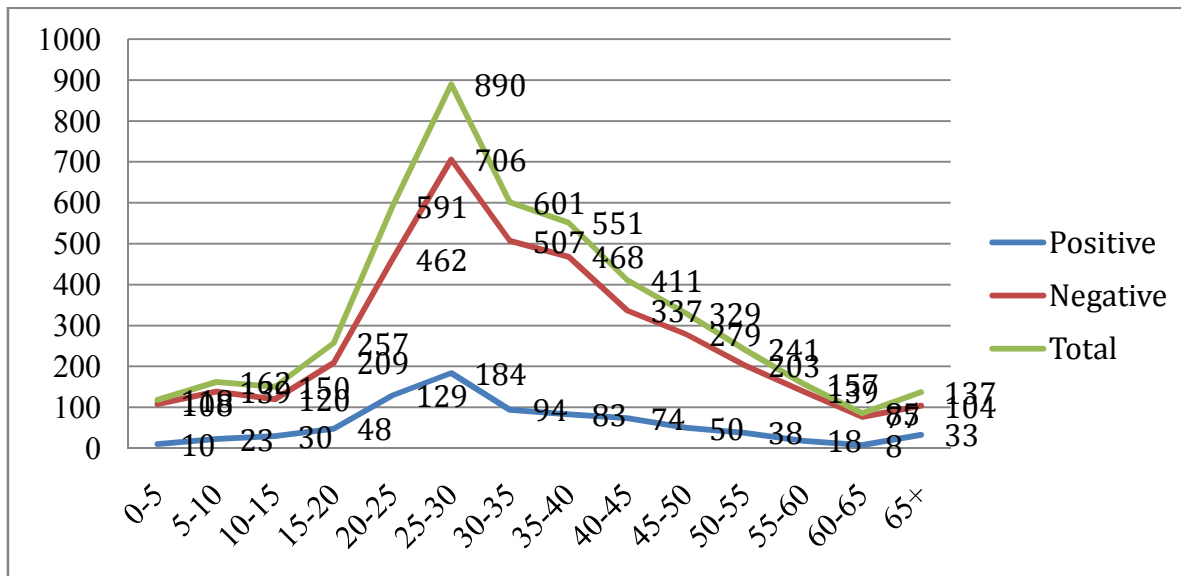
Figure 5: Typhus fever serodiagnosis against each age category

Table1: Two way frequency distribution of Weil Felix titre by sex

Sex	Titer, n (%)									
	1:320	1:180	1:160	1:100	1:80	1:60	1:40	1:20	NG	Total
Female	6(0.29)	2(0.1)	165(7.9)	1(0.05)	280(13.4)	5(0.2)	92(4.4)	27(1.3)	1517(72.4)	2,095(100)
Male	6(0.21)	2(0.07)	99(3.5)	1(0.05)	307(10.9)	3(0.1)	114(4.1)	45(1.6)	2239(79.5)	2,8115(100)
Total	12(0.12)	4(0.08)	264(5.4)	2(0.02)	587(12)	8(0.2)	206(4.2)	72(1.5)	3,756(76.5)	4,910(100)

NB: NG=Negative result

4. Discussion

Many reports showed that rickettsial diseases are among the most covert re-emerging infections of the present times globally, especially   in developing tropical and sub-tropical countries. Rickettsial infection has been one of the great scourges of mankind, occurring in devastating epidemics where there are unhygienic conditions.[10,11] Data from this study suggested that typhus fever generally estimated to be significant health problem in consistent with this report. Multiple infections of febrile illnesses are also commonly diagnosed from acute febrile patients. In many instances, febrile illnesses clinical pictures are closely related in a way that sometimes care-providers fail to identify one from another. Clinically, typhoid fever suspects in early stage are not clearly identifiable from malaria and typhus fever patients in area where both are endemically affecting populations and potentially lead to misdiagnosis.[12,13] As a result, parasite isolation methods are more reliable to manage acute febrile illness in early onset of diseases and may also result in multiple infections. In most countries where environmental sanitation standard is very poor, like Ethiopia, typhoid and typhus fever cause considerable episodes of morbidity and mortality year round.

In this study, almost all typhoid fever suspects were serologically diagnosed for typhus fever with similar protocol and antigenic commercial kits with package size of eight in one. Generally, sera of patients were comparatively more positive for typhus fever (rickettsia species) than for typhoid fever in this study; somewhat contrasting with other studies in Ethiopia.[9,14] Data from this study showed that epidemiologically these febrile illnesses were similarly distributed. In contrast, previous studies in regions of Ethiopia documented that typhoid fever was more prevalent and differently distributed in epidemiology than typhus fever.[9,14] In this study, the titer cut value in diagnosis of Typhus fever also agrees with typhoid fever which was 1 in 80μl and that also agrees with many previous studies in developing countries.

A third of the patients were co-infected with typhoid and typhus fevers showing coexistence of them in study population. Most patients with suspicion of typhoid fever were either co-infected with typhus fever or some had only typhus fever seropositive results. It was suggested to result from environmental sanitation standards that cause co-prevalence of these febrile illnesses in many highland areas of Ethiopia. Co-infection with typhoid fever and typhus fever were predominantly observed compared to other febrile illnesses like malaria and relapsing fever in this study but that is less common in previous studies from Ethiopia. Such findings in this study greatly contrasted with reports from different African countries in which typhomalarial infections predominantly detected.[15] In fact,

the louse borne and flea borne rickettsial infections in this study were reported crudely without identification of species involved in disease causation. Hence, the estimates in this study indicate the prevalence of endemic and epidemic typhus fevers.

Over all, majority of patients with co-infections were aged from 25 to 35 years but the prevalence declined in either age categories. Sera of females were more positive showing higher prevalence rate of typhus group rickettsial diseases in them. The rise in agglutinin titer also showed that higher agglutination reaction detected in middle age group and in females patients. Previous study in Egypt suggested that median age of 26 years were predominantly co-infected comparatively and male sera showed more positive results in contrast to these findings.[16] Report from Los Angeles County revealed that prevalence in age group from 34 to 64 were generally highest over 2000-2012 years indicating age pattern difference with this observation.[17] In this stud, limited data were applied to investigate variation among different study and countries because of lack of previous studies reporting epidemiological characteristics of typhus fever.

In this study, temporal pattern analysis showed that the cases of febrile illness were reported in all months with peak incidence rate in May and October but falling in February and July. However, the diseases significantly remained in public health concern throughout the year in this study. Many previous reports from different developed and developing countries also revealed similar temporal pattern to this finding but with slight variation in extent of incidence rate in month and number of rising to peak. In nearly all reports, typhus fever estimates rise to peak from May through August that partly observed in this study. But all previous studies showed that significant cases continuously occurred in year round.[17]

5. Conclusion

Acute febrile diseases remain known enemies of human globally where substandard living conditions predominantly occur. The consequences from multiple febrile illnesses always result in holding communities back from becoming self-supporting than one infection at a time. Communities need the capacity to manage these severe but treatable and preventable impacts on the lives of their members. It requires extensive efforts to bring down the current level of co-infection of Typhus fever and Typhoid fever in this area. Many difficulties in early diagnosis of febrile illness also need efforts to increase quality of laboratory analysis. The current rapid serological methods cannot reliably detect IgM antibodies upto days after disease progress which might lead to underreporting.

Findings of this study emphasize on serological estimates that considered significant to exclude presence of Typhus fever that disregards so many tests in which lower reaction detected. National guideline in cut value of titer needs special attention to increase accuracy of reporting of findings. In this thematic area further evaluation of co-infections of acute febrile illness is necessary to measure the degree of problem in regions of Ethiopia and give accounts of epidemiology of concurrent infections. In general, the problem considerably high in comparison of many countries that needs professional attention to control.

6. Acknowledgement

7. Author contribution

The author is the only person greatly endeavored from proposal development to manuscript preparation.

8. Conflict of interest

The author declares that there is no any conflict of interest.

List of Abbreviations

AFI=Acute febrile illness
ICL=International Clinical Laboratory
WHO=World Health Organization
ALIBP=Aklilu Lemma Institute of Pathobiology
ID= Identification

References

[1] Longo et al.Harrison's Principles of Internal Medicine 18th edition
[2] Dumler JS, and Walker DH (7th ed) (2009).Rickettsia typhi (Murine typhus). In: Mandell GL et al. Principles and Practice of Infectious Diseases; Philadelphia, Pa: Elsevier Churchill Livingstone
[3] Guerrant LR et al.(3rded).Tropical Infectious Diseases:Principles,Pathogens,and Practices
[4] Alizon S and Baalen MV. Multiple Infections, Immune Dynamics, and the Evolution of Virulence.Am Nat,2008;172(4):E150–E168
[5] Dill T et al, High Seroprevalence for Typhus Group Rickettsiae, Southwestern Tanzania. J Emerg Infect Dis,2013;19(3)
[6] Mazumder RN et al. Typhus Fever: An Overlooked Diagnosis. J Hlth Popul Nutr. 2009; 27(3):419-421
[7] Deressa W. et al.Household and socioeconomic factors associated with childhood febrile illnesses and treatment seeking behaviour in an area of epidemic malaria in rural Ethiopia. Trans Ro Soc Trop Med Hyg (2007)
[8] Azad AF. Flea-borne rickettsioses: ecologic considerations. Emerg Inf Dis,1997; 3:319–27
[9] Tadesse H and Tadesse K. The Etiology of Febrile Illnesses among Febrile Patients Attending Felegeselam Health Center, Northwest Ethiopia.AJBLS, 2013; 1(3): 58-63
[10] Bitam I et al.Fleas and flea-borne diseases.J Inf Dis,2009;14: e667–e676

[11] Rathi N and Rathi A.Rickettsial Infections: Indian Perspective. Indian Ped, 2010; 47

[12] Nsutebu EFet al.Shortcommunication:Prevalenceoftyphoid feverinfebrilepatients with symptoms clinicallycompatiblewithtyphoidfeverin Cameroon.TropMed Int Hlth(2003);8(6):575–578

[13] PradhanP.Coinfectionoftyphoidandmalaria.J Med Lab Diagn(2011); 2(3):22-26

[14] Animute A et al.Febrile illnesses of different Etiology among outpatients in four health center in Northwestern Ethiopia.Jpn J Inf Dis,2009;62:107-110

[15] Reyburn H et al.Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study.BMJ,2004

[16] Parker TM et al. Concurrent Infections in Acute Febrile Illness Patients in Egypt. Am. J. Trop. Med Hyg, 2007;77(2):390–392

[17] Los Angeles County Department of Public Health (LAC). Acute Communicable Disease Control.2012 Annual Morbidity Report, http://www.publichealth.lacounty.gov/ha/hasurveyintro.htm