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Assessing Clot Culture Technique and Relationship between Typhoid Antibodies and Red Blood Cell Indices

Gabriel Lankaji Binda[1*] and Francis Britwum[2]

[1]Hematology Department, Cape Coast Teaching Hospital, Ghana

[2]Department of Education and Psychology, University of Cape Coast, Ghana

[*]Corresponding e-mail: lankajibinda@gmail.com

ABSTRACT

Enteric or typhoid fever is a systemic infection mostly caused by Salmonella typhi and Salmonella paratyphi A. Blood culture and rapid diagnostic antibody detection is widely used in the diagnosis of enteric fever in Ghana. Isolation of Salmonella from blood culture is challenging. The study accessed clot culture technique in diagnosing typhoid fever. The correlation between parameters of red blood cell indices and IgM IgG was also assessed. 400 patients were suspected of having typhoid fever. 94 were not on medication or treatment for typhoid fever. 43 out of the 94 were typhoid IgM positive. Clot culture and the conventional culture techniques were used to isolate Salmonella from the 43 patients. Complete blood counts were obtained from 94 patients. The results showed no bacteria growth for both clot culture and conventional blood culture techniques. The presents of typhoid IgG in the blood is weakly associated with decreasing levels of red blood cell count irrespective of age and sex. For every unit increase in mean cell hemoglobin concentration and hematocrit, the odds of not detecting the presents of typhoid IgM is 0.56 and 0 respectively at a 95% confidence interval. Therefore increasing concentrations of mean cell hemoglobin and hematocrit will result in a false-negative IgM RDT test which is not a result of the faulty Test kit or test procedure. Based on the findings of this research, diagnosing typhoid fever with blood culture remains a challenge as well as IgM detection using RDT test kitsn.

Keywords: Immunoglobulin M (IgM), Immunoglobulin (IgG), Red blood cells, Hematocrit, Mean cell hematocrit concentration, Mean cell volume, Rapid Diagnostic Test (RDT)

INTRODUCTION

Typhoid fever, also called enteric fever, or simply 'typhoid', is a systemic illness caused by a bacterial infection with *Salmonella enterica* subspecies *enterica* serotype *typhi* or serotypes *paratyphi* A, B, or C [1]. Typhoid infects the intestinal tract and occasionally the bloodstream [2]. An infected person can spread typhoid fever to others as long as *Salmonella typhi* bacteria are passed in his/her stool and urine [3]. There is a vaccine for typhoid fever, but it is not recommended for routine use. The vaccine is given to people who work in jobs that put them at risk for infection (such as laboratory workers), people traveling to countries where typhoid is common, or have a family member who is ill with typhoid [4].

Typhoid can be prevented by washing hands thoroughly with soap and warm water before preparing food, before eating, and before feeding children. Washing hands thoroughly with soap and warm water after changing diapers and using the toilet. Anyone who has diarrhoea should not prepare food for others. Ensuring all bodily wastes are properly discarded. This includes the washing or disposal of soiled diapers. Avoid drinking untreated water and consuming only pasteurized milk and dairy products, avoid eating raw or undercooked fish and shellfish. Food should be boiled, cooked, or peeled before eating when traveling to areas with high typhoid prevalence, this will help prevent typhoid infection [5]. Typhoid can be transmitted usually from the first week of illness throughout convalescence. Chronic carrier state (<5% of the population) is usually linked to the biliary or urinary tract and should be distinguished from the short-term fecal carriage. Approximately 10% of untreated patients will shed for 3 months after the onset of

symptoms. Typhoid fever is diagnosed with Stool, urine, bone marrow, or blood for culture. Treatment may include antibiotics or corticosteroids [6].

Background

12.5 million new cases of typhoid were recorded worldwide in 2015 [7]. The global cases of typhoid have plateaued with 11 million to 18 million illnesses and 128,000 to 190,200 deaths per year [8]. The places with a high incidence of typhoid fever (>100/100,000 cases/year) are south-central Asia and south-east Asia and places of medium incidence (10-100/100,000 cases/year) include the rest of Asia, Africa, Latin America, and the Caribbean, and Oceania, except for Australia and New Zealand. Europe, North America, and the rest of the developed world where there is a low incidence of typhoid fever (<10/100 000 cases/year). It is estimated that typhoid fever caused 21,650,974 illnesses and 216,510 deaths in the year 2000 and paratyphoid fever caused 5,412,744 illnesses [9].

It is estimated that 17.8 million cases of typhoid fever occur each year in low and middle-income countries (95% credible interval: 6.9-48.4 million). Central Africa was predicted to experience the highest incidence of typhoid, followed by select countries in Central, South, and Southeast Asia. Incidence typically peaked in the 2-4-year-old age group. Models incorporating widely available economic and environmental indicators were found to describe incidence [10].

Since 1900, improved sanitation and successful antibiotic treatment have steadily decreased the incidence of typhoid fever in the United States. In 1920 there was 35,994 cases of typhoid fever were reported. In 2006, there were 314. It was estimated that 79% of typhoid fever cases occurred in patients who had been outside of the United States [11].

The updated mean annual typhoid fever incidence per 100 000 people decreased from 537 to 348 in East Africa and increased from 160 to 422 in West Africa. The data for West Africa represented six countries, which were Burkina Faso (two sites), Guinea-Bissau, Ghana, Tanzania (two sites), Kenya, and Madagascar [12]. In 2003 the outpatient morbidity patterns in Ghana continue to show a high incidence of communicable diseases which is typical of the disease profile of a developing country. A critical analysis of the morbidity data showed that typhoid fever incidence is 2.1 percent [13].

Statement of the Problem

Enteric fevers are severe systemic forms of Salmonellosis. The best-studied enteric fever is typhoid fever and it is caused by the organism *Salmonella typhi*, and *Salmonella paratyphi* A, B or C. *Salmonella* organisms penetrate the mucosa of the small and large bowel, where there is intracellularly proliferation of the organism which tend to cause ulceration of lymphoid follicles. Initially *Salmonella typhi* proliferates in the second part of the Payer's patches of the lower small intestine from where systemic dissemination occurs, to the liver, spleen, and reticuloendothelial system. For a period varying from 1 to 3 weeks, the organism multiplies within these organs. Rupture of the infected cell occurs, liberating organisms into the bile and for a second time cause infection of the lymphoid tissue of the small intestine, particularly in the ileum. It is this phase of heavy infection that brings the classical bowel pathology of typhoid in its train. Invasion of the mucosa causes the epithelial cells to synthesize and release various pro-inflammatory cytokines including IL-1, IL-6, IL-8, TNF-β, INF, GM-CSF, etc. [14]. Human infections with *Salmonella enterica* results in two major groups of diseases: gastroenteritis and typhoid fever. Clinical observations suggest that gastroenteritis, caused by non-typhoidal *Salmonella* serovars, is characterized by a massive neutrophil influx, which keeps the infection localized to the intestinal mucosa. In contrast, the absence of neutrophilic intestinal infiltrate in the acute phase of typhoid fever suggests a propensity for typhoidal *Salmonella* serovars (*S. typhi*, *S. paratyphi A*, *S. paratyphi B,* and *S. paratyphi C*) to evade aspects of the innate immune response and cause a systemic infection. The fact that there are no virulence genes shared by typhoidal *Salmonella* serovars that are absent from non-typhoidal *Salmonella* serovars, suggests that this innate immune evasion is mediated by different mechanisms in different typhoidal serovars [15]. The presents of Immunoglobulin M in qualitative RDT is used in the diagnosis of typhoid fever in community hospitals and health centers where blood culture diagnosis of enteric fever cannot be performed [16]. The top twenty causes of outpatient morbidity from the year 2002 to 2017 in Ghana include enteric fever which had a total case of

36,5148 in 2017 making 1.2% of the total number of cases of outpatient diseases. There is a decline in the incidence of *Salmonella typhi*. The true isolation of *Salmonella typhi* from routine blood cultures is still challenging [17]. Blood culture sensitivity for *Salmonella typhi* cannot be used as a representation of the proportion of *Salmonella typhi* cases detected in disease burden measures as it underestimates the real problem. It has been proposed that future research should focus on measuring the proportion of typhoid fever cases detected by blood culture based on standardized existing blood and bone marrow culture or another reasonable reference standard in the field settings which will help in understanding the true proportion of *Salmonella typhi* cases identified by blood culture [18]. The challenge in the isolation of *Salmonella typhi* from routine blood cultures and the influence of typhoid fever on hematological parameters necessitated this study [19].

Aim of the Study

To access clot culture technique for isolating *Salmonella* and determine the relationship between typhoid antibodies and red blood cell indices.

Objectives

- To isolate and identify the agents causing enteric fever (*Salmonella typhi*, *Salmonella paratyphi* A, B, and C) using clot culture and conventional blood culture method
- To determine the prevalence of typhoid fever concerning antibody Rapid Diagnostic Testing (RDT) testing
- To establish the correlation of typhoid antibodies and red blood cell indices

Justification

The best laboratory diagnosis of enteric fever is PCR-based amplification of bacteria DNA in the blood of typhoid fever patients. This technique is not available where it is most needed [20]. Due to the unavailability of PCR-based amplification in cape coast teaching hospital The findings of this study will help solve the challenge of isolating *Salmonella typhi* from routine blood cultures and will increase the diagnosis of enteric fever in cape coast teaching hospital. *Salmonella typhi* is a frequent cause of bloodstream infections and it has high rates of antibiotic resistance and blood culture is the best sample for the isolation of *Salmonella* species [21,22].

MATERIALS AND METHODS

Study Area

The study will be conducted on Cape Coast in the Central Region of Ghana. The traditional name of Cape Coast 'Oguaa' originates from the Fante word 'gua' meaning market. It was raised to the status of municipality in 1987 by LI 1373 and upgraded to metropolitan status in 2007 by LI 1927. The location of Metropolis is bounded to the South by the Gulf of Guinea, to the West by the Komenda Edina Eguafo Abrem Municipality (at Iture bridge), to the East by the Abura Asebu Kwamankese District, and to the North by the Twifu Heman Lower Denkyira District. It is located on longitude 1° 15'W and latitude 5°06'N. It occupies an area of approximately 122 square kilometers, with the farthest point at Brabedze located about 17 kilometers from Cape Coast, the Central Regional capital. The Cape Coast Metropolis experiences high temperatures throughout the year. The hottest months are February and March, just before the main rainy season, while the coolest months are June, July, and August. The variability in climate in the Metropolis is influenced more by rainfall than temperature. The Metropolis has a double maximal rainfall, with an annual rainfall total between 750 mm and 1,000 mm. Cape Coast is endowed with many schools across the length and breadth of the Metropolis, ranging from basic to tertiary institutions. These schools attract people from all over the country and the West Africa Sub-region, who pursue various levels of academic and professional education. The Metropolis is endowed with a regional hospital, a district hospital, and various clinics that provide health care to the population. The regional hospital, one of three such facilities in the country serves as a referral center for the region. The 2010 National Population and Housing Census results put the district's total population at 169,894 distributed across all ages and different sexes. The total population consists of 87,084 females and 82,810 males [23].

Study Population

The study population will consist of patients who come to the cape coast teaching hospital for health care.

Sample Size

The sample size will be determined by using a sample population formula considering the following assumptions. Za/2=1.96 for the standard scale of 95% level of confidence, Response distribution, P of 50%, and level of precision of 5%. Given the formula N= [Z2 (P) (1-P)]/(Error)2] Where N=Sample size, Z=1.96, Error=5%, P=50%

A sample size of 400 will be representative of the cape coast population of 169894.

Study Design

A cross-sectional study will be used to determine *Salmonella* in Patients who have tested positive for typhidot IgM or IgM IgG from all age groups at the cape coast teaching hospital.

Criteria for Inclusion

- Patients visiting the laboratory with suspected typhoid enteric fever of all age groups were included in identifying the relationship between the presents of antibodies and red blood cell indices.
- Patients positive for IgM typhoid rapid diagnostic test and not on medication or treatment were included in accessing the clot culture technique.

Criteria for Exclusion

- All patients visiting the laboratory who are not suspected of having typhoid enteric fever.

Sample Collection

A convenience sampling technique was used to collect 400 samples from patients included in the study. Enteric fever suspected patients visiting the laboratory were sampled for typhoid Immunoglobulin qualitative testing. 10 ml of venous blood specimens were collected following the standard phlebotomy protocol into brain heart infusion broth (4.5 ml of blood incubated at 37°C), the sterile red-top test tube containing sterile glass beads (4.5 ml of blood and immediately mixed with glass beads until the clot is formed) and an EDTA tube (1 ml).

Laboratory Analysis

EDTA sample was used for complete blood count analysis using Sysmex XN350 analyzer and qualitatively test for IgG and IgM using JusChek typhoid rapid test cassette following the SOP as indicated by the manufacturer. The clotted sample in the red-top test tube was centrifuged for 5 minutes at 3000 rpm after which serum will be removed aseptically by using a sterile Pasteur pipette. The clot was vortexed for 15 mins and a dissolved clot with hemolysed red blood cells was observed. A well-hemolysed clot showed a bright red liquid sample. The hemolysed clot and the blood culture sample in brain heart infusion were all incubated at 37ºC. After 24hours I subcultured the samples on blood agar, chocolate agar, and McConkey agar for 24 hrs. A no bacteria growth outcome of the subculture necessitated a repetition of subculture for five consecutive days (as the subculture continued to show no growth).

Data Management and Analysis

The data collected from the microbiological, hematology, and serology analysis, full blood count data were analyzed using Microsoft excel 16 professional and IBM SPSS version 26.

RESULTS

Demographic Characteristics of Patients with Suspected Enteric Fever Diagnosis

This study aimed to access clot culture technique for isolating *Salmonella* and determine the relationship of red blood cell indices and typhoid antibodies. A total of 400 patients were suspected of having typhoid enteric (Table 1).

Table 1 Demographic data and typhoid prevalence

Typhoid IgM		NEG	POS	% Prevalence n=400
Age (years)	9 or less	5	2	0.5
	12-19	18	11	2.75
	20-29	70	29	7.25
	30-39	67	29	7.25
	40-49	45	22	5.5
	50-59	41	9	2.25
	60-69	16	11	2.75
	70-79	16	6	1.5
	80 and above	2	1	0.25
Gender	Female	193	90	22.5
	Male	87	30	7.5
IgG	Negative	265	48	12
	Positive	15	72	18
Typhidot	Negative	269	2	0.5
	Positive	11	118	29.5
Conventional culture	No bacteria growth	0	43	10.75
Clot culture	No bacteria growth	0	43	10.75

A total of 400 patients were involved in the study with 283 (70.8%) being females and 117 (29.3%) being males. 90 (22.5%) females and 30 (7.5%) males tested typhoid positive whiles 193 (48.25%) females and 87 (21.75%) males tested negative for typhoid. Ages between 20 years and 39 years were the age group with the highest prevalence of typhoid enteric fever of 7.25%. A total of 120 patients tested positive for typhoid IgM.

Isolating *Salmonella typhi* from the blood sample

The study also aimed to isolate *Salmonella typhi* bacteria from a blood sample of all participants that tested positive for typhoid IgM who were not on *Salmonella typhi* medication or treatment. A total of 400 participants were involved in the study. 120 of them tested positive for typhidot, Out of these 48 patients tested positive only for IgM while 72 patients tested positive for both typhidot IgM and IgG. 43 patients out of the 400 patients tested positive for IgM and were not on typhoid medication or treatment and hence their blood samples were used for the isolation of *Salmonella typhi* with conventional blood culture technique and blood clot culture technique.

Salmonella typhi bacteria were not isolated from all 43 blood culture samples in both blood culture techniques.

Complete Blood Count Relationship with typhidot

A total of 94 patients who tested positive for typhidot IgM also had complete blood count analysis performed.

Bivariate Correlation Analysis between Red Cell Parameters and Typhoid IgM

Qualitative Typhoid rapid diagnostic testing is used in the diagnosis of enteric fever. Complete Blood Count (CBC) was performed on samples from 94 patients out of 400 patients involved in the study. The 94 patients were not on medication for typhoid fever. 43 out of the 94 patients tested positive for typhoid IgM (current infection) and 51 patients out of the 94patients tested negative for typhoid IgM and positive for IgG (no current infection). A bivariate correlation was performed to explore the relationship between red cell parameters and typhoid IgM (Table 2).

Table 2 Bivariate correlation between IgG, IgM, and Red cell parameters

	IgM	IgG
RBC (10^6/UL)	-0.165	-0.223*
HGB (g/dl)	-0.069	-0.159
HCT%	-0.18	-0.196
MCV (fl)	0.045	0.111
MCH (pg)	0.151	0.112
MCHC (g/dl)	0.239*	0.048
Note N=90, *: Correlation is significant at the 0.05 level (2-tailed).		

The relationship between typhoid IgM and red blood cell count was investigated using Pearson product-moment correlation coefficient. Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity, and homoscedasticity. There was a small negative correlation between typhoid IgG and red blood cell count estimation where r= -0.223, $p<0.05$, n=90. Where typhoid IgG is associated weakly associated with lower levels of Red blood cell count estimation. There was a weak positive correlation between typhoid IgM and mean cell hemoglobin concentration where r=0.239, $p<0.05$, and n=90. IgM is weakly associated with increasing the concentration of mean cell hemoglobin concentration. A positive correlation exists between typhoid IgM and means cell hemoglobin concentration where r=0.239, $p<0.05$, and R^2=0.0571. Mean cell hemoglobin has a mean and standard deviation of 32.62 and 1.443315 respectively.

Partial Correlation of FBC Parameters and Typhoid Antibody

A partial correlation was used to explore the relationship between typhoid IgG, IgM, and red blood cell parameters while controlling for scores on the Marlowe-Crowne Social Desirability Scale. Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity, and homoscedasticity. There was a weak, positive, partial correlation between MCHC and IgM controlling for sex and age, r=0.265 n=90, $p<0.011$, results of the zero-order correlation is r=239, n=92, $p>0.021$ indicating that age and gender have little effect on the strength of the correlation between MCHC and IgM in suspected cases of typhoid enteric fever.

There was a negatively weak partial correlation with IgG and RBC controlling for age and sex, r= -0.150, a p-value of 0.153 (not significant). The results from the zero-order partial correlation is r= -223 indicating that age and gender do not affect the strength of the relationship between IgG and IgM. There was a positively weak partial relationship between IgG and absolute basophil count controlling for age and sex, r=252, $p>0.015$ but the results for zero-order partial correlation is r=0.262, $p>0.011$. This indicates that age and gender do not affect the strength of the relationship. This is well represented in the table below (Table 3).

Table 3 Partial correlation of CBC variables to IgM (typhoid)

		IgG	IgM
		RBC	**Mchc**
None	Correlation	-0.223	0.239
	Significance (2-tailed)	0.031	0.021
	df	92	92
Age and Sex	Correlation	-0.15	0.265
	Significance (2-tailed)	0.153	0.011
	df	90	90
Note: N=94 Cells contain zero-order (Pearson) correlations			

Logistic Regression of Typhoid IgM Test and Red Blood Cell Indices

Direc t logistic regression was performed to assess the impact of red blood cell indices (in a suspected case of typhoid fever) on the likelihood that suspected cases of typhoid fever infection would show a positive IgM test result. The model contained six independent variables (hemoglobin, percentage hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and red blood cell count). The full model containing all predictors was statistically significant, χ^2 (6, N=94)=22.901, p=0.001, indicating that the model was able to distinguish between patients who tested positive and negative for typhoid IgM. The model as a whole explained between 18.8% (Cox and Snell R square) and 25.2% (Nagelkerke R squared) of the variance in red cell indices, and correctly classified 63.8% of cases. As shown in the table below, only three of the independent variables made a unique statistically significant contribution to the model (hemoglobin concentration, hematocrit and mean cell hemoglobin).

The strongest predictor of typhoid IgM was hemoglobin concentration, recording an odds ratio of 4.47 with a 95% confidence interval (1.7 to 17.81), the large confidence interval indicates a large margin of error in the odds ratio. The odds of hematocrit and mean cell hemoglobin was less than 1, this indicates that for every unit increase in hematocrit or mean cell hemoglobin concentration the odds of detecting the presents of typhoid IgM is 0 and 0.56 respectively (Table 4).

Table 4 Binary logistic regression of CBC and IgM test result

Variables		B	S.E.	Wald	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
Step 1a	RBC/l	0.006	0.069	0.008	0.929	1.006	0.878	1.153
	HB/gl	1.715	0.596	8.27	0.004	5.554	1.726	17.871
	HCT	-563.924	193.724	8.474	0.004	0	0	0
	MCHC	-0.581	0.214	7.382	0.007	0.56	0.368	0.851
	Gender	-0.012	0.008	2.474	0.116	0.988	0.973	1.003
	Age	0.004	0.014	0.068	0.794	1.004	0.977	1.031
	Constant	193.374	70.062	7.618	0.006	9.58E+83		
a: Variable(s) entered on step 1: rbc /l, hb/gl, hct, mchcg, gender, age								

DISCUSSION

Prevalence of Enteric Fever

The prevalence of enteric fever concerning typhoid antibody RDT test among participants involved in the study was 0.3 (30%) due to 120 patients testing positive for typhoid IgM. The study was conducted at the cape coast teaching hospital in Ghana, which provides health care services to the people of Cape Coast metropolis and its environs. Immunoglobulin M indicates current infection and is hence suitable to determine the prevalence of typhoid enteric fever at a particular time [24,25]. The prevalence of typhoid enteric fever in the Cape Coast Metropolis is 300 per 1000 persons.

Typhoid enteric fever had a higher occurrence in females (22.5%) than in males (7.5%). This is in line with a study in Nigeria that determined the prevalence of typhoid among females and males to be 45% and 42% respectively [26]. The study involved administration of questionnaires to patients diagnosed with typhoid fever but another study in Pakistan involving diagnosing enteric fever with qualitative typhoid antibody RDT determined 20.58% and 14.33% respectively in females and males, the study concluded that the prevalence of enteric typhoid fever was higher in males of school-going age [27]. A study conducted in Ghana stated that 16.6% of typhoid enteric fever occurred in males and 24.6% in females, this is in line with my finding that the prevalence of typhoid enteric fever in females is higher than in males [28].

The ages of the patients are between 8 years to 81 years with the majority of the patients at (mean age=38.91 years).

The ages between 20 years to 39 years recorded the highest number of typhoid-positive cases of 58 (14.5%). A study conducted in Pakistan determined the prevalence of typhoid fever to be 22.01% in participants less than 15 years of age [27]. Pakistan has similar environmental sanitation conditions with Ghana but a study conducted in Ghana determined that the prevalence of typhoid fever was highest between the ages of 21 years to 30 years with a prevalence of 31%. The findings of this study relate to the findings of my study where participants between the ages of 20 years to 29 years had the highest prevalence of 7.25% and ages between 30years and 39years had the second-highest prevalence of 7.2.5%.

Most of the participants resided in the Abura community and these participants had the highest prevalence of typhoid fever of 8.5%. This may be because the people of Abura in the cape coast do not have a good source of drinking water, poor sanitation, and poor waste disposal [29].

Isolation of *Salmonella typhi* from Blood Sample

Blood culture using both conventional culture and clot culture techniques were unsuccessful in isolating *Salmonella typhi* from the 43 patients who tested positive for typhoid IgM and were not on any medication or treatment for typhoid. All 43 were out-patient cases and hence had no critical illness from typhoid fever that required hospitalization. Research conducted to evaluate the efficiency of blood culture concluded that blood cultures are inefficient in detecting bacteremia [30]. Even dough blood culture remains the primary means of establishing a diagnosis for *Salmonella typhi* it has been found that the technique is slow and insensitive [31]. The Ghana Ministry of Health indicates that the absence of a national antibiotic use policy that guides the use and control of resistance is also contributing immensely to the upsurge in the abuse of antibiotics in Ghana [32]. A study published in 2020 by the pan African journal indicates there is a high antibiotic misuse in teaching hospitals in Ghana [33]. Inappropriate antibiotic use was influenced by a general lack of knowledge on antibiotics [34]. A study conducted in Cape Coast metropolis established antibiotic abuse among the people living a Cape Coast. The study involved 530 patients from 15 years and above patronizing 11 pharmacy shops in the Cape Coast Metropolis participated in questionnaires and interviews. 59.9% of the interviewees were aware of the harmful potential of antibiotics abuse yet a significant number (71.5%) purchase antibiotics without prescriptions with 69.9% personally requesting specific drugs without seeking advice from the pharmacists ($p<0.01$) [35].

The Relationship between Qualitative Typhoid Test Parameters and Complete Blood Count Parameters

My study established that there is a negative point bivariant correlation between typhoid IgG and red blood cell count where $r= -0.223$, $p<0.05$ with $R^2=0.050$. The presents of typhoid IgG in the blood of the patients is weakly associated with decreasing levels of red blood cell count irrespective of age and sex. A publication by the American Society of Hematology indicates that IgG is gradually accumulated on the surface of red blood cells in pathologic states and *Salmonella typhi* pathology hemolysis of red blood cells occurs as a result of *Salmonella typhi* Ty 2 which is a membrane protein of *Salmonella typhi* [36,37].

Also, logistic regression analysis detected that for every unit increase in mean cell hemoglobin concentration the odds of not detecting the presents of typhoid IgM is 0.56 at a 95% confidence interval and for every unit increase in hematocrit levels there are no odds of detecting the presents of typhoid IgM at a 95% confidence interval and this finding is confirmed by research accessing the changes in some hematological parameters in typhoid fever patients attending Landmark University Medical Center in Nigeria concluded that there is a significant reduction in the hematocrit values in typhoid positive males and females when compared to typhoid negative males and female [19]. Another study on the effect of typhoid or paratyphoid fevers (enteric fever) on basic hematological parameters of the patient, hematocrit, Hemoglobin estimation, and reticulocytes count was carried out a comprehensive study of 200 samples collected from culturally confirmed *Salmonella* patients and healthy individuals obtained a result showing that there was a significant decrease in the hematocrit and hemoglobin [38].

CONCLUSION

Based on the findings of this research, diagnosis of enteric fever with blood culture remains a challenge. The presents of typhoid IgG in the blood is weakly associated with decreasing levels of red blood cell count irrespective of age and sex. For every unit increase in mean cell hemoglobin concentration and hematocrit, the odds of not detecting the

presents of typhoid IgM is 0.56 and 0 respectively at a 95% confidence interval. Therefore increasing concentrations of mean cell hemoglobin and hematocrit will result in a false-negative IgM RDT test which is not a result of the faulty test kit or test procedure.

Recommendation

Further studies using a large sample size should be conducted to bring a clearer picture of diagnosing typhoid fever with blood culture and also establishing the level of false-negative test outcomes of typhoid rapid diagnostic testing by performing ELISA or PCR antibody detection test for patients suspected of having typhoid enteric fever and normal or increased concentrations of mean cell hemoglobin and hematocrit.

DECLARATIONS

Conflicts of Interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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