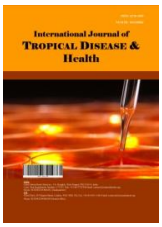

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Blood Cellular Changes Associated with Bacteremia and Malaria Co-morbidity among Children in Western, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. Authors GO and SM conceived and designed the study protocols, performed statistical analysis and wrote the first draft of the manuscript. Authors MM and CO managed the analyses of the study, proofread the manuscript and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Malaria and bacteremia co-morbidity in children cause changes in blood cellular components. Complete blood count from children whose haemoglobin genotypes and bacteremia tests are not known, greatly influence clinical management and interpretation of the haematology results in resource limited healthcare facilities.
Objectives: We investigated cellular components from children with bacteremia and malaria co-morbidity. We also analysed the haemoglobin genotypes and bacteria isolates from children with haemoglobin AA, SS and AS in western Kenya.
Methods: A total number of 384 children were recruited and complete blood counts done with an automated cell counter. Microscopy was used to determine malaria infections, while bacteremia was determined by blood culture. The haemoglobin genotypes were analysed using the electrophoresis technique.

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Results: Children with haemoglobin AA and AS had elevated granulocyte counts. Most of the bacteria isolates were from children with malaria and haemoglobin AS. The bacteria isolated from blood culture included non-typhi salmonella, *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Streptococcus pyogenes* and *Viridans*.
Salmonella species and staphylococcus aureus were the most prevalent bacteria isolates associated with bacteremia in children with haemoglobin AS and malaria positive.
Conclusion: Children with Hb AS have higher chances of malaria and bacterial co-infection which leads to lymphocytopenia, erythrocytopenia and thrombocytopenia. Bacteria responsible for most of malaria co-infections in this region are Salmonella species and *Staphylococcus aureus*. The malaria and bacterial co-infection in pre-school children initiate differential cellular changes which should be investigated further.

Keywords: Bacteremia; haemoglobin AA; AS; malaria.

1. INTRODUCTION

In Africa malaria and bacterial diseases are the most prevalent cause of death and morbidity in children less than five years old [1]. Non-Typhi *Salmonella* (NTS) is the common cause of bacteremia in adults and children in sub-Saharan Africa [2]. Adults infected with human immunodeficiency virus (HIV) and children under 3 years old carry the burden of invasive disease [3]. Malaria and bacteremia co-morbidity is common in African children and presents with fever of temperature above 37°C.

Streptococcus pneumonia and *Salmonella species* have been consistently reported as causes of bacteremia in children [4]. More than half of all pediatrics admissions in hospitals in Africa are due to malaria and bacteremia [5]. About 81% of malaria sickness happens in the African region with the highest mortality and causes grave illness, particularly in youngsters and expectant women [6]. In Kenya, complicated malaria and bacteremia co-infection in children have been shown to pose a bigger challenge to clinicians [7]. Clinical malaria in endemic areas may be asymptomatic in some children or severe disease which can be fatal and life-threatening due to the destruction of the host red blood cells and eventual reduction in the haemoglobin levels that lead to severe anaemia [8].

Occasionally, invasive bacteria have been reported to cause most of the bacteremia cases in young children who present with symptoms like malaria infection [9]. A study in Kilifi county referral hospital in Kenya showed that bacterial diseases in children and infants are the principal cause of hospital admissions in the area and most of the deaths are linked to malaria and bacteremia [3] Clinically, children diagnosed with malaria and bacterial co-infection have signs of splenomegaly [10]. Severe malaria anemia is the foremost cause of sickness and death in children in areas where *P. falciparum* malaria is holoendemic [11].

People who suffer from sickle cell disease have abnormal diminished spleen function that makes them more susceptible to infections [12]. Haemoglobin S (Hb S), have been linked to protection from malaria parasite infection or the clinical manifestation of malaria [13]. Studies on haemoglobinopathy concluded that Hb S plays a big role in the way the parasite infect and survive in the infected red blood cells [14]. The high frequency of the haemoglobin variant gene especially *Hb S* in malaria-endemic regions is thought to give a selective advantage due to its effects on the malaria parasites once they enter the red blood cells [15].

Malaria and bacterial co-infections are quite challenging because both diseases cause similar severe illnesses and life-threatening conditions in children. Due to undeveloped immune responses in infants and young children, they respond with high body temperatures to different diseases [4]. Most bacterial infection occurs during high malaria transmission seasons in the pediatrics population hence complicating the management of malaria infections [16]. In areas where haemoglobinopathies are common, especially in parts of western Kenya, haematological parameters in children with malaria and bacteremia co-infections remain unknown.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in the Siaya County referral Hospital in western Kenya. The patient catchment area for this hospital is largely rural

population within Siaya County and the neighboring counties (Fig. 1). Siaya County experiences an equatorial climate at an altitude between 1145 m – 1420 m above the sea level, it borders the eastern shores of Lake Victoria. The lowest temperatures vary between 18.0°C in July and 21.0°C in December, while the highest temperature may reach 30°C in August. This climatic condition offers a favorable environment for *Anopheles gambiae* and *Anopheles funestus* the vector for the protozoan *Plasmodium falciparum.*

2.2 Study Design and Participants

2.2.1 Inclusion criteria

Children under the age of 3 years presenting with fever were enrolled in a cross-sectional study. Children whom their guardians gave written consent during the hospital visit. Only children who had malaria parasitemia and Hb <11.0 g/dl, aged between 3 to 36 months were enrolled in the study. Children were examined for any illness by a trained clinical officer and all severe cases reported to the hospital for treatments.

2.2.2 Exclusion criteria

Children with complicated malaria associated with hypoglycemia, history of any AIDS-related illness and evidence of acute respiratory infections we're not allowed to participate in the study.

2.3 Blood Collection

About 5 ml of venous blood was collected in a syringe for culture, a complete blood count, blood smear malaria parasites test, HIV rapid test, and Hb genotype determination. Blood for bacteremia test was first put into bacteria growth media (BACTEC Peds Plus, Becton Dickinson) and later transferred to an automated system (BACTEC 9050, Becton Dickinson) for screening.

2.4 Hematological Measurements

Complete blood count (CBC) was performed using Beckman Coulter AcT diff. 2 analyser (Beckman-Coulter Corporation, Miami, USA). The blood parameters analysed included white blood cells count (Wbcc), red blood cells count (Rbcc), haemoglobin (Hb), packed cell volume (PCV), lymphocytes (Lymph), Monocytes (Mono) Granulocytes (Gran), platelets count (Plts) and red blood cells indices.

2.5 Haemoglobin Phenotype Determination

Alkaline cellulose acetate electrophoresis with Titan III plates was used for haemoglobin electrophoresis. (Helena Biosciences, Oxford, UK). Hemolysates prepared from blood samples. Hemo controls were dispensed onto the acetate paper alongside participant children's blood (hemolysate), and haemoglobin variants were separated by electrophoresis with an alkaline buffer at pH 8.6. The plates were then stained using Ponceau S stain, and haemoglobin types scored using the Hemo control run on test Titan 111 plate.

2.6 Bacteriology Methods

About 3 ml of blood was inoculated in a BD bacteria inoculation bottle (BD Bactec peds plus/f) for blood culture. The inoculated bottle was incubated in a BD automated bacteria growth system (Bactec 9050; Becton Dickson) for a minimum of five days. The positive blood samples were plated on sheep blood agar, chocolate agar and MacConkey agar and incubated at 37°C aerobically. If no growth was detected after 18–24 hrs incubation, an additional incubation period of 4 days was employed with daily inspection for growth. Suspected bacteria colonies were identified with Grams stain, colonial characteristics, and API biochemical panels (Bio Mérieux, USA).

2.7 Malaria Diagnosis

Giemsa stained blood smears were examined for the presence or absence of malaria parasites. The prepared smears were dried, and thin blood smears fixed with methanol 10 seconds. The smears were examined by a trained medical laboratory technologist. If no malaria parasite or hemozoin found after examining thick smears, the participants were categorized as malaria negative. A thin blood film was examined to confirm *plasmodium* species.

2.8 Data Analysis

Data was analysed using SPSS software version: 18 (SPSS Inc). Kruskal-Wallis tests were applied to compare group differences. Haematological measurements across study groups were compared using Mann-Whitney U tests. Tabulation of data was done using Graph Pad Prism version 5.0 (GraphPad Software, Inc), Statistical significance was considered for probability values (P) less than 0.05.

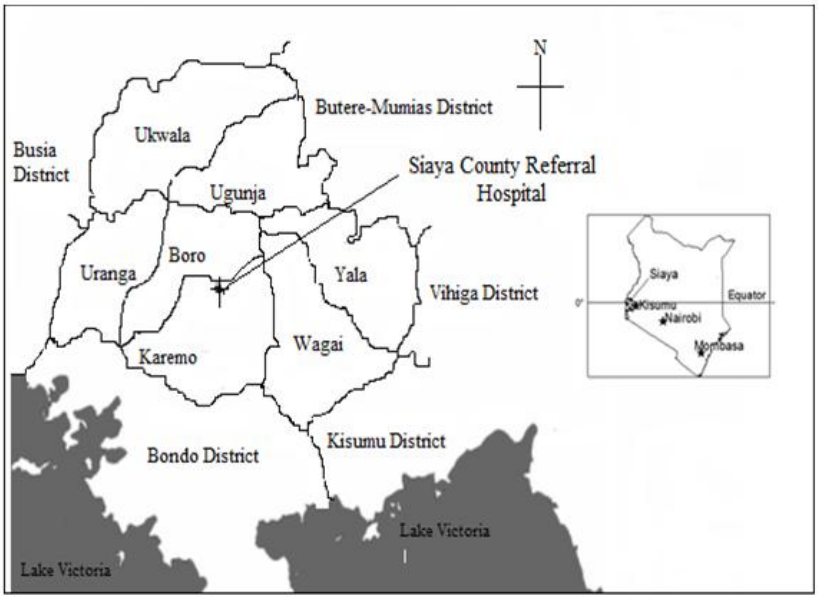

Fig. 1. Siaya county map showing the location of Siaya county referral hospital

3. RESULTS

3.1 General Characteristics Participants

The average age of all participants was 2 years old. Three hundred and eighty-one participants were mono-infected with malaria parasites while the rest were not infected as confirmed by microscopy. The participants were stratified into four study groups (Table 1). Group 1 comprised of children with haemoglobin genotype AA (Hb AA) and Malaria infection (80.2%); group 2 comprised of children with haemoglobin genotype AS (Hb AS) and Malaria infection (11%); group 3 children had haemoglobin genotype AS (Hb AS), malaria infection and bacteremia (8%); group 4 comprised children with haemoglobin genotype SS (Hb SS) with no malaria infection and bacteremia (0.8%).

3.2 Blood Cellular Changes

To identify the blood cellular components, blood was analysed immediately after collection (Table 2). There was intergroup difference in Lymphocytes count (Lymph, *P* = 0.02) and granulocytes count (Gran, *P* = 0.05), however the most significant difference was in red blood cells count (Rbc, *P* = 0.001) and platelets count (Plts, *P* = 0.002). There was no significant difference in total white blood cell count (WBC, *P* = 0.16) and Monocytes (Mon, *P* = 0.86). Lymphocytopenia, erythrocytopenia and thrombocytopenia were observed in group 3 children with malaria and bacteremia co-morbidity when compared to other study groups.

3.3 Bacteria Isolates from Blood Samples

To investigate the bacterial infections, we carried out a series of blood cultures. The predominant bacteria were *Staphylococcus aureus* (38.7%) isolates. Among gram-negative bacterial isolates, *Salmonella spp* was the prevalent (32.2%) isolates. Other gram-positive bacteria isolated included; *S. pyogenes* 3.2% isolates, *streptococci* 6.5% isolates (Table 3). The table shows the number of bacteria isolated from each group after the blood culture at 37°C aerobically. Plates with no growth at 24 hours were incubated for 2 more days. Only group 3 children with haemoglobin genotype AS had a bacterial infection.

4. DISCUSSION

This study describes cellular alterations in children with malaria and bacteria co-infection in a region where malaria is a burden throughout the year. Only children with Hb AS were associated with malaria and bacteremia co-morbidity (Table 2). A study in another region in Kenya investigated immune responses to bacteremia in children with Hb AS indicated that bacteremia occurred with malaria infection, but the sickle cell trait was more protective [17]. Here, we describe malaria and bacteremia in children with Hb AS, as a common finding in the

study area (Table 1). The prevalence of bacteremia was high in children with Hb AS infected with malaria parasites compared to children with Hb AA and Hb SS with no bacteremia (Table 3). Similarly, malaria and bacteremia co-infection have previously been reported in African children [3].

We characterized bacteria isolates according to gram reactions and the results showed many of the isolates were gram-positive bacteria (Table 3). This implied possibly the causes of upper respiratory tract infections (URTI) among young preschool children in Siaya County. In this area, URTI is not given much attention due to malaria infection dominance. The previous study reported an increased occurrence of bronchial and throat infections in children infected with malaria parasites [18]. The data also indicate that most notable isolates are child pathogens such as *E. coli* and *Salmonella spp*, which may be explained to non-susceptibility to self-prescription first-line antibiotics sold over the counter. The current study shows a higher rate of *S. aureus* isolates which is consistent with other studies done elsewhere in African children [19].

There were elevated granulocytes and total white blood count in children with bacteremia and malaria co-infection (Table 2). This is an important immune response in children under the age of 5 years due to their immature cellular immune responses [12]. Therefore, it is important to analyze haemoglobin genotypes in children with bacteria and malaria co-infection, to rule out sickle cell disease as well.

Granulocyte cells which include neutrophils, eosinophils, and basophils are important immune cells that can indicate parasitic or bacterial infections. In a situation where individual granulocyte parameters are not enumerated on hematology machines counters; it is important to perform peripheral blood film examination to get a picture of cell distribution. A previous study in Malawi and Kenya showed that bacteremia and malaria parasitemia was associated with increased granulocytes counts [20]. Group comparison in children with Hb AS, malaria, and bacteremia co-morbidity showed statistically significant thrombocytopenia (Table 2) which is attributed to the destruction of circulating platelets during malaria infections.

There was erythrocytopenia in children with Hb AA and AS respectively (Table 2). Malaria and bacteremia co-morbidity in children with Hb AS could be the cause of erythrocytopenia. Increased monocytes count was prominent in children with Hb AS, malaria, and bacteria co-infection (Table 2). The genesis of elevated monocytes counts could not be explained, although it could be linked to increased levels of *plasmodium* pigments that aggravate monocytes adhesions with infected red blood cells.

Following the characterization of malaria mono-infection and bacteria co-infection, the results showed both gram-positive and gram-negative bacteremia is a common occurrence in preschool-going children in this region (Table 3).

Table 1. General characteristics of participants

	Haemoglobin genotype	Parasitemia	Bacteremia	Participants
Group 1	Hb AA	Positive	Negative	308 (80.2)
Group 2	Hb AS	Positive	Negative	42 (11)
Group 3	Hb AS	Positive	Positive	31 (8)
Group 4	Hb SS	Negative	Negative	3 (0.8)

Figures n (%)

Table 2. Cellular parameters of children with malaria and non-malaria infected

Study groups	Group 1 (n = 308)	Group 2 (n =42)	Group 3 (n = 31)	Group 4 (n=3)	Normal reference	P- Value
WBC($\times10^3$/µl)	12.9	11.7	12.7	8.8	9.8	0.16
Lymph (%)	42.3	37.6	37.8	60	56.8	0.02
Mon (%)	7.28	7.0	6.8	7.9	7.7	0.86
Gran (%)	50.4	55.4	55.3	31.7	35.2	0.05
Plts ($\times10^3$/µl)	176.6	204	155	430	301	0.002
Rbc ($\times10^6$/µl)	2.3	3.0	3.9	3.2	4.02	0.001

Note: WBC- white blood cells, Lymp=Lymphocytes, Mon=Monocytes, Gran=Granulocytes, Plts=Platelets, Rbc= Red blood cells.

P-Value generated by Chi-square analyses

Table 3. Bacteria distribution in participants

	Group 1 (n=308)	Group 2 (n=42)	Group 3 (n=31)	Group 4 (n=3)	percentage of isolates
Gram-negative isolates					
E. cloacae	0	0	2	0	6.5
E. coli	0	0	1	0	3.2
Salmonella spp.	0	0	10	0	32.3
Gram positive isolates					
S. aureus	0	0	12	0	38.7
L. monocytogenes	0	0	3	0	9.7
S. pyogenes	0	0	1	0	3.2
Streptococcus	0	0	2	0	6.5
Total isolates	0	0	31	0	100

Staphylococcus aureus was the most common gram-positive isolates from blood culture, which is similar to a previous study on pediatrics subjects [21]. All bacterial isolates were from children with Hb AS (Table 3), which confirms children with Hb AS and malaria infection are predisposed to invasive bacterial infections [22]. Non-*Typhi Salmonella spp* was the most common gram-negative isolates in children with Hb AS (Table 3). These findings support a similar study done in Kilifi district hospital in Kenya, where they highlighted that Hb AS correlated with improved protection from both gram-positive and gram-negative bacterial infections [23]. All cases of invasive bacteremia infection were in Hb AS genotype that is normally thought to correlate with lower carriage of malaria parasites. *Streptococcus pneumonia* has been the most prevalent gram-positive isolates from previous studies of malaria and bacteremia [24] but in our current study, there was no isolate of the same in blood culture (Table 3). The difference is likely due to the increased uptake of *streptococcal* pneumonia vaccine among the children in Kenya.

Though previous studies observed increased protection of malaria infection in children with Hb AS, our results showed increased cases of bacteremia and malaria infection. This could be partly because malaria infection causes increased red cell haemolysis resulting in a mechanism believed to potentiate the vulnerability to salmonellosis.

5. CONCLUSION

This study highlights lymphocytopenia, erythrocytopenia and thrombocytopenia in children with Hb AS in malaria and bacteremia holoendemic area. The isolated bacteria, *Salmonella spp* and *Staphylococcus aureus* were the most prevalent isolates in children with Hb AS genotype. These bacteria co-infection is believed to contribute to severe illness in malaria-infected children.

6. RECOMMENDATION

The link between cellular dysfunction, malaria and bacteria co-infection should be investigated further because malaria increases erythrophagocytosis by immune cells.

CONSENT

Participation in the study was voluntary and informed consent was obtained from parents/guardians before conducting any study-related activity.

ETHICAL APPROVAL

Ethical approval was granted by Kenyatta University Ethics Review Committee (KUERC No. KU/R/COMM/51/624). Administrative authorization was obtained from the hospital medical superintendent and the paediatrics ward head nurse.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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