

everywhere but occasionally males are more affected by infection due to travel. Eventhough both sexes are equally susceptible to typhoid fever infections, sometimes sera of males are found more positive than females are in widal examination (Okonko et al., 2010).¹¹Our observations contrast another in that sera of females were more positive to slide agglutination tests compared to males in this retrospective study. The difference in our findings might be resulted from behavioral factors of sexes and/or geographical variability (Figure 3).

Many previous studies reported incidence of typhoid fever in three countries where it was known to be endemic.¹⁸,¹⁹In their report Salmonella Typhi isolates were very common in age less than 10 years in three of them and in Indonesia and New Dehli, children less than five years were more susceptible to typhoid fever than adult populations. But typhoid fever incidence rise in school-aged children compared to lower and higher age categories throughout five years in Chile and Vietnam. Different from these, Widal seropositivity rate of this study rise in age between 20-40 years and falls otherwise. Eventhough tests with varying diagnostic value are correlated, our findings are not coinciding in peak positive result distribution in all age groups of different countries. Many studies result shows that age pattern of typhoid fever almost consistent with one another in worldwide and not attack only specific age group. In other words, general populations where underlying causes largely exist are at high risk of having typhoid fever out of age predilection (Singh, 2001).

5. Conclusion and recommendations

In conclusion, serological estimate of typhoid fever was considered to be significant among study populations involved and had prominent epidemiological profiles in Ethiopia. Widal agglutination test has long been used for diagnosis of typhoid fever from suspected febrile patients. Still it is widely used over all part of Ethiopia as it is most practical laboratory tool but reading of Widal test result varies greatly. Rapid slide quantitative test is non-specific type of serological tests in population with unknown baseline titer and superimposed by typhoid fever endemicity. The background antibody in Ethiopian populations has not been yet determined to know diagnostic reliability of Widal test and also non-typhoidal Salmonella species serogroup A, B, and D which have O antigenic determinants are very common. Attention should be given as in most patients immunity production impaired or delays due to host factors and history of antibiotic initiation that lead to false negative. As well, laboratory confirmation of rapid slide test positive by isolating diseases-producing organisms and/or Widal tube confirmatory test should be performed to definitely diagnosis typhoid fever.

Scientifically it is proved that in apparently health person serum titer can rise up to 1 in 60 μ l due to history of immunization with TAB vaccines, Malaria infection, and other febrile illnesses. Many reports of study involving slide semi-quantitative test agree with cut-off titre at equal to 1 in 80 μ l or more but cut-off titre equal to 1 in 160 μ l or

more used second to that titre. Titers of agglutination greater than 1 in 80 μ l to 1 in 160 μ l with tube test or 1 in 8 to 1 in 16 μ l with the rapid slide tests are usually significant indicative of an acute infection. The detection of specific antibodies produced against febrile antigens clearly suggests valuable diagnostic information on potential disease. In unpaired serum slide agglutination test, the final diagnosis whether widal positive or negative, however, should take into consideration the patient's history, antibiotic administration, and additional clinical investigations. Generally speaking, fourfold or more rise of H or O agglutinins in paired sera of 7-10 days apart is strongly indicative of typhoid fever and more meaningful than single slide agglutination test.

Data from this study showed that typhoid fever is still a major public health problem next to malaria among febrile illnesses in Ethiopia. The findings really inspire further studies to make sure the epidemiological patterns and level of occurrences of Typhoid fever are consistent throughout regions. Further studies also should focus largely on test validies by incorporating multiple laboratory tests. As it is highly affecting school-aged children and young adults who are not fully developed resistance against invasion of organisms and productive populations respectively, greater emphasis should be given to improve per capita water supply and sanitary standards. To fully protect community, it would be better if immunization programs are given as supplementary measures to sanitary standards all over regions. Other important measures to consider are regular control of food establishment and implementation of integrated sanitary control programs in the areas.

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7. Authors' Contribution

Author endeavored to this thesis research from proposal to manuscript preparation.

8. Conflict of Interest

The authors declare that there is no any conflict of interest.

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screening test, and confirmatory tube titrations are performed in specific procedures as follows.

A. Slide qualitative test

One drop (50 µl) of Serum sample was placed into individual cells of slide with 6 reaction cells. Next, a drop of IgM somatic O antigen, IgG flagellar H antigen, and combined paratyphi A, B and C antigens 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 kit reagent-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 resumed after observation of significant reactive antigens.

B. Semi-qualitative slide test

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.

C. Interpretation

Within 1 minute, the characteristic pattern of H and O antigens agglutination for Salmonella Typhi and Salmonella Paratyphi were examined under bright artificial light with naked eye. Usually, the somatic O antigens reaction was identified as coarse, compact agglutination that don't easily be dispersed. In case of flagellar H antigen, the loose flocculent agglutination which suggests presence of corresponding antibody was reported. The titration reading was taken at highest serum dilution in which still reaction was visibly observed. The result was reported in tube equivalent dilution as 1 in 20, 1 in 40, 1 in 80, 1 in 160, and 1 in 320.

Annex: Serological Procedures

Bio-merieux technology product used for both slide agglutination and tube titration tests in this laboratory. The serum of patients that well processed is tested for detections of evidence for presence of antibody against febrile pathogens. The antisera of patients that mounted against somatic O antigen and flagellar H antigens are measured by their agglutination reactions. Each slide