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Comparative Evaluation of Haematological Parameters in Malaria Infected Children and Teens Age Group Attending State Hospital, Ijebu-Ode, Ogun State

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ABSTRACT

Background: *Plasmodium* parasites, which cause the acute fever sickness known as malaria, are transmitted to people through the bites of infected female *Anopheles* mosquitoes. *Plasmodium falciparum* and *Plasmodium vivax* are two of the five parasite species that cause malaria in humans and are also the two most hazardous.

Methods: A total of 200 subjects were recruited for this study and were grouped into pre-teen age group (1-12 years) and teen age group (13-19 years), among which 50 were control and 50 were malaria infected subjects for each group. Standard laboratory procedure was employed for blood collection and analysis. Parasitological and haematological parameters such as malaria parasite, pack cell volume (PCV), white blood cell (WBC) total and differential count and platelet counts were carried out from the blood samples.

Results: From the 200 subjects recruited for this study, 88 (44%) were males and 112 (56%) were females. The analysis of the haematological parameters between the two groups (preteens and teen) revealed that there was no statistical significant difference among some of the examined haematological parameters except PCV ($p < 0.01$), total WBC ($p < 0.01$), platelet ($p < 0.01$) and haemoglobin ($p < 0.05$). While for other parameters, the differences are not statistically significant; neutrophil ($p > 0.05$), lymphocyte ($p > 0.05$), monocyte ($p > 0.05$), eosinophil ($p > 0.05$), basophil ($p > 0.05$) and platelet ($p > 0.05$) between the preteen and teen age group.

Discussion: These changes in haematological parameters can become profound as the infection's severity rises. As a result, early diagnosis of malaria infection is necessary for proper treatment.

Keywords: *Plasmodium falciparum*, Malaria Parasite, Haemoglobin, Platelet, Monocyte.

INTRODUCTION

Plasmodium parasites, which cause the acute fever sickness known as malaria, are transmitted to people through the bites of infected female *Anopheles* mosquitoes. *Plasmodium falciparum* is the deadliest malaria parasite and the most frequent on the African continent¹. There are five parasitic species that cause malaria in humans and two of these species, *Plasmodium vivax* and *Plasmodium falciparum*, pose the greatest threat. The most common malaria parasite outside of sub-Saharan Africa is *Plasmodium vivax*. The initial signs of malaria, including fever, headache, and chills, can be mild and challenging to diagnose. They typically show 10 to 15 days after the infecting insect bite. *Plasmodium falciparum* malaria can develop to severe disease and death in less than 24 hours if untreated^{2,3}. *Plasmodium knowlesi*, a type of malaria that naturally affects macaques in Southeast Asia, also infects people, resulting in "zoonotic" malaria¹.

Malaria is a serious public health concern. Families are caught in a cycle of illness, pain, and poverty due to the condition and the price of its treatment. Nearly 50% of the world's population, the majority of whom reside in sub-Saharan Africa, are currently at risk of contracting malaria and dealing with associated economic difficulties¹. Malaria kills a child under the age of five almost every day¹. Numerous of these deaths can be avoided or treated. There were 247 million cases of malaria worldwide in 2021, which resulted in 619,000 fatalities. Children under the age of five

made up 77% of these fatalities. This equates to a daily toll of more than 1,000 children under the age of five.

Nearly half of the world's population was susceptible to malaria in 2020¹. Infants, children under five, pregnant women, persons with HIV/AIDS, and anyone migrating to places with high malaria transmission but low immunity, such as migrant workers, mobile communities, and travelers, are at significantly increased risk of contracting malaria and developing severe disease¹. Any disease condition, including endemic illnesses like malaria that might have an impact on human health and appear in a variety of clinical manifestations, is likely to have an impact on changes in haematological parameters. In the tropics, malaria is one of the leading causes of death. In 2010, there were around 219,000,000 cases recorded worldwide⁴. One of the most typical malarial consequences is hemorrhaging, and this plays a significant part in the pathogenesis of malaria⁵. The main cell types that are affected by these alterations include leucocytes, thrombocytes, and red blood cells (RBCs)^{6, 7}. Patients with malaria typically have significantly higher monocyte and neutrophil counts compared to non-malaria infected patients⁸, while patients with malaria typically have significantly lower platelets, WBCs, lymphocytes, eosinophils, RBCs, and Hb levels^{8,9}. Thrombocytopenia is the most typical consequence from malaria infection^{10,11,12}. People with platelet counts below 150,000/L are 12–15 times more likely to contract malaria than people with platelet counts above 150,000/L¹². Findings have demonstrated a correlation between the ratio

of monocytes to lymphocytes and the probability of developing clinical malaria over time¹³. The majority of the five million people in Thailand who live in high malaria transmission areas reside around the border with Burma¹⁴. Clinical diagnosis is widely performed to determine the presence of malaria, especially in these areas. Fever and other signs and symptoms are known to be sensitive indicators of malaria infection, but they lack specificity and positive predictive values, particularly in regions where malaria is less common¹⁵. Additionally, it may be challenging to differentiate between the signs and symptoms of the disease from other viral or bacterial infections¹⁶. The most frequent test and gold standard for identifying malaria infection is often microscopic slide examination of peripheral blood¹⁷. In smear examinations, it is time-consuming and demands technical knowledge. It is widely known that malaria infection causes haematological abnormalities such as leucocytosis and thrombocytopenia. People who live in malaria-endemic areas may find it simple and helpful to obtain these haematological changes, which have diagnostic value. The enormous burden of sickness in early children has largely eclipsed the issue of malaria in adolescents. Although a large number of teenagers are at risk of contracting malaria, little is known about the disease's prevalence and effects in this age range. According to studies, even in regions with steady malaria transmission, malaria is a common cause of clinical disease in adolescents and a preventable cause of mortality. Due to immunological and hormonal variables, younger adolescents may be more at risk than older adolescents¹⁸. Comparing the haematological parameters in malaria patients of the children and adolescent age groups at State Hospital, Ijebu-Ode, Ogun State, was the aim of this study.

Materials and Methods

Study Area

This study was conducted in Ijebu-Ode, Ogun State, Nigeria.

Study Population

This study was carried out among children between the ages of 1 year to 12 years and teens who are between the ages of 13 years to 19 years in Ijebu-Ode, children with malaria infected were recruited from State Hospital, Ijebu-Ode, while control samples (malaria non-infected) were recruited randomly from subjects outside the hospital who were tested to be negative to malaria. 200 sample size of participants of children were selected. The participants were divided into two; preteen (age 1-12) and teen (13-19). 50 participants were selected for control in each group of the participant. The participants were selected within the target population.

Research Design

This is a cross-sectional descriptive study of comparative assessment of haematological parameters in malaria patients of children and teens age group attending State Hospital, Ijebu-Ode Ogun State.

Ethical Consideration

The protocol for this study was sought and approved by the ethics and research committee of Lead City University, Ibadan, Oyo State, with the approval number LCU-REC/22/072 dated 31st January, 2022.

Inclusion criteria

- Children and teens attending State Hospital, Ijebu Ode, Ogun State.
- Children between the ages of 1 year to 12 years and teens between the ages of 13-19 years
- Both male and female subjects
- Those that consented to participate in the study.

Exclusion criteria

- Subjects younger than 1 year old and older than 19 years old
- Those that did not consent to this study
- Sick cell patients

Sampling Technique

The blood samples from children and teens attending State Hospital Ijebu-Ode, were selectively collected for laboratory tests to determine malaria infection as the evidence of infection before proceeding to haematological parameters.

Sample Collection, Processing and Analysis for malaria parasite

A venous blood sample of 2mls was collected into EDTA bottle from each of the children/teens using needle and syringe. Standard and careful laboratory procedures were adopted in collecting blood samples from the subjects. Thick and thin blood films of the blood samples were made on clean dry grease free slides, labelled and allowed to dry. The thin films were fixed with methanol to avoid lysis, allowed to dry for 30 seconds. The thin and thick films were stained with freshly prepared 10% working solution of Giemsa stain for 8-10 minutes. The stain is gently flush by dropping clean water over it and allowed to dry. The films were examined under the light binocular microscope using x100 objective lens, with a drop of oil immersion¹⁹. The presence of malaria parasite in either of the films is regarded as positive, either with scanty, one plus (+), two pluses (++) or three pluses (+++).

Procedure for Packed Cell Volume Estimation

Micro-haematocrit method for packed cell volume estimation: this involves the use of micro haematocrit centrifuge to spin blood in a capillary tube at 12,000rpm for 5 minutes and read using the micro-haematocrit reader.

The capillary tube was filled to about three quarter ($\frac{3}{4}$) with well mixed EDTA

anticoagulated blood. The unfilled end was sealed using a sealant material. The capillary tube was carefully placed in one of the numbered slots of the microhaematocrit centrifuge with the sealed end against the rim gasket (to prevent breakage). The inner lid was carefully positioned to avoid dislodging of the tube and centrifuged at 12,000rpm for 5minutes. Immediately after centrifuging, the packed cell volume was read.

The PCV was read using micro-haematocrit reader with the base of the red cell column (above the sealant) on the 0 line and the top of the plasma column on the 100 line. The PCV was read from scale. The reading point was the top of the red cell columns just below the buffy coat layer (consisting of white blood cell and platelet).

Procedure for Total White Blood Cell Counts

The method for total white blood cell counts involved the use of Neubauer counting chamber and Turks solution using the microscope which is the manual method of counting. About 0.38ml of diluting fluid was measured and dispensed into a small container or tube, 0.02ml of well mixed EDTA anticoagulated venous blood was added and mixed. The counting chamber was assembled, the center grid area of the chamber and

the haemocytometer cover glass was completely clean and dry. The cover glass was slide into position over the grid area and it was pressed down on each side until rainbow colors (Newton's rings) are seen. The solution was remixed by using capillary tube. At an angle of about 45°, the grid area of the chamber was filled with the solution, the filled chamber was left undisturbed for 2 minutes to allow the white cells to settle. To prevent drying of the fluid, the chamber was placed in a Petri-dish on dampen tissue and it was covered with a lid. The underside of the chamber was dried and it was placed on the microscope stage using x10 objective lens with condenser iris closed, the ruling was focused and the white cell. It was focus until the white blood cells appear as small black dot. The cells were counted in the four large corner squares of the chamber

Statistical Analysis

Statistical analysis for Social Sciences (SPSS) version 25 was the statistical package used in analyzing all data obtained. Frequency table, Bar chart and Pearson Chi-Square were used to compare the means of the different analytes at $p < 0.05$ statistical significance.

RESULT

Table 1.0: frequency distribution according to gender and malaria status.

		<i>Frequency (%)</i>
<i>Gender</i>	Male	88 (44)
	Female	112 (56)
	Total	200 (100)
<i>Malaria status</i>	Negative	100 (50)
	Positive	100 (50)
	Total	200 (100)

This shows the frequency and the percentage frequency of the study subjects based on gender and the malaria status. From the 200 participant, it is seen that females constituted the larger percentage of the study (Table 1.0).

Table 2.0: Mean value and standard deviation of haematological parameters across groups

Parameters	Preteen (1-12yrs) (mp -ve) n=50	Preteen (1-12yrs) (mp +ve) n=50	Teen (13- 19yrs) (mp -ve) n=50	Teen (13- 19yrs) (mp +ve) n=50	p-value
Age Mn±SD	6.48 ±3.530	6.12 ±2.974	15.92 ±1.627	15.74 ±1.736	0.023
haemoglobin Mn±SD	10.84 ±1.9104	10.59 ±2.2439	11.84 ±1.616	11.20 ±1.8066	0.031
PCV Mn±SD	32.52 ±5.726	31.78 ±6.741	35.50 ±4.841	33.58 ±5.406	0.001
Total WBC Mn±SD	8683.80 ±4728.266	10152.80 ±5873.058	7156.40 ±3104.843	8157.80 ±6495.080	0.001
Neutrophil Mn±SD	57.78 ±14.966	53.68 ±10.374	53.18 ±12.676	55.94 ±10.135	0.678
Lymphocyte Mn±SD	33.50 ±12.981	37.20 ±10.319	38.02 ±11.741	34.00 ±11.308	0.878
Monocytes Mn±SD	6.74 ±3.294	6.74 ±3.932	6.58 ±2.726	6.70 ±3.358	0.678
Eosinophil Mn±SD	1.96 ±2.040	2.36 ±2.164	2.16 ±2.132	2.44 ±2.681	0.455
Basophil Mn±SD	0.02 ±0.141	0.02 ±0.141	0.04 ±0.283	0.10 ±0.364	0.344
Platelet Mn±SD	256.64 ±129.332	211.96 ±123.757	243.10 ±124.215	180.22 ±80.394	0.001

This shows the mean value and standard deviation of haematological parameters across groups. The groups include Preteen 1-12 years (mp -ve), Preteen 1-12 years (mp +ve), Teen 13-19 years (mp -ve), Teen 13-19 years (mp +ve). Each group consist of 50 subjects (N) (Table 2.0).

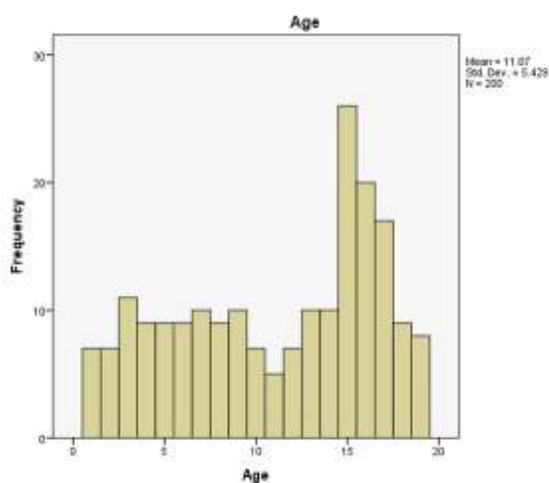


Figure 1.0: Age Frequency of the study

The above chart shows the age frequency of the subjects with age 15 occurring most.

Table 3.0: Mean and standard deviation of haematological parameters for preteen subjects with malaria parasite.

Malaria parasite	Packed cell volume	Total WBC	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil	Platelet	p-value
N	38	38	38	38	38	38	38	38	
% of Total N	76.0%	76.0%	76.0%	76.0%	76.0%	76.0%	76.0%	76.0%	0.677
1+ Mean	31.61	11665.79	55.55	35.34	6.21	2.05	.00	141.13	
Std. Deviation	6.352	9003.769	15.076	14.964	3.042	1.845	.000	44.734	
N	10	10	10	10	10	10	10	10	
% of Total N	20.0%	20.0%	20.0%	20.0%	20.0%	20.0%	20.0%	20.0%	
2+ Mean	17.30	18480.00	55.10	36.40	8.50	1.00	.00	147.40	
Std. Deviation	8.795	11200.74	19.105	15.650	6.096	1.247	.000	61.815	
N	2	2	2	2	2	2	2	2	
% of Total N	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%	
3+ Mean	23.00	9200.00	64.00	24.50	10.00	1.50	.00	114.00	
Std. Deviation	8.485	2828.427	4.243	3.536	7.071	.707	.000	36.770	

This shows the mean and standard deviation of haematological parameters for preteen subjects that are positive to malaria parasite (1+, 2+ and 3+). There are changes in the haematological values of 1+ parasitemia, 2+ parasitemia and 3+ parasitemia. Although the changes were not statistically significant ($p>0.05$). Out of the 50 subjects that are malaria positive in the age group of 1-12 years, 38 of the subjects were certified with 1+ parasitemia, 10 were certified with 2+ parasitemia and 2 were certified with 3+ parasitemia. (Table 3.0).

Table 4.0: Mean and standard deviation of haematological parameters for teen subjects with malaria parasite.

Malaria parasite	Packed cell volume	Total WBC	Neutrophil	Monocyte	Lymphocyte	Eosinophil	Basophil	Platelet	p-value
N	44	44	44	44	44	44	44	44	
% of Total N	88.0%	88.0%	88.0%	88.0%	88.0%	88.0%	88.0%	88.0%	0.455
1+ Mean	32.93	10315.45	65.95	6.20	25.84	2.23	.00	123.48	
Std. Deviation	6.711	7645.991	14.338	3.651	13.362	2.133	.000	32.205	
N	6	6	6	6	6	6	6	6	
% of Total N	12.0%	12.0%	12.0%	12.0%	12.0%	12.0%	12.0%	12.0%	
2+ Mean	35.17	7050.00	69.50	8.50	21.33	.67	.00	98.33	
Std. Deviation	4.215	4332.090	15.110	5.541	12.770	.816	.000	38.229	

This shows the mean and standard deviation of haematological parameters for teen subjects with malaria parasite (1+ and 2+). There are changes in the haematological value of 1+ parasitemia and 2+ parasitemia. Although the change was not statistically significant ($p>0.05$). out of the 50 subjects that are malaria positive in the age group of 13-19, 44(88%) of the subjects were certified with 1+ parasitemia while 6(12%) of the subjects were certified with 2+ parasitemia (Table 4.0).

Discussion

In sub-Saharan Africa, particularly Nigeria, malaria is a major health concern that can be prevented and treated^{2,3}.

Plasmodium falciparum is the most prevalent malaria parasite in the WHO African region, accounting for 99.7% of estimated malaria cases in 2017, *Plasmodium vivax* is the predominant (74.1%) parasite in the WHO region of the Americas². *Plasmodium vivax* contributed 4% of the total global cases in 2015, but outside Africa the proportion was 41% among all malaria infections. Its high burden of disease is maintained in part due to dormant liver stage parasite forms known as hypnozoites which can induce clinical relapse episodes¹⁷.

In this study, 200 subjects were recruited, out of which 88 (44%) were males and 112 (56%) were females. The females constituted the larger percentage of the study. The subjects were divided into pre-teen age group (1-12 years) with a mean age of 6.3 years (SD= 3.25 years) and teen age group (13-19 years) with a mean age of 15.83 years (SD=1.68 years), among which 50 were control and 50 were malaria infected subjects for each of the two groups. The analysis of the haematological parameters between the two groups (preteens and teen) revealed that there was no statistical significant difference among some of the examined haematological parameters except haemoglobin ($p<0.05$), platelet ($p<0.01$), PCV ($p<0.01$), and total WBC ($p<0.01$). While for other parameters, the differences are not statistically significant; neutrophil ($p>0.05$), lymphocyte ($p>0.05$), monocyte ($p>0.05$), eosinophil ($p>0.05$), basophil ($p>0.05$) and platelet ($p>0.05$) between the preteen and teen age group (table 2.0).

Thrombocytopenia was the most common haematological abnormality observed in malaria subjects, both pre-teen and teen age groups (Table 2.0). This is in tandem with Abro et al.,¹⁹ who carried out a study on malaria and "haematological changes, the results indicated that 83% of the subjects recruited for the study had thrombocytopenia. Malaria, which is frequently accompanied by palpable splenomegaly and circulating immune complexes²⁰, is thought to promote platelet destruction and shorten the lifespan of the cells, which is thought to be the cause of thrombocytopenia. Reduced platelet synthesis due to an increase in megakaryocytes in the bone marrow may result in thrombocytopenia in malaria patients¹¹. The immune system's destruction of platelets has been theorized as the cause of the thrombocytopenia seen in malaria infection. Patients with malaria have higher blood levels of immunoglobulin G (IgG), which binds to malaria antigens that are attached to platelets and causes more thrombocytes to be destroyed²¹. According to one study, aggregated platelets are mistakenly counted by analyzers as a single platelet, which results in pseudo-thrombocytopenia¹¹.

Due to its considerable decrease when compared to the control, anemia was the second most often seen haematological abnormality in malaria-infected participants. This suggests that anaemia and *P. falciparum* infection are more frequently linked. This is in line with research by Jain et al.,²² in which anaemia was found in 66 patients (94.28%), of which 37 (56.06%) had *Plasmodium falciparum* infection. According to Abro et al.,²³ who carried out a study on malaria and "haematological changes, the results indicated that 64% of the subjects recruited for the study had anaemia, 24% lymphopenia and 10% monocytosis. Also, the findings of Bawal et al.,²⁴ which observed a reduced PCV among children infected with malaria parasite in Kisumu, western Kenya. Anaemia is

brought on by high parasitaemia, especially when *P. falciparum* is present. *P. falciparum* causes increased red blood cell death. This could be the result of parasitized red blood cells being hemolyzed, the clearance of parasitized red blood cells being expedited, or inefficient erythropoiesis²⁵. A number of variables, including mechanical destruction of the parasitized red blood cells, decreased RBC synthesis in the bone marrow, and phagocytosis of parasite-infected RBC, have been proposed to play a role in the complex and poorly understood pathophysiology of anemia in malaria²¹. One of the most frequent side effects of malaria, particularly in young children and pregnant women in areas with high transmission rates, is anemia.²⁶

According to this study, both *P. falciparum* infected groups had a little lower Hb level than those who weren't infected (Table 2). This is in line with the findings of Maina et al., 2009, who noted a decrease in the level of hemoglobin among *P. falciparum*-infected kids residing in western Kenya. This is also consistent with a study by²⁴, which discovered a substantial rise in the haemoglobin and WBC count in children under five who had malaria compared to the control in a cross-sectional study at the HO Municipality of Ghana.

This study revealed that there was significant difference in total white blood cell count in malaria-infected children (teen and preteen age group) when compared with their controls. This is in tandem with the findings of Maina et al.,²¹ who revealed significant increase in WBC count in children infected with malaria when compared to the control in a cross-sectional study among children in Kisumu, western Kenya, also with the study of Francis et al., 2014 who observed a significant difference the WBC counts of malaria infected subjects when compared with the control. White blood cell alterations caused by malaria can vary based on host immunity, parasitemia, and co-infection levels. Neutrophils, macrophages, and natural killer (NK) cells are examples of effector cells that are stimulated as part of the body's immunological response to infections²⁷.

Conclusion

This study established that there are changes in haematological profile of malaria infected children (pre-teen and teen age group) when compared with non-infected children. Although some of the haematological profile changes are not statistically significant, while some are significant such as the platelet. The common thing that was noticed are thrombocytopenia, reduced packed cell volume and reduced total white blood cell.

Therefore this study concluded that malaria tends to cause reduce platelet, packed cell volume and total white blood cell count.

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The Assessment of The Effect of Plasmodiasis On Some Haematological Parameters of Patients in Rivers State University Teaching Hospital (RSUTH) Port Harcourt

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ABSTRACT

Malaria is a major mosquito-borne public health challenge with various haematological consequences affecting approximately 229 million people worldwide with 409,000 deaths most of which occurred in Africa, followed by South East Asia. These haematological changes caused by plasmodium involves the major cell types such as the Red Blood Cells, White Blood Cells and Platelets. The aim of this study was to assess the effect of plasmodiasis on some haematological parameters of patients attending Rivers State University Teaching Hospital (RSUTH), Port-Harcourt. This was a cross sectional study involving 200 subjects within the age range of 10-90 years of which 125 (62.5%) were males and 75 (37.5%) were females. The presence of plasmodium falciparum malaria was determined by staining a thick and thin blood film with Giemsa stain and viewing under a microscope. Haematological parameters of the patients was analyzed by auto analyzer method using Sysmex XN-550 haematology analyzer. It was shown from the result gotten that male (65.5%) are more exposed to malaria in Port Harcourt, Rivers State than female (35.5%). The study also showed a statistical significance in the Haemoglobin of Male (8.81 ± 3.61) as compared to that of the female (11.62 ± 3.77) ($p=0.0097$) while other parameters showed no statistical significance at $p<0.05$. The result showed a lower mean value for RBC, PCV, HB, MCV, MCH and MCHC of plasmodium parasitized patients when compared with the non- parasitized patients. Higher mean value for WBC, Lymphocytes, Monocytes, and Eosinophils where statistically significant when compared with the control subjects. The result gotten emphasised the need for proper management of *P. falciparum* malaria. It is also recommended that full blood count should be carried out on a malaria parasitized patients in order to guide treatment.

Key Words:

INTRODUCTION

Plasmodiasis is a disease transmitted by a bite of infected mosquito. It occurs in Humans and other animals. Severe disease is largely caused by *Plasmodium falciparum*, while the disease caused by *Plasmodium Vivax*, *Plasmodium ovale* and *Plasmodium Malariae* are generally milder and rarely fatal. *Plasmodium knowlesi* a zoonotic Malaria parasite that causes Malaria in macaques but can also infect Humans, these species of Plasmodium differ in morphology, immunology, geographical distribution, relapsed pattern, incubation time and in drug response (American Mosquito Control Association, 2016). The World Health Organization (WHO) estimates that there were 229 million cases of malaria in worldwide 2019, with 409,000 deaths, most of which occurred in Africa, followed by South East Asia. 67% of all malaria deaths were in children under 5 year of age. (World Health Organization, 2015) Thus, malaria continues to be viewed as a highly significant disease of global public health importance (World Health Organization, 2020).

There are six major Plasmodium species infecting humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale curtisi*, *Plasmodium ovale wallikeri* and *Plasmodium knowlesi* (Sutherland *et al.*, 2010). *Plasmodium falciparum* and *P. vivax* are the two main causes of human malaria infections. *Falciparum* malaria poses a risk of severe complications and contributes to the majority of deaths (White *et al.*, 2013). However, evidence have shown that Nigeria has the highest population at risk of Malaria in Africa and therefore most vulnerable to the risk of missing millennium development goal target (World Health Organization, 2020).

Changes in Haematological parameters are likely to be influenced by any disease condition including endemic diseases, such as Malaria, that can affect health of mankind with various clinical presentations. Malaria is a major cause of deaths in the tropical area of the world. Two hundred and nineteen million cases were reported worldwide in 2010.

Haematological alterations that are thought to characterize malaria are related to the overt biochemical changes that occur during the asexual stage of the life cycle of the malaria parasite (Alexandra *et al.*, 2017).

Haematological changes are some of the most common complications in Malaria and they play a major role in Malaria pathogenesis. These changes involve the major cell types such as RBCs, leucocytes, and thrombocytes. Malaria infected patients tend to have significantly lower platelets, WBCs, lymphocytes, eosinophils, RBCs and Hb level, while monocyte and neutrophil counts were significantly higher in comparison to non-Malaria infected patients (Facer 214). One study showed patients with higher WBCs count compared with community controls. The most common complication during Malaria Infection is thrombocy -topenia. Persons with platelet counts $< 150,000/\mu\text{L}$ were 12-15 times more likely to have Malaria Infection than persons with platelet counts $> 150,000/\mu\text{L}$. (Deng *et al.*, 2016) A previous study found that the ratio of monocytes to lymphocytes correlated with risk of clinical Malaria during follow-up (Kotepui *et al.*, 2014)

MATERIALS AND METHODS

2.1 Study Design

This was a cross sectional study where the subjects were randomly selected. The study was carried out among

patients attending Rivers State University Teaching Hospital in PortHarcourt, Rivers State. Two hundred (200) subjects were recruited for this study and their blood samples were collected to screen for malaria and analyzed for haematological parameters.

2.2 Study Area

This study was carried out in RSUTH, Port Harcourt, River state, Nigeria. Rivers State is situated in the South-South region of Nigeria with a population of 5,198,716 according to 2006 Census report and is located at coordinates, 4° 55' N, 6°50'E. Rivers State is bound at the south by Atlantic Ocean, the North by Imo, Abia, and Anambra States, to the the East by Akwa-Ibom State and to the West by Bayelsa and Delta States. Two hundred (200) subjects were recruited for this study.

2.3 Eligibility Criteria

2.3.1 Inclusive criteria

The subjects included in the study include those within the age range of 10-90years who had malaria parasitaemia with no history of other underlying illness and were not on any anti-malarial drug. Controls group were apparently healthy individuals who were not infected by malaria parasite and other underlying illness.

2.3.2 Exclusive criteria

Those who were excluded from this study include all malaria infected subjects on anti-malaria medication or other medications prior to commencement of this study, Smokers, and alcoholics.

2.4 Ethical Approval and Informed Consent

The Ethics Committee of the Rivers State University, Port Harcourt, Nigeria approved the protocol for this study. A written Informed consent was obtained from the subjects and the institutional authorities.

2.5 Subject Selection

Subjects were selected randomly from the RSUTH having ensured that they met the criteria that made them suitable for inclusion and exclusion.

2.6 Sample Collection and Preparation

5ml of venous blood was antiseptically collected from each individual involved in this study both for the test and control. Each sample was collected and dispensed into ethylenediamine tetra-acetic acid (EDTA) contained bottles and mixed properly for malaria malarasite test and assessment of haematological parameters.

2.7 Laboratory Analysis

2.7.1: Blood film preparation (World Health Organization, 1999; Cheesbrough, 2006)

2.7.1.1: Thin and thick film preparation

Each blood was mixed thoroughly and gently. A drop of blood was placed at the centre of a clean grease free glass slide and another drop of blood at the right side of the same slide with the aid of a Pasteur pipette. Using a smooth edged slide spreader, the drop of blood was spread to make a thin film. The end of a Pasteur pipette was used to spread the drop of blood to make an even thick smear. This was allowed to air dry before staining.

2.7.2: Staining Thin and Thick films using Giemsa's stain

The dried thin film was fixed in methanol. This was then stained with the thick film using Giemsa's stain. 3% solution of Giemsa stain was made and the films were stained for 30 minutes. The back of each slide was blotted with cotton wool and place in the draining rack for drying. The films were examined using x100 Objective lens, (Oil immersion). The presence of malaria parasite was done using the thick blood films while the identification of the species was done using thin blood films. A slide was scored as no parasite seen when 100 per high power fields had been examined without seeing any malaria parasite.

2.9 Statistical Analysis

The data obtained from the study were analysed using the GraphPad Prism Version 8.0.2.263. The data were expressed as mean and standard deviation. Comparison of the means was done using the one-way analysis of variance (ANOVA). The Tukey comparison test was used to verify significant differences between the groups at $P < 0.05$.

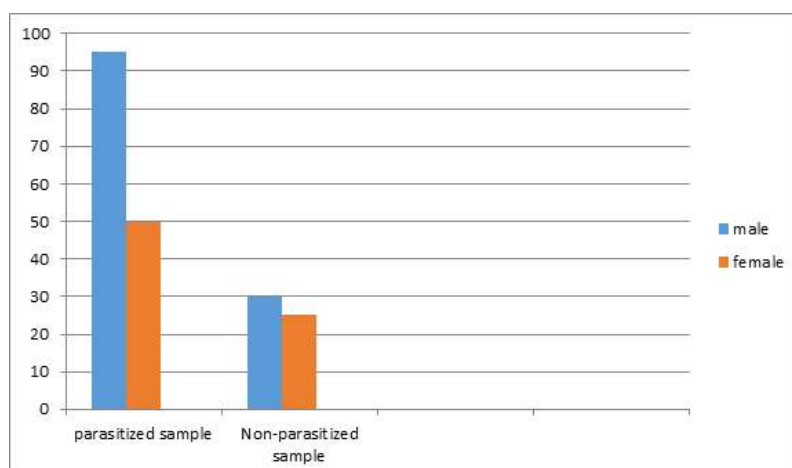
RESULTS

3.1 The Total Number of Malaria Parasitized and Non Parasitized Subjects in relation to gender.

A total of 200 samples were collected for this study of which 145(72.5%) were confirmed to be parasitized by Malaria while 55 (27.5%) samples were non parasitized. Out of this 200 samples 125(62.5%) were from male and 75 (37.5%) were from female. Of the 145 parasitized samples, 95 (65.5%) were from male and 50 (34.5%) were from female. And for the 55 non parasitized samples 30 (54.5%) were from male and 25 (45.5%) were from female.

Table 3.1: The Total Number of Malaria Parasitized and Non Parasitized Sample in relation to gender.

Subject	Male (%)	Female (%)	Total (%)
Parasitized samples	95 (65.5)	50 (34.5)	145 (50)
Non-parasitized samples	30 (54.5)	25 (45.5)	55(50)
Total	125(62.5)	75 (37.5)	200 (100)

Figure 3.1: Chart showing the Total Number of Malaria Parasitized and Non Parasitized Sample in relation to gender

3.2 Comparative Analysis of Haematological Parameters of Malaria Parasitized Subjects Against Non-parasitized subjects

Comparing the haematological parameters of the parasitized and non parasitized sample it was shown that the White Blood Cell had a statistical significance with mean \pm STD of 5.95 ± 3.38 and 4.93 ± 1.17 respectively with a P-value of 0.0457, the Red blood cell had a significance value with mean \pm STD of 3.07 ± 1.42 and 4.63 ± 0.62 respectively with a P-value of 0.0001, the Haemoglobin was statistically significant with mean \pm STD of 10.16 ± 3.92 and 13.47 ± 1.11 respectively with P-value of 0.0001, the Haematocrit was statistically significant with mean \pm STD of 28.64 ± 4.73 and 39.72 ± 3.58 respectively and p-value of 0.0001, Mean Corpuscular Volume was significant with mean \pm STD of 69.52 ± 10.8 and 83.81 ± 8.08 respectively with p-value of 0.0001, Mean Corpuscular Haemoglobin was significant

with mean \pm STD of 23.98 ± 4.90 and 33.63 ± 1.58 respectively with p-value of 0.0001, Mean Corpuscular Haemoglobin Concentration was also significant with mean \pm STD of 28.37 ± 2.99 and 33.63 ± 1.58 respectively with p-value of 0.0001, Lymphocyte was significant with mean \pm STD of 49.09 ± 29.68 and 33.68 ± 5.31 respectively with p-value of 0.0005, monocyte was also significant with mean \pm STD of 10.11 ± 4.04 and 5.20 ± 2.33 respectively with p-value of 0.0001 and Eosinophils was also significant with mean \pm STD of 5.16 ± 0.69 and 3.02 ± 1.36 respectively with p-value of 0.0001. Other parameters like the platelet, Neutrophil and Basophils were statistically non-significant all set at a significant level of $p < 0.05$ as shown in table 4.2 below.

Table 3.2: Comparative Analysis of Haematological Parameters of Malaria Parasitized subjects Against Non-parasitized Subjects

Parameters	Parasitized subjects	Non-parasitized subjects	p-value	T-value	Remark
WBC($\times 10^3$ /ul)	5.95 ± 3.38	4.93 ± 1.17	0.0457	2.024	S
RBC($\times 10^6$ /ul)	3.07 ± 1.42	4.63 ± 0.62	0.0001	7.117	S
HB(g/dl)	10.16 ± 3.92	13.47 ± 1.11	0.0001	5.75	S
HCT(%)	28.64 ± 4.73	39.72 ± 3.58	0.0001	13.21	S
MCV(fl)	69.52 ± 10.8	83.81 ± 8.08	0.0001	7.49	S
MCH(pg)	23.98 ± 4.90	33.63 ± 1.58	0.0001	11.01	S
MCHC(g/dl)	28.37 ± 2.99	33.63 ± 1.58	0.0001	11.01	S
PLT($\times 10^3$ /ul)	210.7 ± 80.94	196.1 ± 39.37	0.2541	1.147	NS
NEUT(%)	59.96 ± 12.07	56.72 ± 6.10	0.0935	1.694	NS
LYM(%)	49.09 ± 29.68	33.68 ± 5.31	0.0005	3.614	S
MONO(%)	10.11 ± 4.04	5.20 ± 2.33	0.0001	7.457	S
EOSINO(%)	5.16 ± 0.69	3.02 ± 1.36	0.0001	9.905	S
BASO(%)	0.29 ± 0.26	0.40 ± 0.49	0.1847	1.336	NS

KEYS: S=Significant, NS=Not Significant. WBC=White Blood Cell, RBC= Red Blood Cell, HCT= Haematocrit, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration, PLT= Platelet, NEUT= Neutrophil, LYM= Lymphocytes, MONO= Monocytes, EOSINO= Eosinophils, BASO= Basophils

3.3 Comparative Analysis of Haematological Parameters of Malaria parasitized Subjects Based on Gender

Comparing the haematological parameters of male and female malaria parasitized subjects, it was shown that only the haemoglobin value was statistically significant with mean \pm STD of 8.81 ± 3.61 and 11.62 ± 3.77 respectively with p-value of 0.0097 while other parameters were statistically non-significant at a significant level of $p < 0.05$

Parameters	Male	Female	p-value	T-value	Remark
WBC($\times 10^3$ /ul)	6.56 ± 3.61	5.29 ± 3.05	0.1872	1.338	NS
RBC($\times 10^6$ /ul)	2.79 ± 1.20	3.39 ± 1.60	0.1357	1.518	NS
HB(g/dl)	8.81 ± 3.61	11.62 ± 3.77	0.0097	2.695	S
HCT(%)	28.00 ± 5.07	29.34 ± 4.34	0.3232	0.9982	NS
MCV(fl)	66.94 ± 11.27	72.32 ± 9.74	0.0781	1.800	NS
MCH(pg)	24.30 ± 4.27	23.64 ± 5.58	0.6421	0.4677	NS
MCHC(g/dl)	28.10 ± 3.13	28.66 ± 2.86	0.5145	0.6567	NS
PLT($\times 10^3$ /ul)	209.1 ± 75.48	212.5 ± 88.07	0.8858	0.1444	NS

KEYS: S=Significant, NS=Not Significant. WBC=White Blood Cell, RBC= Red Blood Cell, HCT= Haematocrit, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration, PLT= Platelet.

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion

This study was carried out to investigate the haematological parameters of malaria parasitized individuals in Rivers State University Teaching Hospital (RSUTH), Nigeria.

A total of 200 samples were collected for this study of which 145(72.5%) were confirmed to be parasitized by Malaria while 55 (27.5%) samples were non parasitized. Out of this 200 samples 125(62.5%) were from male and 75 (37.5%) were from female. Of the 145 parasitized samples, 95 (65.5%) were from male and 50 (34.5%) were from female. And for the 55 non parasitized samples 30 (54.5%) were from male and 25 (45.5%) were from female.

Our findings showed that male (65.5%) are more exposed to malaria in Port Harcourt, Rivers State than female (35.5%). Which is in agreement with a study carried out by Mana et al., (2020) which indicated in their study that male are more exposed to malaria than female in Port-harcourt, Nigeria. This might be because male do more menial jobs than females and these jobs exposes them to a non-hygienic environment exposing them to mosquito bites and consequently malaria infestation. The study also showed a statistical significance in the Haemoglobin of Male (8.81 ± 3.61) as compared to that of the female (11.62 ± 3.77) ($p=0.0097$) while other parameters showed no statistical significance. This is in agreement with a study carried out by Menendez et al., (2000) but in discord with another study carried out by Price et al., (2001), they stated in their study that there is no statistical significance in the Haemoglobin of male and female. This disparity might be due to the difference in sample size for both male and female. In their study they had more male female participants than male, rather in this study we had more male participants.

From the result it is shown that the White Blood Cell had a statistical significance with values of 5.95 ± 3.38 and 4.93 ± 1.17 respectively with a P-value of 0.0457, the Red blood cell had a significance value of 3.07 ± 1.42 and 4.63 ± 0.62 respectively with a P-value of 0.0001, the Haemoglobin was statistically significant with values of 10.16 ± 3.92 and 13.47 ± 1.11 respectively with P-value of 0.0001, the Haematocrit was statistically significant with values of 28.64 ± 4.73 and 39.72 ± 3.58 respectively and p-value of 0.0001, Mean Corpuscular Volume was significant with values of 69.52 ± 10.8 and 83.81 ± 8.08 respectively with p-value of 0.0001, Mean Corpuscular Haemoglobin was significant with values of 23.98 ± 4.90 and 33.63 ± 1.58 respectively with p-value of 0.0001, Mean Corpuscular Haemoglobin Concentration was also significant with values of 28.37 ± 2.99 and 33.63 ± 1.58 respectively with p-value of 0.0001, Lymphocyte was significant with values of 49.09 ± 29.68 and 33.68 ± 5.31 respectively with p-value of 0.0005, monocyte was also significant with values of 10.11 ± 4.04 and 5.20 ± 2.33 respectively with p-value of 0.0001 and Eosinophils was also significant with values of 5.16 ± 0.69 and 3.02 ± 1.36 respectively with p-value of 0.0001. Other parameters like the platelet, Neutrophil and Basophils were statistically non-significant all set at a significant level of $p < 0.05$ as shown in table 4.3. this result is in agreement with previous studies carried out by Ktra et al., (2017) and Abdala, (2019).

Comparing the haematological parameters of male and female malaria parasitized subjects it was shown that only the haemoglobin value was statistically significant with values of 8.81 ± 3.61 and 11.62 ± 3.77 respectively with p-value of 0.0097 while other parameters were statistically non-significant at a significant level of $p < 0.05$ which agrees with previous studies carried out by Mana et al., (2010) and Abdala (2019).

The result from this study as seen in Table 4.3 shows a lower mean value for RBC, PCV, Hb, MCV, MCH and

MCHC of malaria parasitized subjects when compared to the non-parasitized subjects. Higher mean value for WBC, Lymphocytes, Monocytes, and Eosinophils were statistically significant when compared with the non-parasitized subjects which is in agreement with studies carried out by Prce et al., (2001) and Mana et al., (2020). This can be attributed to increased consistent hemolysis caused by malaria paritaemia.

This study confirms that haematological abnormalities in malaria infection are common. Leukocytosis was frequently seen in the malaria-infected patients which was confirmed by another study by Mana et al., (2010) that have demonstrated leukocytosis and contrast with other studies that had demonstrated leucopenia such as those done by Menendez et al., (2018) and Prce et al., (2001). Neutrophil and lymphocyte counts were the most important leukocytic changes associated with malaria infection. The increase in neutrophil count associated with malaria infection is in agreement with previous studies by Mana et al., (2020) which suggested that this increase might be due to activated neutrophil production or release from the marrow or suppressed peripheral removal (Mana et al., 2020).

The observation of increased monocytes counts in this Study is also in agreement with previous studies by Abdala, (2019) and that carried out by Mana et al., (2020). Mononuclear cells, which are activated by Plasmodium during malarial infection, produce inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin-1 (IL1) and interleukin-6 (IL6). These cytokines stimulate the hepatic synthesis of acute phase inflammatory proteins, including CRP, which increase during malaria infection (Abdala, 2019).

Anaemia is one of the most common complications in malaria infection especially in younger children and pregnant women in high transmission areas (Menendez et al., 2018). The pathogenesis of anaemia during malaria infection is not clearly understood. However, It is thought to result from the parasite's primary target in the red blood cell resulting in RBCs destruction, accelerated removal of both parasitized and non-parasitized (Prce et al., 2001), bone marrow dysfunction (Abdala, 2019) and the level of parasitemia (Ktua et al., 2017). This study, reported a significant reduction of Hb level, RBC count, MCV, MCH, and MCHC levels in Patients infected with malaria as compared To non-malaria infected group.

Platelet abnormalities in malaria are both qualitative and quantitative change. In this study, platelet counts was not statistically significant.

4.2 Conclusions

In conclusion, the association of haematological parameters of plasmodium parasitized individuals attending Rivers State University Teaching Hospital was determined. The most commonly changed parameters were Hb, RBC, MCV, MCH, MCHC, WBC, neutrophil, and lymphocyte counts. Presence of anaemia in people from endemic areas may be useful as supportive diagnostic criteria for malaria in case with low level of parasite number. Therefore, when used with other clinical and microscopy parameters, it can significantly improve malaria diagnosis and timely further treat for malaria infection.

4.3 Contribution to Knowledge

This study has contributed the following to knowledge:

1. It has been established from the result of this study that male are more exposed to malaria than female.
2. It has also shown from the result of this study that the most commonly affected parameters by plasmodiasis were Hb, RBC, MCV, MCH, MCHC, WBC, neutrophil, and lymphocyte counts.

4.3 Recommendation

It is therefore recommended that proper management of *P. falciparum* malaria should be carried out in order to know when to administer treatment. It is also recommended that full blood count should be carried out on a malaria parasitized patients in order to guide treatment. Further study should be carried out for determine the effect of malaria on the haematological parameters of individuals.

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Fast Food Consumption and Dental Caries among Teenagers

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ABSTRACT

Background: Dental caries in teenagers is a significant public health concern. Fast food consumption among teenagers in Bangladesh is steadily increasing. The objectives of the study were to determine the association between fast food consumption and dental caries among teenagers.

Methods: This cross-sectional study was conducted from January to December 2022. The calculated sample size was 345 teenagers from two secondary schools in Dhaka city. The convenience sampling technique was used to collect data through face-to-face interviews with a pretested, semi-structured questionnaire. A checklist was also used to collect data through clinical examination of the oral cavity. By double-checking, data were altered for quality control. Statistical software was used to analyze the data.

Results: Among 345 teenagers, the mean \pm SD age was 14.88 ± 1.17 years. The majority of the teenagers (53.6%) were female and Muslim (95%). Around 59.4% of the teenagers' fathers were business owners, while 37.4% were government employees. The majority of respondents (73.3%) ate breakfast before going to school and 52.2% took food from the school canteen. The majority (93.3%) ate at outside restaurants, with 90.4% eating fast food. 59.7% of people ate fast food at least once a week. Dental caries affected 36.8% of the teenager who ate fast food ($p < 0.05$). A significant association was found between dental caries and the frequency of fast-food consumption. Among the respondents, 4.8% had dental caries who ate fast food daily ($p < 0.05$).

Conclusion: To prevent dental caries, teenagers should avoid eating fast food and snacks between meals and brush their teeth on a regular basis.

Keywords: Dental caries; fast food; teenagers.

INTRODUCTION

Globalization has resulted in a nutritional shift away from home cooking and toward fast food, which has contributed to an increase in dental caries among teenagers [1,2]. Fast food lacks micronutrients such as vitamins, minerals, amino acids, and fiber. Fast food refers to foods that are perceived to have little or no nutritional value. It contains a lot of refined sugar, white flour, trans fat, poly saturated fat salt, and a lot of food additives. It also lacks proteins, vitamins, minerals, and fiber [3]. Diet and nutrition impact oral cavity growth, health, and disease development, affecting tooth integrity, pH, saliva, and plaque makeup [4,5]. The frequency, with which cariogenic foods are consumed, particularly between meals, is strongly related to the risk of tooth decay [6]. Dental caries is a multifaceted disease. Dental caries remains a major public health issue around the world. Dental caries is increasing in both developed and developing countries. Caries prevalence has been reported to range up to 12% in developed countries, whereas it has been reported to be as high as 70% in less developed countries, particularly among disadvantaged groups [7,8]. In Bangladesh, dental caries is a prevalent oro-dental issue. Dental caries affects almost 40% of children under the age of five. It has been observed that around 88% of people aged 13 to 22 are missing one or more permanent teeth as a result of dental caries or a related condition [9]. The aim of the study was to determine the association between fast food consumption and dental caries among teenagers.

METHODS

This cross-sectional study was carried out from January 2022 to December 2022. The study place was P.M Pilot

boys' school, Zinzira, Keranigong, Dhaka. And P.M Pilot girls' school, Zinzira, Keranigong, Dhaka. The study population comprised of teenagers aged 13 to 17 years from above mentioned institutions in Dhaka district.

Convenience sampling technique was used for sample selection. Face-to-face interviews were conducted using a semi-structured questionnaire and a clinical examination checklist. Before data collection pretesting was done among 35 teenagers at Shahid Cadet Academy School, Keranigonj, Dhaka to check the accuracy and degree of reliability of the questionnaire. To ensure consistency, the data were manually checked and edited after collection, and then coded, entered and analyzed with SPSS 25. The descriptive analysis made use of frequency, mean, standard deviation, and percentage. Chi-square test used to determine the association between fast food consumption and dental caries among teenagers.

RESULTS

In our study, among 345 students, 125 (36.3%) respondents belonged to 13-14 years and 220 (63.7%) belonged to 15-17 years. Among them 53.6% were male and 46.4% were female. The religion of the majority of respondents were Islam (95%) and 59.4% of the respondents were businessmen (Table 1). Whereas the majority of respondents (322 (93.3%)) ate at a restaurant, 23 (6.7%) did not eat at a restaurant (Table 2).

Most of the respondents 312 (96.9%) liked to eat fast foods in restaurants, 21 (6.5%) of the respondents ate Chinese food in restaurants, 8 (2.5%) of the respondents ate Indian food in restaurants, 19 (6%) of the respondents ate Hossain

Bangla food in outside restaurants (Table 3). Table 4 also revealed that majority of the respondents 206 (59.7%) ate fast food weekly, 16 (4.6%) respondents ate fast food daily, 38 (11.0%) respondents ate fast food monthly, 52 (15.1%) respondents ate fast food occasionally. Among the respondents, 73.3% took breakfast before going to school and 52.2% took food from the school canteen (Table 5).

According to Table 6, the majority of the respondents, 218

(63.2%), had no caries, while 127 (36.8%) had caries. Table 7 shows the relationship between dental caries and respondents' preference for eating out. The findings revealed a significant relationship between dental caries and respondents' preference for eating out. ($p < 0.05$). Table 8 shows the relationship between dental caries and the frequency of fast-food consumption. The results showed that there was a significant relationship between dental caries and the frequency of fast-food consumption ($p < 0.05$).

Table 1. Distribution of the respondents by Socio-demographic status (n-345)

Socio-demographic status	Percentage (%)	Total (%)
Age		100
13 - 14 Years	36.3	
15- 17 Years	63.7	
Sex		100
Male	53.6	
Female	46.4	
Religion		100
Islam	95	
Hinduism	5	
Occupation		100
Service	37.4	
Business	59.4	
Day labor	2	
Farmer	1.2	

Table 2. Respondent distribution at an outside restaurant (n-345)

Eating in outside Restaurant	Frequency	Percentage
Yes	322	93.3
No	23	6.7
Total	345	100.0

Table 3. The distribution of respondents based on the types of foods consumed in outside restaurants (n-322)

Types of Outside Restaurants	Frequency	Percentage
Chinese food	7	2.2
Indian food	2	0.6
Fast food	312	96.9
Bangla food	1	0.3
Total	322	100.0

Table 4. Distribution of respondents based on frequency of fast-food consumption (n-312)

Frequency of fast-food consumption	Frequency	Percentage
Daily	16	4.6
Weekly	206	59.7
Monthly	38	11.0
Occasionally	52	15.1
Total	312	100.0

Table 5. The distribution of respondents according to the food habit during school time (n-345)

Category	Frequency	Percentage (%)
Breakfast		
Taking breakfast before going to school	253	73.3
Taking breakfast at school	92	26.7
Take foods from the school canteen		
Yes	180	52.2
No	165	47.8

Table 6. The distribution of respondents according to the presence of dental caries (n=345)

Dental caries	Frequency	Percentage
Present	127	36.8
Absent	218	63.2
Total	345	100.0

Table 7. Association between dental caries and the respondents' preference to eat at an outside restaurant (n=345)

Preference to eat at an outside restaurant	Dental caries		Total f(%)	Statistics
	Yes f (%)	No f(%)		
Yes	126(36.5%)	196(56.8%)	322(93.3%)	Chi-square test $\chi^2 = 11.165$ $p=0.000$ ($p<0.05$)
No	1(0.3%)	22(6.4%)	23(6.7%)	
Total	127	218	345	

*Statistically significant

Table 8. Association between dental caries and frequency of eating fast foods of the respondents (n=312)

Frequency Eating fast foods	Dental caries		Total f(%)	Statistics
	Yes f(%)	No f(%)		
Daily	154(4.8%)	1(0.3%)	16(5.1%)	Chi-square test $\chi^2 = 24.256$ $p=0.000$ ($p<0.05$)
Weekly	80(25.6%)	126(40.4%)	206(66.0%)	
Monthly	10(3.2%)	28(9.0%)	38(12.2%)	
Occasionally	16(5.1%)	36(11.5%)	52(16.7%)	
Total	121	191	312	

*Statistically significant

DISCUSSION

The respondents' age distribution revealed that a sizable proportion were between the ages of 13 and 17. Specifically, 36.3% of the participants were aged 13 to 14, while the vast majority, 63.7%, were aged 15 to 17. This distribution is consistent with previous research indicating that teenagers constitute a significant demographic in terms of fast-food consumption [10,11]. Furthermore, an overwhelming majority (90.4%) of respondents said they ate fast food, with only a minority (9.6%) saying they didn't. These figures highlight the pervasiveness of fast-food consumption among teenagers, correlating with previous research highlighting fast food's popularity among this age group [12]. The analysis of respondents' fast food consumption patterns revealed varying levels of frequency. The majority (59.7%) reported eating fast food at least once a week, with a smaller percentage eating it on a daily (4.6%), monthly (11.0%), or occasional (15.1%) basis. Furthermore, a small percentage of respondents (9.6%) said they avoided fast food entirely. These findings reflect the diverse nature of fast-food consumption habits among the adolescent population, reflecting a range of preferences and behaviors [13]. Importantly, the study found a link between dental caries and respondents' preference for Hossain et al.; Asian J. Med. Health, vol. 21, no. 10, pp. 204-209, 2023; Article no.AJMAH.104770 208 eating out and frequency of fast-food consumption. The significant association between dental caries and restaurant preference highlights the potential impact of restaurant-based meals on oral health outcomes. Similarly, the link between dental caries and frequent fast-food consumption emphasizes the potential role of diet in the development of oral health problems [14].

CONCLUSION

Oral health is an important aspect of overall health and well-being. The study highlights the link between fast food

consumption and dental caries in teenagers, emphasizing the importance of addressing dietary habits to reduce oral health issues. The study reveals the need for public health initiatives to raise awareness and develop targeted interventions to promote better oral health outcomes among teenagers. Addressing dietary habits is crucial for a healthy mouth, allowing individuals to speak, eat, and socialize without fear of disease or discomfort.

CONSENT

All authors declare that "written informed consent from the participant was obtained for publication of this research report and accompanying images."

ETHICAL APPROVAL

Ethical approval was taken from the IRB of National Institute of Preventive and Social Medicine (NIPSOM) Bangladesh.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Evaluating the Impact of the GeneXpert Technique on Case Detection and Management of Tuberculosis in Ghana

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ABSTRACT

Background: Rapid and more sensitive diagnostic assays have seen substantial investment globally intending to improve Tuberculosis (TB) diagnosis towards treatment. This study evaluated the impact of the GeneXpert intervention on TB case detection and management in Ghana.

Methods: The study made use of an interrupted time series design. Data from Ghana's District Health Information Management System (DHIMS II) was used to evaluate the impact of the intervention. A cross-section of DHIMS II data was analyzed repeatedly using 2017 and 2022 as baseline and end lines, respectively. Descriptive analysis and interrupted time series models were used.

Results: No immediate impact was observed on bacteriologically confirmed TB, however, 5 years following the intervention, the number of bacteriologically confirmed TB cases increased by an average of 562 cases per year (step and ramp (coefficients of pre- and post-intervention) variables being -278.5 (95% CI): -1118.3, 561.3) and 561.9 (95% CI: 352.3, 771.6), respectively. The cure rate decreased on average by 32.8% at the onset (step coefficient of -32.8 (95% CI: 40.5 - 25.0), however, there were no significant changes in the pre-and post-intervention cure rates (ramp coefficient = 1.5 (95% CI: -4.0, 7.0). Subsequently, the treatment completed rate increased sharply in the year of implementation but did not significantly differ from the pre-and post-intervention period (step and ramp coefficients were 29.4% (95% CI: 27.0, 31.9) and 1.5% (95% CI: -1.8, 0.4). Moreover, the intervention did not have a significant sudden or gradual impact on adverse treatment outcomes; step and ramp coefficients were 3.0% (95% CI: -4.5, 10.6) and 0.2% (95% CI: -5.1, 5.5)

Conclusions: While bacteriologically diagnosed TB cases improved during the implementation period, clinical outcomes were not significantly impacted. To achieve recommended global targets, there is a need to address treatment follow-up challenges and enhance monitoring as part of addressing implementation challenges.

Keywords: Tuberculosis; GeneXpert; intervention; interrupted time series; impact.

INTRODUCTION

Tuberculosis remains a global public health concern, specifically in low and middle-income countries [1]. An estimated 10.6 million people were infected with TB worldwide in 2021 [2]. Without effective treatment strategies and improvement in diagnostic techniques, the goal to achieve a 90% and 95% reduction in TB incidence and mortality by 2035 will not be possible [3,4]. Given that barely two-thirds of the expected number of TB cases are diagnosed or reported to health authorities, diagnostic improvement is of the utmost importance [3].

The aim of tuberculosis control is early detection and management. The gold standard for tuberculosis testing is sputum culture, but its turnaround time is too long and thus delays the treatment of patients infected with TB (Parsons et al., 2011). Sputum smear microscopy, the most widely used diagnostic tool has been shown to have low sensitivity ranging from 30-70 % (Ejeta et al., 2018; Pinyopornpanish et al., 2015; Steingart et al., 2014). Rapid and more sensitive diagnostic assays, including GeneXpert MTB/RIF®, have seen substantial worldwide and national investment to improve the diagnostic cascade of treatment for patients. There has been a significant global scale-up of the GeneXpert technique since the World Health Organization

(WHO) endorsed it in 2010. The advent of GeneXpert epitomizes a paradigm shift in the diagnosis of TB and drug-resistant as well as simplified molecular testing worldwide (Pinyopornpanish et al., 2015). The GeneXpert assay has shown improved sensitivity (77-99%) than sputum smear microscopy and is comparable to sputum culture and produces results in less than 2 hours making it a better diagnostic tool (Ejeta et al., 2018; Moussa et al., 2016; Pinyopornpanish et al., 2015) [5]. Rapid and accurate diagnosis of TB and timely initiation of appropriate treatment and management are key to curbing the spread and elimination of the disease (Dravid et al., 2019).

Previous studies have shown that Ghana has made progress in its quest to eliminate TB and achieve the global target by 2035 but poor health infrastructure, and low TB case detection and management have hampered its progress over the years [6]. The disease remains a significant public health problem in the country despite the several efforts that have been made over the years to curb its incidence. In the last quarter of 2017, the NTP adopted the GeneXpert as the initial diagnostic tool for TB testing across the country [7]. The introduction of the GeneXpert showed a significant improvement in the coverage of testing among all notified cases in 2018 at 58% and 20% coverage in 2017. The

number of GeneXpert testing facilities over the years has increased from 126 sites to 143 GeneXpert testing sites at the close of 2021. These facilities have been linked to over a thousand TB requesting sites through the spoke and hub system of sputum sample transportation [7]. However, very little information is available to ascertain the impact of this strategy in improving case detection and management of TB in Ghana. The current study seeks to understand what impact GeneXpert intervention is having on case detection and management of TB and help Sorvor and Ewusie; Asian J. Med. Health, vol. 21, no. 10, pp. 241-252, 2023; Article no. AJMAH.104892 243 inform future decisions regarding improvement as well as further rollout.

METHODS

2.1 Study Design and Setting

The study made use of an interrupted time series design, in which the same group is observed repeatedly across time, both before and after the intervention [8]. Interrupted time series is robust and regarded as one of the best designs for determining causality when randomized controlled trials (RCTs) are not possible [9,10]. Administrative and clinically generated data collected routinely using Ghana's District Health Information Management System (DHIMS II) was used to evaluate the impact of the intervention. A cross-section of the DHIMS data was analyzed repeatedly using 2017 and 2022 as baseline and end lines, respectively. The Ghanaian health system is made up of three levels namely; national, regional, and district, however, service delivery is carried out at the zonal level: national, regional, district, sub-district, and Community Health Planning and Services (CHPS). The NTP is a Public Health Division's Disease Control and Prevention Department programme. The National Tuberculosis Control Programme's major activities are coordinated through Ghana's five-tier health system.

The Ghana Health Service, as well as the Ministry of Health and its agencies, have a nationwide network of health facilities (hospitals, health centres, clinics, and maternity homes) spread over the country's sixteen regions and 261 districts (as of 2022). There are currently 405 hospitals, 822 health centres, 1006 clinics, and 601 medical laboratories in operation in Ghana, accounting for 83 per cent of all health facilities, 9% of faith-based institutions, and 7% of the commercial sector. The National Tuberculosis Control Program operates within the Ministry's structures.

2.2 Data Analysis

Data were extracted in an Excel file (Microsoft Excel 16) double-checked and analyzed using STATA and R statistical soft wares. The years 1997-2017 were selected to study trends before, and 2018–2022 after, the rollout of GeneXpert as a routine tuberculosis diagnostic test. Different indicators such as the number of bacteriologically confirmed cases, cure rate, treatment completion rate and adverse outcome rates were retained for analysis.

For time series analysis to be properly specified, the model assumes the data to be stationary, meaning it has a constant mean and variance; without seasonal variation, and is not correlated with its values (autocorrelation). To be sure these assumptions are not violated, we chose the autoregressive integrated moving average (ARIMA) model using the Box-Jenkins method to select the model

parameters. ARIMA models an outcome variable as a function of past values of the outcome variable and the error term. It consists of an autoregressive (AR) model, a moving average (MA) model and a part that integrates the two by inducing stationarity through differencing. Differencing means computing the difference between adjacent values of the observed data. The AR model has the outcome variable predicted by lagged values of the outcome (values of the outcome shifted forward by one or more time points) whereas the MA models the outcome by lagged values of the error term. Thus, an ARIMA model is characterized by p, the number of lags of the AR model, q, the number of lags of the MA model, and d, the order of differencing required to induce stationarity. This is typically written as ARIMA (p,d,q) [10].

In our analysis of the data, we assessed for the violation or otherwise of the assumptions. For stationarity, we plotted the trend line for visual assessment and confirmed using the augmented Dickey-fuller test. The plot was considered stationary at $p < 0.05$. If not stationary, the first or second-order differencing was done to induce stationarity. We cross-checked visually and statistically as discussed above for stationarity. For autocorrelation, we used the autocorrelation function (ACF) and partial autocorrelation (PACF) plots for visual assessment. The plots suggested autocorrelation if the lags in the ACF plot exceeded the upper bound or lower bound of the confidence interval. After- fitting the ARIMA models, we checked for autocorrelation using the Box-Ljung test. A p-value great than or equal to 0.05 was considered insufficient evidence against autocorrelation. The above procedures are presented for only the impact of GeneXpert on the number of bacteriologically confirmed cases but were followed for all other outcomes.

To fit the ARIMA models, we used the "auto. arima()" command in the R package "forecast" to determine the parameters for the best model with the Akaike Information Criteria as the basis for model selection. We sought to evaluate the effect of GeneXpert on the following outcomes: the Sorvor and Ewusie; Asian J. Med. Health, vol. 21, no. 10, pp. 241-252, 2023; Article no. AJMAH. 104892 244 number of bacteriologically confirmed cases and the treatment outcome rates (the cure rate, the rate of treatment completion, the successful outcome rate, and the adverse outcome rate). We did this by forecasting (using an ARIMA model) each outcome from 2017 to 2021 (2017 to 2022 for the number bacteriologically confirmed) based on the prevailing trend before the national implementation of GeneXpert for TB diagnosis (before 2017). This forecast, being the counterfactual is compared with the observed data to provide an eyeball assessment of GeneXpert's impact. We fit the ARIMA model with a covariate that predicts a sudden and sustained change at implementation (step) and another that predicts a change in slope that occurs after the implementation of the intervention (ramp). These two were used to show statistically, the impact of GeneXpert on the outcomes. The model fitness based on the procedures described was assessed using plots of the residuals.

RESULTS

3.1 Descriptive Summary

The median number of bacteriologically confirmed TB cases from 1997 to 2022 was 7713 (interquartile range 7400.8-8006.8), the minimum (6877) number of

bacteriologically confirmed cases was observed in 1999 and the maximum number (11713) was observed in 2022. Similarly, the median cure rate was 63.6% (IQR 45.9-74.8), the lowest (36.7%) and the highest (79.1%) cure rate was recorded in 1997 and 2009 respectively. On the other hand, the median treatment completed rate was 7.8% (IQR 6.4 - 13.1), the minimum (5.3) was recorded in 2005 and the maximum (41.9) was recorded in 2017. The median adverse outcome was 15.6%, the highest (55.3%) was observed in 1997 and the lowest (11.9%) was observed in 2014 (Table 1). 3.2 Impact on the Number of Bacteriologically Diagnosed TB Cases

We postulate that the introduction of the GeneXpert to diagnose TB in Ghana will not result in an immediate increase in bacteriologically confirmed numbers since its availability was staggered across the country. Over time, we expect to see a gradual increase after the intervention.

The plot of the data is shown in Fig. 1. The plot shows an uptrend, particularly noticeable at the start of the trend and after the implementation of the intervention. This suggests non-stationarity in the data, a violation of a key assumption for time series data analysis. A confirmation using the augmented Dickey-fuller test yielded $p = 0.99$, indicating insufficient evidence against non-stationarity.

We determined the second difference as being necessary to induce stationarity. The plot is presented in Fig. 2. This was statistically confirmed with the augmented Dickey-fuller test with $p < 0.01$. Fig. 3 presents a chart of the autocorrelation function (ACF) and partial autocorrelation (PACF) plots before (plot 1) and after (plot 2) differencing to enable visual assessment of autocorrelation in the data.

The first 2 lags exceeded the upper bound for the ACF as well as the first for the PACF in the plots before differencing, suggesting autocorrelation in the data. After differencing, only the first lag in the ACF plot exceeded the upper bound and none in the PACF plot.

Using the "auto. arima()" command in the R package "forecast" to determine the best ARIMA model the resulting model was a regression with ARIMA(0,0,0) errors. Plots of the residuals are presented in Fig. 4. The residuals are normally distributed with a reasonably equal variance around the mean. Also, there is no autocorrelation in the residuals from the ACF plot. The Box-Ljung test for the residuals was $p = 0.490$, meaning insufficient evidence for autocorrelation.

The coefficients of the step and ramp variables were -278.5 (95% confidence interval (CI): -1118.3, 561.3) and 561.9 (95% CI: 352.3, 771.6), respectively. These indicate that there was no immediate impact on the number of bacteriologically confirmed cases of TB at the time of implementation. However, over the 5 years following its implementation the number of bacteriologically confirmed cases increased by an average of 562 cases per year.

A trend of the annual number of bacteriologically confirmed TB cases in Ghana from 1997 to 2021 with an overlay of the counterfactual (red line) after GeneXpert implementation is presented in Fig. 5. Our model predicts 7559.7 (95% CI: 6947.1, 8172.3) as the number of bacteriologically confirmed cases for each of the years following the implementation of the GeneXpert. The sharp increase in observed value of 11713 in 2021 owes to the GeneXpert implementation for the diagnosis of TB in Ghana.

Table 1. Summary statistics of various tuberculosis indicators used in the study

Variable	Median	IQR	Minimum	Maximum
Number bacteriologically confirmed	7713	7400.8 – 8006.8	6877	11713
Cure rate	63.6	45.9 - 74.8	36.7	79.1
Treatment completed	7.8%	6.4 – 13.1	5.3	41.9
Adverse outcomes	15.6	13.5 – 38.5	11.9	55.3

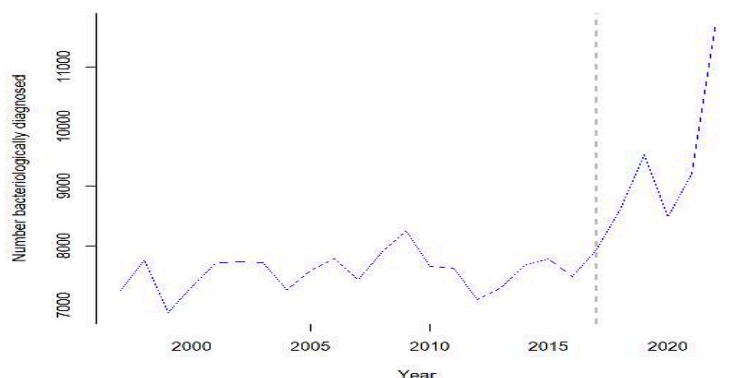


Fig. 1. Trend of the annual number of bacteriologically confirmed TB cases in Ghana from 1997 to 2021

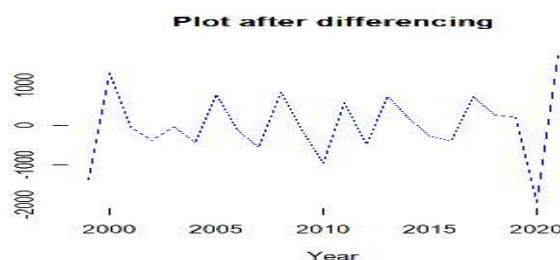


Fig. 2. Trend of the annual number of bacteriologically confirmed TB cases in Ghana after second-order differencing

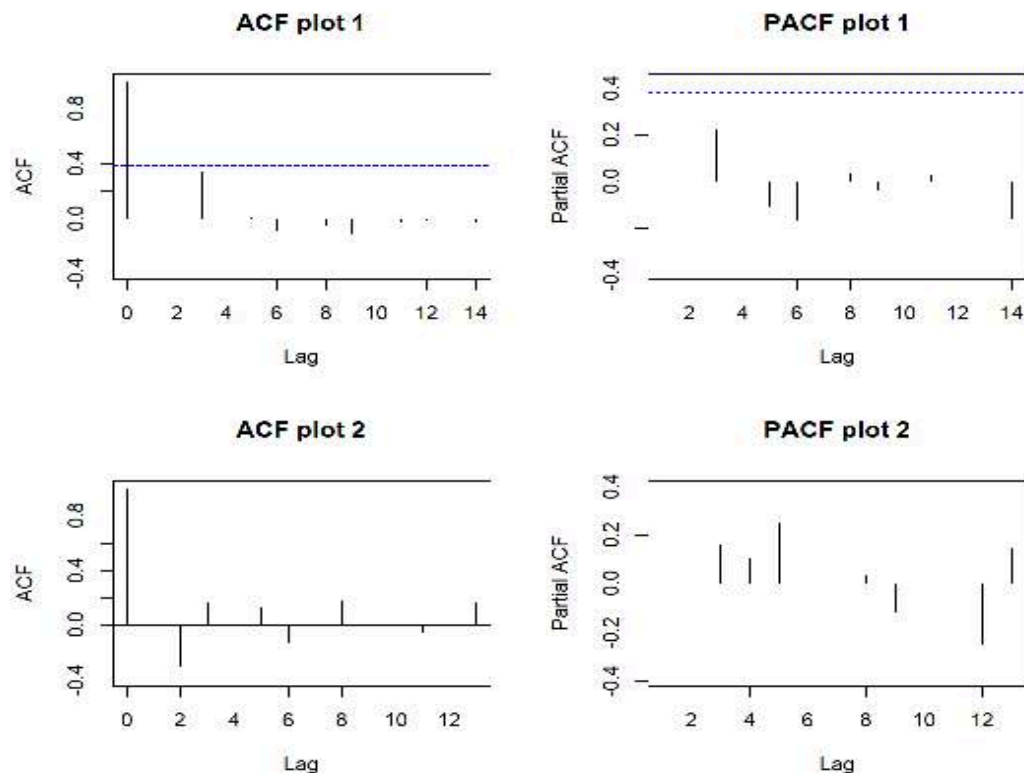


Fig. 3. Autocorrelation function (ACF) and partial autocorrelation function (PACF) plots before differencing (plot1) and after differencing (plot2)

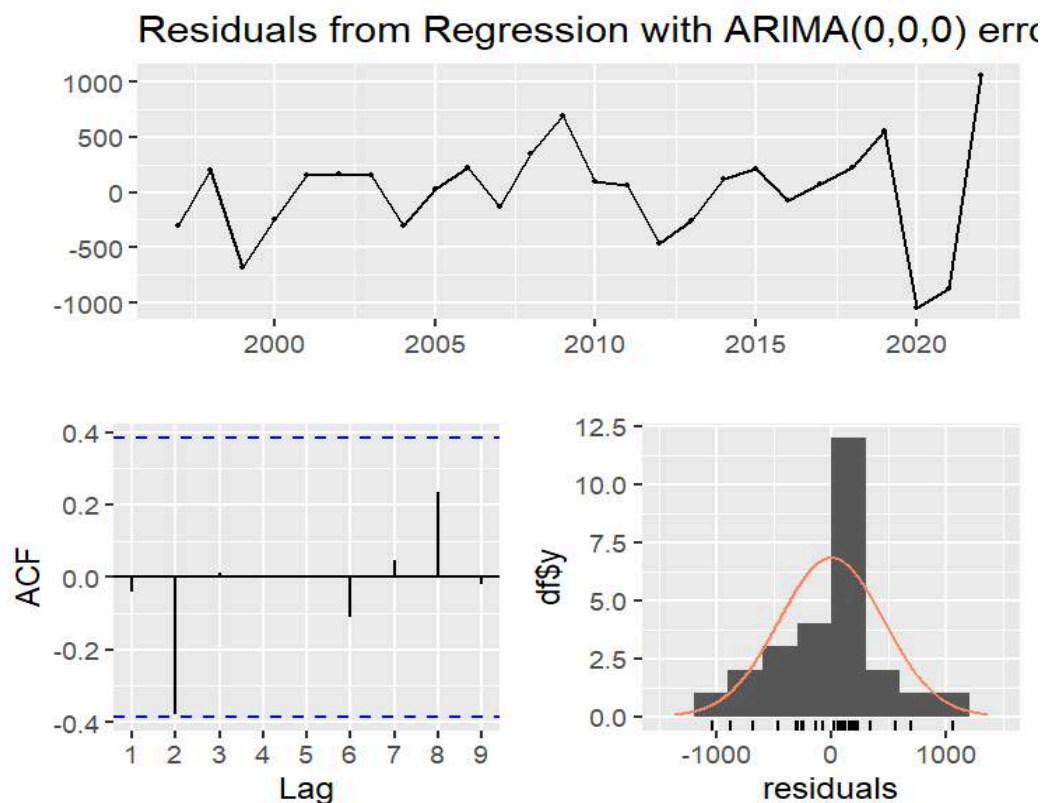


Fig. 4. Residual plots of the best ARIMA model (regression with ARIMA (0, 0, 0) errors) for the number of bacteriologically confirmed

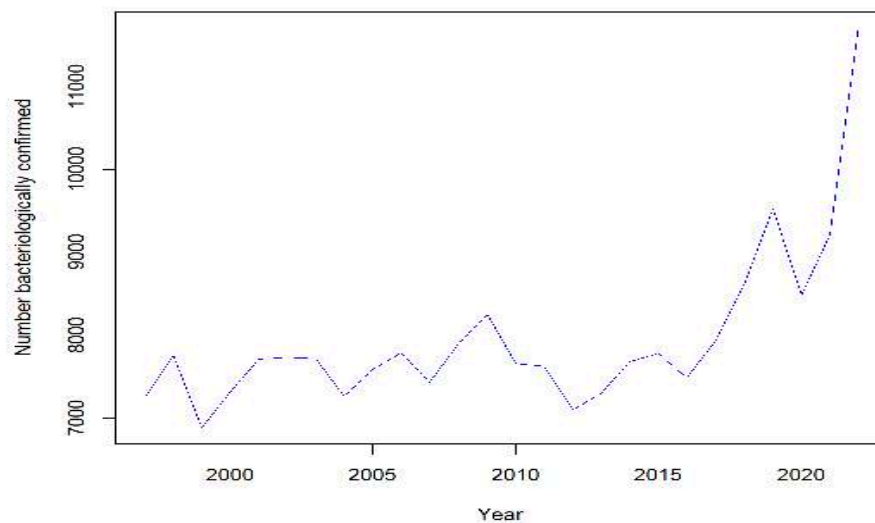


Fig. 5. Trend of the annual number of bacteriologically confirmed TB cases in Ghana from 1997 to 2021 with an overlay of the counterfactual (red line) after gen expert implementation

The findings show that the treatment completed rate increased sharply in the year of implementation but did not significantly differ in trend afterwards in comparison with the trend

before. The observed increase in treatment completed rate from 13.2% to 41.9% is due to the intervention.

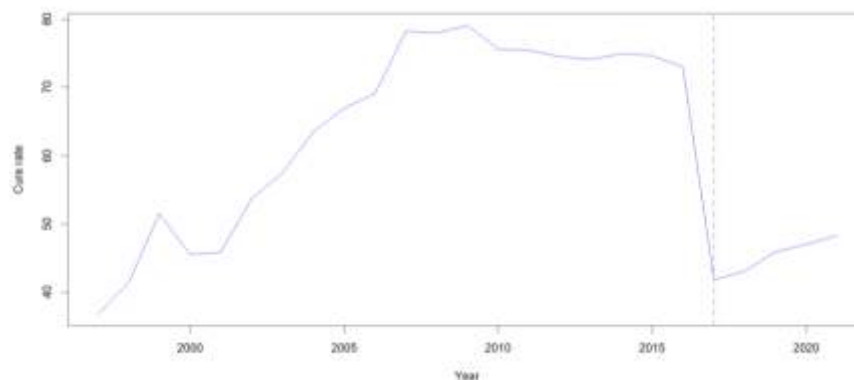


Fig. 6. Trend of the annual cure rate of TB cases in Ghana from 1997 to 2021

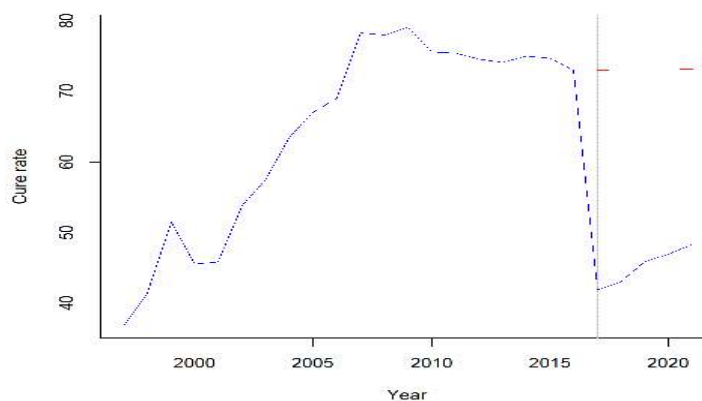


Fig. 7. Trend of annual TB cure rate in Ghana from 1997 to 2021 with an overlay of the counterfactual (red line) after GeneXpert implementation

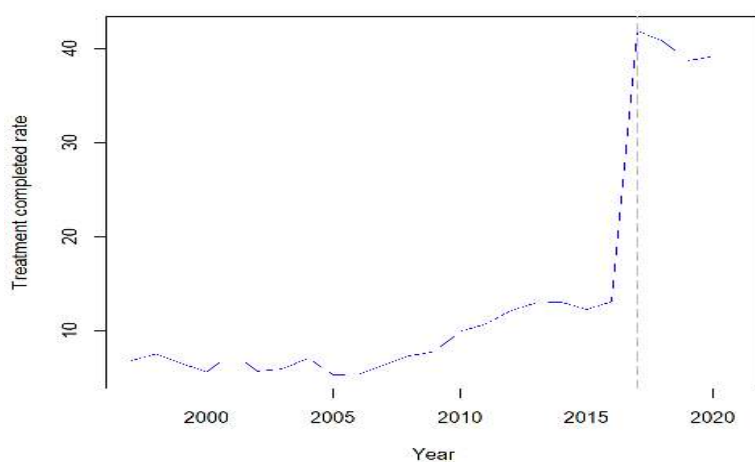


Fig. 8. Trend of annual treatment completed rate of TB cases in Ghana from 1997 to 2021

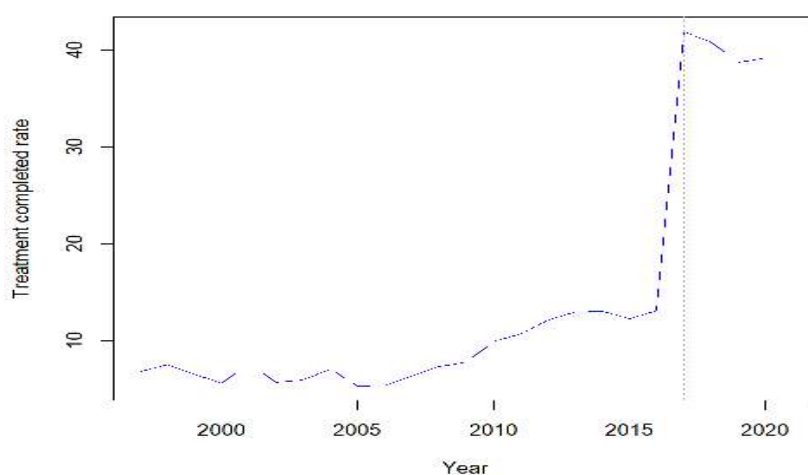


Fig. 9. Trend of annual treatment completed rate in Ghana from 1997 to 2021 with an overlay of the counterfactual (red line) after GeneXpert implementation

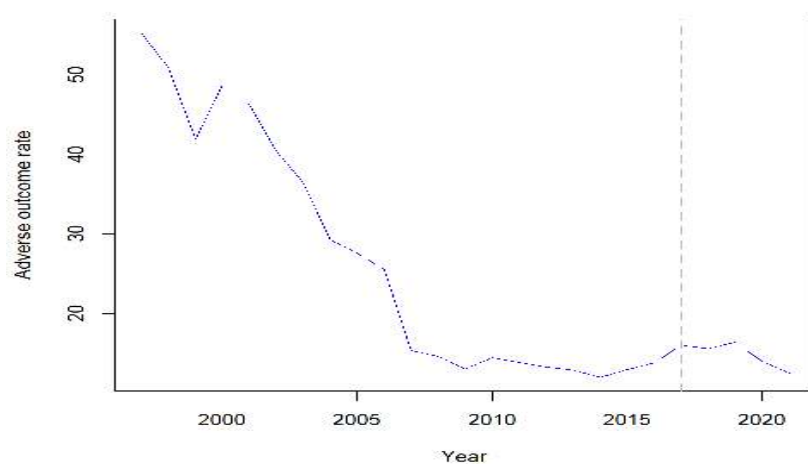


Fig. 10. Trend of annual adverse outcomes rate of TB cases in Ghana from 1997 to 2021

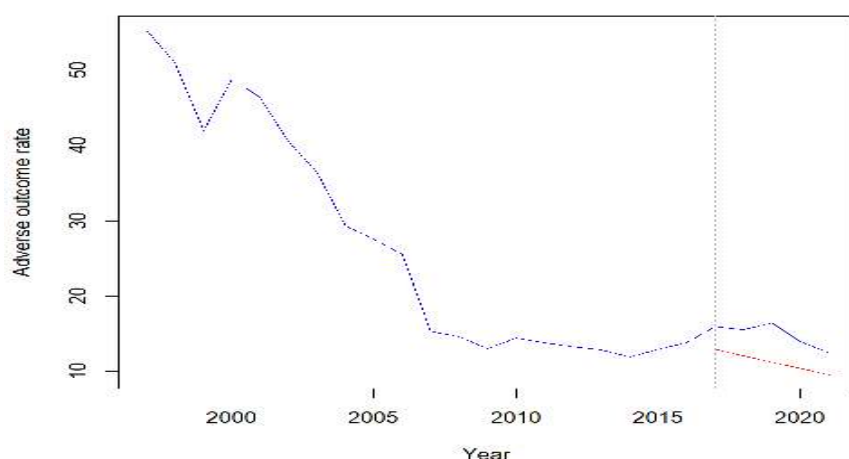


Fig. 11. Trend of annual adverse TB outcomes rates in Ghana from 1997 to 2021 with an overlay of the counterfactual (red line) after GeneXpert implementation

3.3 Impact on Cure Rate

We postulate that the introduction of the GeneXpert to diagnose TB in Ghana will increase the cure rate. This is based on the knowledge that GeneXpert is more sensitive than sputum microscopy which was mainly used for diagnosis [11,12]. Having fewer people likely to be misdiagnosed will lead to improvement in the cure rate.

The trend plot is shown in Fig. 6. The plot shows an uptrend in the cure rate until the year before the implementation of the intervention. The cure rate fell sharply in the year of the implementation and then resumed the uptrend thereafter. The plot appears non-stationary. This was confirmed with the augmented Dickey-fuller test with $p = 0.93$. The second difference induced stationarity with an augmented Dickey-fuller test showing evidence of stationarity ($p = 0.028$).

Using the "auto. arima()" command in the R package "forecast" we determined the best ARIMA model with the information criteria as the basis for model selection. The resulting model was a regression with ARIMA (0, 2, and 1) errors. The Box-Ljung test of the residuals confirmed the goodness of fit at $p = 0.670$.

The step coefficient of -32.8 (95% CI: 40.5 – 25.0) indicates that the implementation of the GeneXpert had an immediate effect of reducing the cure rate by an average of 32.8%. The period after the implementation (2018 – 2021) did not see a change in trend different than that before the implementation (ramp coefficient = 1.5 (95% CI: -4.0, 7.0)). The observed drop in cure rate to 41.9% in 2017 owes to the implementation of GeneXpert.

3.4 Impact on Treatment Completed

We postulate that the introduction of the GeneXpert to diagnose TB in Ghana will reduce the proportion of treatment completed. This is based on the knowledge that GeneXpert is more sensitive than sputum microscopy which was mainly used for diagnosis [11]. Assuming no change in the general rate of success, an improvement in the cure rate will mean fewer people being categorized as treatment completed.

Fig. 8 presents the treatment completed trend plot from 1997 to 2021. A gradual up trend is observed up to 2016 after which a sharp rise is seen. The trend shows a mild decline

from 2017. The trend is non-stationary. The augmented Dickey-fuller test confirmed this $p = 0.780$ and second order differencing induced stationarity (augmented Dickey-fuller test $p = 0.018$).

The "auto. arima()" command in the R package "forecast" determined regression with ARIMA(0,1,0) errors as the best ARIMA model using the information criteria for model selection. The step and ramp coefficients were 29.4% (95% CI: 27.0, 31.9) and 1.5% (95% CI: -1.8, 0.4), respectively.

The findings show that the treatment completed rate increased sharply in the year of implementation but did not significantly differ in trend afterwards in comparison with the trend before. The observed increase in treatment completed rate from 13.2% to 41.9% is due to the intervention.

3.5 Impact on the Rate of Adverse Outcomes

We postulate that the introduction of the GeneXpert to diagnose TB in Ghana will improve adverse outcomes. The plot of the trend of the adverse outcomes rate from 1997 to 2021 shows a steady drop from 1997 to 2007 after which it plateaued until 2021. The sharp fall in the rate makes the trend non-stationary (augmented Dickey-fuller test $p = 0.973$).

Regression with ARIMA (0,2,1) errors was the best model and it was selected using the "auto. arima()" command in the R package "forecast" based on having the smallest Akaike Information Criterion value of all the potential models. The step and ramp coefficients were determined using this model as 3.0% (95% CI: -4.5, 10.6) and 0.2% (95% CI: -5.1, 5.5), respectively.

From the findings, the implementation of GeneXpert as the diagnostic mechanism for TB in Ghana did not have a significant sudden or gradual impact on adverse treatment outcomes.

DISCUSSION

Generally, the number of bacteriologically confirmed TB cases improved over time between 2017 and 2021 following the implementation of the GeneXpert intervention. However, there was no significant change in the cure rate during the implementation of the GeneXpert intervention (2018 -2021). Similarly, the findings show that the implementation of the intervention did not have a significant impact on the treatment completion rate and adverse treatment outcomes.

Previous studies showed that GeneXpert has superior sensitivity compared with the conventional sputum smear microscopy in the diagnosis of TB [11, 5, 12]. It is therefore not surprising that the bacteriologically confirmed cases increased over time during the implementation of the intervention. Our model predicts that if the intervention is implemented optimally, the number of bacteriologically confirmed tuberculosis cases per year would increase by 7559.7. Similar findings have been reported showing that GeneXpert's technique is a game changer in the diagnosis and control of TB globally [13-17]. The microbiological detection of TB is critical because it enables accurate diagnosis and ensures that the most effective treatment regimen is administered as soon as possible [2].

While TB cases increased significantly with the implementation of the intervention, cure rates reduced by an average of 32.2% in the year of implementation (2017) and there was no difference in the cure rate before implementation. The highest (79.1%) cure rate was observed in 2009, implying that the cure rate worsened with the implementation of the intervention, falling below the international recommended benchmark of 85% [18]. This could be attributable to challenges with the sputum smear microscopy which is used for monitoring treatment outcomes. Prior studies have also shown that the GeneXpert intervention did not have any impact on treatment outcomes compared with microscopy except for reduced time to diagnosis and treatment as well as empirical treatment, corroborating what was found in this study [13]. Similarly, other studies have attributed the lack of public health impact of the GeneXpert to implementation barriers [14]. It is therefore imperative to improve and strengthen laboratory capacity and infrastructure to improve TB treatment outcomes. The minor impact of the GeneXpert clinical outcomes seen in this study and previous studies is a major concern and could have dire consequences for TB control globally [14,19]. It is imperative for TB control programmes to institute innovative approaches to deal with barriers to the implementation of the technique.

CONCLUSION

Our findings indicate that, although case detection increased significantly with the rollout of the GeneXpert intervention, clinical outcomes had no impact. There is a need to address implementation challenges that could be accounting for this trend.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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