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Prevalence of typhoid fever and anemia (low PCV) among patients attending University College Hospital Ibadan, Oyo, Nigeria

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Abstract

This study was carried out to determine the prevalence of Salmonella typhi and changes in Packed Cell Volume (PCV) for its possible implication in anemia among patients attending University College Hospital (UCH) Ibadan, Oyo state, Nigeria. Two hundred participants (100 male and 100 female inpatients and outpatients) were used for the study; their blood samples were examined for the presence and level of Salmonella typhi antibodies by widal agglutination technique. Out of the 200 blood samples analyzed, 68% were positive while 32% were negative among male, and 46% were positive while 54% were tested negative among female. This study also shows that typhoid fever decreased significantly the PCV levels of the patients with the highest range obtained as 24 -35% compare to normal healthy adult PCV level 40–48%. The implication of the result is that typhoid fever could lead to anemia. Therefore, this study calls for better personal hygienic living, improved environmental sanitation, and to provide adequate health education programme to the general public on the prevalence of Salmonella typhi and anemia.

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Introduction

Typhoid fever (enteric fever) caused by Salmonella typhi is an endemic disease in the tropic and sub-tropic and has become a major public health problem in developing countries of the world with an estimated annual incidence of 540 per 100,000 (Ibekwe *et al*., 2008). The annual incidence of typhoid is estimated to be about 17 million cases worldwide (WHO, 2008). It is often encountered in tropical countries including Nigeria where they constitute serious causes of morbidities and mortalities (Ibekwe *et al*., 2008). Anemia makes important contributions to disease burden in most low- and middle-income countries. The burden of anemia remains highest in sub-Saharan Africa, with Nigeria also heavily affected.

Enteric fever caused by *Salmonella typhi* is often encountered in tropical countries including Nigeria where they constitute serious sources of morbidities and mortalities (Baver, 1995).

In 2009, over 40,000 cases of *Salmonella* (13.6 cases per 100,000 persons) were reported to the Centers for Disease Control and Prevention (CDC) by public health laboratories across the nation, representing a decrease of approximately 15% from the previous year, but a 4.2% increase since 1996. Overall, the incidence of *Salmonella* in the United States has not significantly changed since 1996 (Philip, 2000). Only a small proportion of all *Salmonella* infections are diagnosed and reported to health departments. It is estimated that for every reported case, there are approximately 38.6 undiagnosed infections.

A 2008 WHO analysis reported that anemia affected 24.8% of the world's population, including 42% of pregnant women, 30% of non-pregnant women, and 47% of preschool children. Most recently, global anemia prevalence was estimated at 29% in pregnant women, 38% in non-pregnant women, and 43% in children, with reductions since 1995 in each group. The Global Burden of Disease (GBD) 2000 report estimated that anemia accounted for 2% of all YLD and 1% of disability-adjusted life-years; the GBD 2004 update had similar findings (Global Burden of Disease Study, 2010).

Anemia makes important contributions to disease burden in most low- and middle-income countries. The burden of anemia remains highest in sub-Saharan Africa, with Nigeria also heavily affected. The global anemia prevalence in 2010 was 32.9%, resulting in 68.4 million years lived with disability (YLD). The results emphasize the important contribution made by anemia to the overall global burden of disease and should help focus attention and resources toward this problem (Kassebaum *et al*., 2014).

This study is aimed at investigating the prevalence of typhoid fever caused by *Salmonella* typhi and anemia among patients in University College, hospital Ibadan, Oyo State, Nigeria, with a view to enlightening the public on how these endemic diseases could be prevented and controlled.

Materials and methods

Study Area

This study was conducted in university college hospital Ibadan, Oyo state Southwest Nigeria. This city has experienced both population and economic growth since the 1950s due to its position between the cities of south west. University college hospital Ibadan is a government -owned federal health care Institution in Nigeria.

Target Population

The target population of this study comprised of male and female adult patients (18- 60 years) in university college hospital Ibadan, Oyo state Nigeria. A sample of two hundred (200) respondents was used. Simple random sampling technique was used to select two hundred (200) respondents from five major departments namely: Medicine, Surgery, Orthopaedics, Gynaecology and Emergency department.

Method of Data Collection (Sample collection)

- A total of 200 blood samples were collected from 200 patients (130 females and 70 males) in university college hospital Ibadan, Oyo state, Southwestern, Nigeria. The samples were collected in sterile containers and transported to the laboratory for analysed.
- Two milliliters of the blood samples were centrifuged at a high speed for 5 min in order to separate the serum from the blood cells.
- The blood culture bottle was incubated at 37oC for 24hrs. 2ml of the cultured blood was taken through needle and syringe, primary inoculum was made and striking was finally done using MacConkey Agar plate.
- This was carried out repeated for seven days till there was growth on the plate. Gram staining and biochemical tests were carried out as a confirmatory test to confirm the organism isolated.
- 2/3 of helparinised tube was filled with patients' blood; one end of the tube was sealed with plasticine and spurned with hematocrits centrifuge at 50000rpm for 5 mins.

Results were later read using hematocrit reader and reported accurately.

Method of Data Analysis

Data collected will be analyzed using both descriptive statistical method of analysis for the purpose of this study. A frequency simple percentage was used to present data. Percentage was calculated as number of responses per total number of respondent multiply by 100

$$\frac{\text{Number of responses}}{\text{Total number of respondents}} \times 100$$

Results and conclusion

Analysis from the Table 1 above revealed the Socio demographic Data of the respondents. Distribution according to age revealed that 44 (22%) are between 18 and 27years, 120 (60%) are between 28 and 37years while 36 (18%) are 38 and above. On level of education, 46 (23%) had primary education, 76 (38%) had secondary education and 78 (39%) had tertiary education. Distribution according to religion revealed that 116 (58%) are Christians, 84 (42%) are Muslims while none is a traditionalist. Also, 55 (27.5%) are from medicine department, 48 (24%) are from surgery department, 45 (22.5%) are from Orthopedics department, 22 (11%) are from gynecology department and 30 (15%) are from emergency department. The results of this study revealed that majority of the respondents are mature and educated.

Table 1. Demographic Data.

Variable	Responses	Frequency	Percentages
Age	18-27	44	22%
	28-37	120	60%
	38 and above	36	18%
	Total	200	100%
Education	Primary	46	23%
	Secondary	76	38%
	Tertiary	78	39%
	Total	200	100%
Religion	Christianity	116	58%
	Islam	84	42%
	Traditional	00	00%
	Total	200	100%
Tribe	Yoruba	166	83%
	Igbo	30	15%
	Hausa	04	02%
	Total	200	100%
Department	Medicine	55	27.5%
	Surgery	48	24%
	Orthopaedics	45	22.5%
	Gynaecology	22	11%
	Emergency	30	15%
	Total	200	100%

Table 2 shows that out of the 200 participants of the study, 100 (50%) are males while 100 (50%) are females. 68% were positive while 32% were negative among males and 46% were positive while 54% were tested negative among female respondents. This shows that samples analyzed for *Salmonella agglutinin* titres were Widal positive among the majority of the participants in this study. This indicates a high prevalence of typhoid fever in the sampled population. However, some of the subjects may not be having the active disease. This is in agreement with the observations of Outi *et al*. (1989) and Adeleke *et al*. (2006) in a similar study on Widal

reaction as being more relevance in diagnosing post-infection complications when *S. typhi* may not be isolated. The Widal test reaction involves the use of bacterial suspensions of *S. typhi* and *S.* Paratyphi `A' and `B', treated to retain only the `O' and `H' antigens. These antigens are employed to detect corresponding antibodies in the serum of a patient suspected of having typhoid fever. The IgM somatic O antibody appears first and represents the initial serologic response in acute typhoid fever, while the IgG flagella H antibody usually develops more slowly but persists for longer (Hoffman *et al*., 1986; Washington and Henry, 1991; Olopoenia *et al*., 2000). While bacteriological culture remains the gold standard for definitive diagnosis of typhoid fever, lack of its immediate availability during the acute febrile illness may limit its use. In an acute febrile illness in an endemic typhoid region where the clinical picture is ambiguous, a rapid, accurate, specific and sensitive test should be used to differentiate typhoidal from non-typhoidal febrile illnesses. Clinicians usually elect to treat, rather than wait for blood or stool culture results, which may take 3-5 days. While there might be some merit in this approach, particularly in areas where culture facilities are either poor or not available, and where Widal testing is the norm, the use of rapid antigen screening directly from the stool of the suspected patient would be more useful (Olopoenia *et al*., 2000).

Table 2. Distribution of respondents according to widal sera (salmonella agglutinin titres) in relation to sex.

Sex	No of sera tested%	No of widal positive (%)	No of widal negative (%)
Male	100 (50)	68 (68)	32 (32)
Female	100 (50)	46 (46)	54 (54)
Total	200 (100)	114 (57)	86 (43)

Also in this study, more sera from males were more Widal positive than sera from females. This is probably as a reflection of different eating habits and level of personal hygiene. This is also in agreement with the findings of Adeleke *et al*. (2006). In 380 males, the titre of Salmonella 'O' were higher than those of the 'H' whereas in 460 females, Salmonella 'H' titres were higher than those of 'O'. This differs from what was reported in a similar study by Ibekwe *et al*. (2008) where 82 apparently normal males had higher titre of Salmonella 'H' and 118 apparently normal females had higher Salmonella 'O' titres (Ibekwe *et al*., 2008).

Furthermore, analysis from table 3 revealed the specie of Salmonellae present in the tested blood samples. 56 (28%) are tested positive to *S. paratyphi* A-O with PCV between 23-30, 48 (24%) are tested positive to *S. paratyphi* B-O with PCV between 22-27, 63 (31.5%) are tested positive to *S. paratyphi* C-O with PCV between 23-30, 34 (17%) are tested positive to *S. typhi* O with PCV between 23-35, 47 (23.5%) are tested positive to *S. paratyphi* A-H with PCV between 22-31, 31 (15.5%) are tested positive to *S. paratyphi* B-H with PCV between 23-30, 28 (14%) are tested positive to *S. paratyphi* C-H with PCV between 23-28 and 40 (20%) are tested positive to *S. typhi* H with PCV between 24-35. The value of Widal test depends upon the standardization and maintenance of the antigens to produce consistent results, and it has become evident from work done in recent years on standardization of the Widal test and interpretation of the results that both the O and H antigens are necessary for proper serologic analysis of the suspected serum. However, according to Welch in 1936 (reviewed in Olopoenia *et al*., 2000), no Widal test, regardless of the composition and standardization of the antigens used, is infallible, and thus it is unlikely that any will be developed that will lower the validity of the isolation of the aetiologic agent. Sansone *et al*. (1972) published a case report where the Widal reaction to typhoid O antigen on admission for an unexposed patient was 1:320, with an increase in titre to 1:20 480 by the fourth day. In an individual with no prior exposure to *S. typhi* infection (either lack of active infection or absence of passive immunization), a higher than

1:50 or 1:100 titre on an initial single test, usually correlates fairly well with exposure to typhoid fever (Olopoenia *et al*., 2000).

Table 3. Frequency distribution of the respondents according to widal sera (salmonella agglutine titre) in relation to its species and PCV levels.

Salmonellae		No of widal positive (%)	PCV
S. paratyphi A-O	200	56 (28%)	23-30
S. paratyphi B-O	200	48 (24%)	22-27
S. paratyphi C-O	200	63 (31.5%)	23-30
S. typhi O	200	34 (17%)	23-35
S. paratyphi A-H	200	47 (23.5%)	22-31
S. paratyphi B-H	200	31 (15.5%)	23-30
S. paratyphi C-H	200	28 (14%)	23-28
S. typhi H	200	40 (20%)	24-35

However, even these single high-value titres in an endemic area where repeated exposures to *S. typhi* may have occurred, do not have any clinical relevance in the absence of a positive isolate of the causative organism or its antigen. A second sample collection will proof useful. But, in a situation where second sample collection is not feasible, knowledge of the agglutinin levels in the sera of normal subjects from the patients' community can form the baseline on which a diagnosis can be made (Opera and Nweke, 1991; Ibekwe *et al*., 2008).

Also, a negative agglutination test may be for one of several reasons which include: 1) absence of infection by *S. typhi*, 2) the carrier state, 3) an inadequate inoculum of bacterial antigen in the host to induce antibody production, 4) technical difficulty or errors in the performance of the test, 5) previous antibiotic treatment and 6) variability in the preparation of commercial antigens.

A negative Widal test result does not therefore necessarily rule out the absence of infection. Such results are best kept as a reference for subsequent comparative analysis (Olopoenia *et al*., 2000). A positive agglutination tests (on two successive occasions) on the other hand, may also be open to several different interpretations. 1) the patient being tested has typhoid fever, 2) previous immunization with *Salmonella antigen*, 3) cross-reaction with non-typhoidal Salmonella, 4) variability and poorly standardised commercial antigen preparation, 5) infection with malaria or other enterobacteriaceae, 6) other diseases such as dengue (Olopoenia *et al*., 2000). This could lead to confusion in the serological diagnosis of typhoid fever. Therefore, serological findings have to be interpreted with a lot of caution particularly in country like Nigeria where there are yet to be laid down standard baseline titres (Ibekwe *et al*., 2008).

More so, in endemic typhoid regions, a single testing of a serum specimen for Widal agglutinin cannot provide a reliable diagnosis due to: repeated exposure to small inocula of *S. typhi* or to other *Salmonella* spp. that contain type 9 or 12 antigens, previous typhoid fever immunization and other infectious agents such as malaria (Olopoenia *et al*., 2000). Although a number of reports from some developing countries have suggested that a single Widal test is sufficient to make the diagnosis of typhoid fever (Mohammed *et al*., 1992; Rasaily *et al*., 1993; Choo *et al*., 1993), others have disputed the usefulness of such a single test result (Hoffman *et al*., 1986; Aquino *et al*., 1991). In some developing countries where the use of a single Widal test appears to be the norm, there has been an increase in the rate of false positive results (Olopoenia *et al*., 2000).

Typhoid and paratyphoid fevers are common in less industrialized countries, principally owing to the problem of unsafe drinking-water, inadequate sewage disposal and flooding. Public health interventions to prevent typhoid and paratyphoid include: 1) health education about personal hygiene, especially regarding hand-washing after toilet use and before food preparation; provision of a safe water supply; 2) proper sanitation systems; 3) excluding disease carriers from food handling. Control measures to combat typhoid include health education and antibiotic treatment. A vaccine is available, although it is not routinely recommended except

for those who will have prolonged exposure to potentially contaminated food and water in high-risk areas. The vaccine does not provide full protection from infection (WHO, 2008).

The review of Olopoenia *et al*. (2000) and Adeleke *et al*. (2006) suggesting Widal agglutination test as being bedeviled with controversies in term of quality of *Salmonella antigens* and interpretation of results is also pertinent. It should be stressed that a single Widal agglutination test has no diagnostic significance.

According to Hoffman *et al*. (1986), the results of a single Widal test, tube dilution, micro-agglutination or slide agglutination are virtually un-interpretable unless the sensitivity and specificity of the test for the specific laboratory and patient population are known, as well as predictive values. Even in the extreme case of a high titre in a single Widal agglutination test, the causative organism may often be due to other species of *Salmonella*, rather than *S. typhi* (Olopoenia *et al*., 2000). Thus, for a more definite diagnosis of typhoid fever, serologic test and ood culture as well as stool culture from every patient are quite relevant. Therefore, efforts must be made however, to confirm the diagnosis by paired sera investigation more than in presently the case.

In conclusion, it is clear that *Salmonella agglutinins* are common among patients. Obviously therefore, the prevalence of typhoid fever and the phenomenon of Multi-Drug Resistance (MDR) of its causative agent are seriously constituting a menace in poor developing tropical countries. The resultant effect on health status would affect productivity, intellectual development and other aspects of life. There is therefore an urgent need, for measures to curtail the spread of the disease such as good sanitary habit and development of more reliable and efficient means of identification of pathogens in the case of typhoid fever as well as development of good dietary habits by the public in the case of anemia caused by diet.

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