International Research Journal of Natural and Applied Sciences
Vol. 3, Issue 3, March 2016 IF- 3.827 ISSN: (2349-4077)
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STUDIES ON THE CONCOMITANT INFECTIONS OF MALARIA, HUMAN IMMUNODEFICIENCY VIRUS AND HEPATITIS B VIRUS AMONG STUDENTS IN ENUGU WEST.

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ABSTRACT

Background*: In the developing countries, concomitant infections are the rule rather than the exception, and the complete clinical picture involves several microorganisms that influence each other as well as the host. Malaria, HIV/AIDS and hepatitis B virus (HBV) are among the most devastating diseases in many low-income countries, particularly in Africa.* ***Objective****: Studies on the concomitant infections of malaria, human immunodeficiency virus and hepatitis B virus among Students in Enugu West was investigated.* ***Materials and Methods:*** *A total of 1500 blood*

specimens were collected and assayed for Hepatitis B (HBsAg) by commercial enzyme-linked immunosorbent assay kits, the Nigerian National HIV testing algorithm recommended by WHO for resource – poor countries was used for HIV and malaria using CareStart malaria HRP2 and Giemsa staining of thick blood film.

Result: *The result revealed that a total of 102 (6.8%) students were positive to all the infections, while malaria parasite was the most prevalent in the study population 63(4.2%), followed by hepatitis B virus 36(2.4%) and the least infection was HIV 3(0.2%), when compared, it was statistically significant at p>0.05 . The gender distribution of the co-infections of malaria/HIV, malaria/HBV, HIV/HBV and malaria/HIV/HBV among the study population showed that males were the most infected to malaria/HBV 10(1.9%) than females 18(1.8%). The comparison between the gender differed significantly at p<0.05. The age group of 21- 25 years was the most prevalent to the co-infections at a prevalent rate of 6.1% to malaria/HIV and malaria/HBV, respectively, while 3.0% to HIV/HBV and malaria/HIV/HBV, respectively. The least prevalent was the age group of 11-15 years which recorded 0.4% to malaria/HBV. The distribution according to LGA in Enugu West Senatorial Zone revealed that females from Oji-river were the most infected to malaria/HBV 6(2.0%), followed by Ezeagu 5(1.7%) and the least was Udi 1(0.3%), at p<0.05, was considered statistically significant. While Males had equal co-infection rate of 2(0.7%) to malaria/HBV across all the LGA's. Only in Agwu LGA where females infected to HIV/HBV and malaria/HIV/HBV at a prevalent rate of 1(0.3%), respectively and it differed significantly at p<0.05.*

Conclusion/Recommendation: *It is important to note that while these rates may be suggesting low endemicity, there is therefore, an urgent need to institute public health measures that will reduce disease burdens and transmission among students in Enugu West.*

key words: Concomitant**,** Malaria**,** Human Immunodeficiency Virus, Hepatitis B Virus and Students.

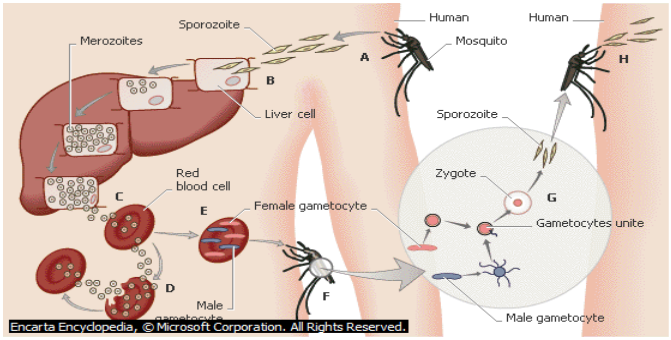
INTRODUCTION

In the developing countries, concomitant infections are the rule rather than the exception, and the complete clinical picture involves several microorganisms that influence each other as well as the host (Lundqvist *et al*., 2010). Malaria is by far the most devastating acute febrile illness in humans. The World Health Organization (WHO) has estimated that this disease is responsible

for 1.5 to 2.7 million deaths annually, and about 1 million of those cases occur in children under the age of 5 years (WHO, 2008).

Malaria, HIV/AIDS and hepatitis B virus (HBV) are among the most devastating diseases in many low-income countries, particularly in sub-Saharan Africa. Due to the overlapping distribution of malaria, HIV and HBV, there is a theoretical possibility of concomitant infection and the potential for immunological interactions between these three infections that farther results T-cell impairment and severe malarial anemia (Lundqvist *et al.*, 2010).

Malaria infection is initiated by the transmission of 5-50 sporozoites to the host by the bite of an infected female Anopheles mosquitoes. Some sporozite will enter the blood stream, reaching the liver. They will invade and then multiply within hepatocytes. Upon maturity, merozites are released back into the bloodstream and invade red blood cell (RBC). After multiplication, new merozites will be formed and will reinvade red blood cell. During the cycle, sexual forms of the parasite are generated and consequently taken up by mosquitoes during feeding. This sexual form will mate and carry on further parasite development in the mosquito mid gut forming new sporozites. The pre-erythrocytic stage of infection which lasts between 5 and 14 days, depending on the human *Plasmodium* species is asymptomatic. The blood stages which are associated with clinical disease can last up to a year with *P. falciparum* infection and close 50 years with *P. malariae*, if not treated. A malarial attack is characterized by recurrent peak of fever during the acute phase and can be associated with diverse range of syndrome, including severe malaria anemia, metabolic acidosis and shock syndrome (Hochman and Kami, 2009). It has been proposed that these syndrome result from differential parasite specificity (for example organ-specific sequestration) of parasite, Infected Red Blood Cell (iRBC), parasite toxins and the host immune response, (including cytokine and chemokine production and recruitment and sequestration of inflammatory cells to target organs) (Dondorp *et al.*, 1999).



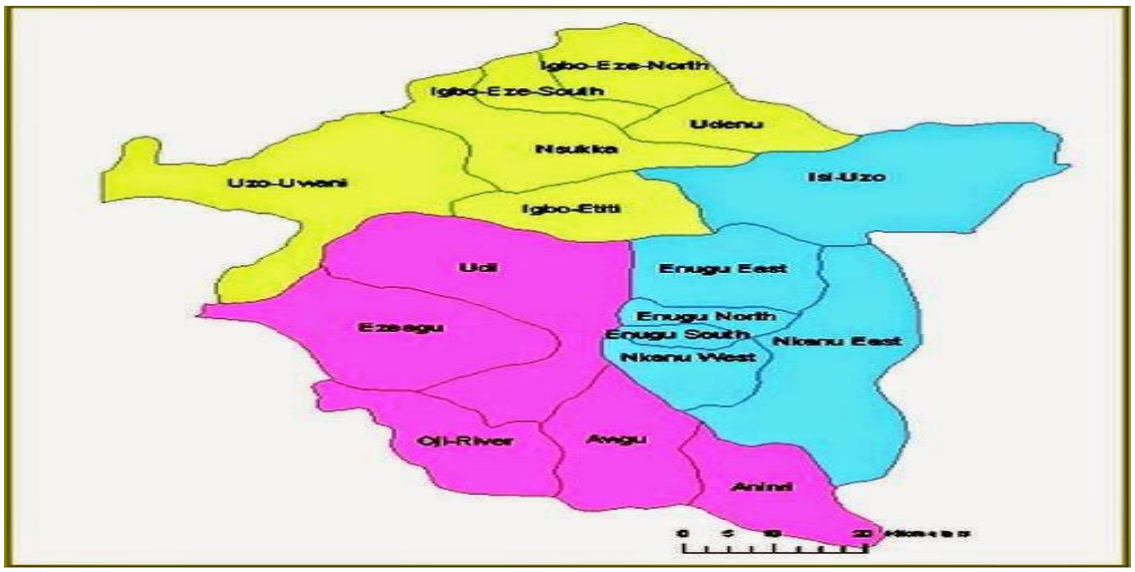
Malaria on HIV/AIDS transmission and progression

Malaria and HIV are among the two most important global health problems of developing countries. They cause more than 4million deaths a year (Abebe *et al*., 2013). Malaria, sometimes called the "king of diseases", is caused by protozoan parasites of the genus *Plasmodium*. It is one of the leading causes of illness and death in the world. Nine out of ten of these deaths occur in Africa and the rest occurs in Asia and Latin America, being the world's most prevalent vector-borne disease. It is the fourth leading cause of death of children under the age of five years and pregnant women in developing countries. The proportion increases each year because of deteriorating health systems, growing drug and insecticide resistance, climate change and natural disasters (Abebe *et al*., 2013). Studies on the concomitant infections of malaria, human immunodeficiency virus and hepatitis B virus among Students in Enugu West was investigated.

MATERIALS AND METHODS

Study Area

This study was carried out at the General Hospitals in Enugu West. **Enugu West district**: The constituency is made up of five Local Government Areas namely, Aninri, Awgu, Ezeagu, Oji-River, and Udi Local Government Area. Aninri Local Government Area has an area of 364 km² and a population of 133,723. Awgu Local Government Area has a population of 390,68. Ezeagu Local Government Area has an area of 633 km² and a population of 169,718. Oji River Local Government Area has an area of 403 km² and a population of 126,587. Udi Local Government Area has an area of 897 km² and a population of 234,002.



Map of Enugu State showing the Local Government Areas

Ethical Clearance

Prior to the commencement of the study, ethical clearance was sought by writing from the Chief Medical Director (CMD) of General Hospitals in Enugu West. This was done in writing explaining the purpose of the study and seeking for permission to use the health facilities as well as the co-operation of their staff. On receiving approval, officers in charge of the Laboratory section were also consulted. The following General Hospitals was used (Aninri, Awgu, Ezeagu, Oji-River, and Udi).

Subjects

The population of Enugu West is predominantly traders while some are public servant, artisans, farmers, and students. Students between 11-15, 16-20 and 21-25 years of age were given special considerations.

Sample Size

An estimated 1500 subjects formed the population size in which 300 subjects were collected from each of the five Local Governments,100 subjects from each of the three Secondary Schools from each LGA. The following Schools were sampled. In Awgu LGA, Boys Secondary School, Mgbowo; Alpha Secondary School, Awgu; Girls Secondary School, Mmaku. Aninri LGA, Community Secondary School Oduma; Community Secondary School, Mpu; Comprehensive Secondary School. Ezeagu LGA, Community Secondary School Akama Oghe; Community Secondary School, Owa; Comprehensive Secondary School, Iwollo. Oji-River LGA, Model Secondary School Inyi; Urban Secondary School Oji river; Anglican Grammer School, Achi. Udi LGA, Paul Secondary School Eke; Community Secondary School Ngwo; Community Secondary School, Amaokwe, Udi.

Collection of Samples

The method of sample collection employed was venopuncture technique as described by Cheesbrough (2006). A tube tourniquet was used to tie the upper arm of the patient to enable the veins to be bulge. The skin was cleaned with ethanol and allowed to dry. New sterile syringe and needle was used to puncture the vein and the plunger was gently withdrawn. Ten milliliters of venous blood was collected. The blood specimens were used for

1. HIV screening based on Nigerian National HIV testing algorithm
2. Malaria screening using test kit and staining method

3. Hepatitis B virus screening using test kit

Experiment 1: **Prevalence of HIV among the subjects.**

Procedure: The Nigerian National HIV testing algorithm recommended by WHO for resource – poor countries was used. It is a serial testing using 3 rapid test kits (RTKs) namely Determine, (Inverness, Japan), UniGold (Trinity, Ireland) and StatPak (Chembo diagnostic system).

General Principle of RTK: It is an immunochromatographic test for the qualitative detection of antibodies to HIV – 1 and HIV – 2. Specimen is added to the specimen pad. As the specimen migrates through the conjugate pad, it reconstitute and mixes with selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient's window site. If antibodies to HIV – 1 and/or HIV – 2 are present in the specimen, the antibodies bind to the antigen – selenium colloid and to the antigen at the patient's window, forming a red line at the patient's window site. If antibodies to HIV – 1 and/or HIV – 2 are absent, the antigen – selenium colloid flows past the patient's window and no red line is formed at the patient's window site. To ensure validity, a procedural colour bar is incorporated in the assay device.

Expected Result: Two red bars in both the patient's window and control indicated a positive result. One red bar in the control window and no red bar in the patients represent a negative result. An invalid test occurs when a red bar develops at the patient window only and none at the control window.

Experiment 2: Prevalence of HBV among the subjects

Procedure: The blood that was collected was placed in a test tube and allowed to coagulate to obtain serum. The test card was placed on a smooth, horizontal clean surface with sticky parts removed to expose the sticky part. The test strip was dipped into the serum for 10 seconds without exceeding the maximum line on the test strip, then they were then read after 15minutes.

Expected Result: Two red bars in both the patient's region and control region indicate a positive result. One red bar in the control region and no red bar in the patient's region represent a negative result. When only one line appears on the control(c) region only, or one line appears on the test region and non on the control region(c) and also when no line appear on the test (T) and control(c), the result is said to be invalid.

Experiment 3: The distribution of malaria among the subjects using CareStart malaria HRP2

Whole blood was used for the diagnosis of malaria using parallel Malaria *Plasmodium falciparum* Rapid Test Device (manufactured by Global device, USA and INDR Diagnostica, USA). The malaria P.f. Rapid Test Device (Whole Blood) is a qualitative, membrane based immunoassay for the detection of P.f antigen in whole blood.

Principle: The principle is based on a rapid chromatographic immunoassay for the qualitative detection of circulating *P. falciparum* antigen in the whole blood. This method utilizes Gold conjugate to selectively detect *Plasmodium* antigen.

Procedure: The procedure was as described by the manufacturer. About 5µl of whole blood was added into sample well by using a pipette and 2 drops of assay buffer were added into assay buffer well. The result was read within 20 minutes.

Expected Result: The presence of two pink lines at the region of the control and test sample signified presence of *P. falciparum* malaria infection while the presence of only one pink line in the control region signified absence of *P. falciparum*. The test device has inherent quality control that validates the result.

Experiment 4: The distribution of malaria among the subjects using Giemsa staining of thick blood film

The method described by Etusim *et al*. (2013) was used. A thick blood film was made on a grease free slide. Giemsa stain was poured on the film and allowed to stay for 30 minutes on the staining rack; the stain was then washed off with running water. Finally, a drop of oil immersion was dropped on the stain and viewed through the objective lens (x100), when the slide had dried.

STATISTICAL ANALYSES

Data generated from this study was analyzed using IBM SPSS version 10.0 statistical software. The degree of correlation between variables was evaluated by using the Spearman correlation analysis method. For all the statistical tests, a two-tailed p-value of < 0.05 was considered statistically significant.

RESULTS

A total of 1500 blood specimens were collected from Secondary School Students in Enugu West and screened for Infections of Hepatitis B Virus (HBV), Malaria parasite (MP) and Human Immunodeficiency Virus (HIV). The result revealed that a total of 102 (6.8%) students were positive to all the infections, while malaria parasite was the most prevalent in the study population 63(4.2%), followed by hepatitis B virus 36(2.4%) and the least infection was HIV 3(0.2%), when compared, it was statistically significant at $p>0.05$ (Table 1).

The gender distribution of the co-infections of malaria/HIV, malaria/HBV, HIV/HBV and malaria/HIV/HBV among the study population showed that 28(1.7%) was the most prevalent to malaria/HBV infection and the least was 1(0.1%) to HIV/HBV and malaria/HIV/HBV, respectively. Males were the most infected to malaria/HBV 10(1.9%) than females 18(1.8%). The comparison between the gender differed significantly at $p<0.05$ and other values are found in Table 2.

The age distribution of the co-infections of malaria/HIV, malaria/HBV, HIV/HBV and malaria/HIV/HBV among the study population showed that the age group of 21- 25 years was the most prevalent to the co-infections at a prevalent rate of 6.1% to malaria/HIV and malaria/HBV, respectively, while 3.0% to HIV/HBV and malaria/HIV/HBV, respectively. The least prevalent was the age group of 11-15 years which recorded 0.4% to malaria/HBV and other values are found in Table 3. The comparison between the age group differed significantly at $p <0.05$.

The gender distribution of the co-infections of malaria/HIV, malaria/HBV, HIV/HBV and malaria/HIV/HBV according to LGA in Enugu West Senatorial Zone revealed that females from Oji-river were the most infected to malaria/HBV 6(2.0%), followed by Ezeagu 5(1.7%) and the least was Udi 1(0.3%), at $p<0.05$, was considered statistically significant. While Males had equal co-infection rate of 2(0.7%) to malaria/HBV across all the LGA's. Only in Agwu LGA were females infected to HIV/HBV and malaria/HIV/HBV at a prevalent rate of 1(0.3%), respectively and it differed significantly at $p<0.05$ (Table 4).

The distribution of the co-infections according to Secondary Schools in Agwu LGA revealed that (B) Alpha Secondary School, Agwu was the most infected to malaria/HIV 2(2.0%), malaria/HBV 3(3.0%), HIV/HBV 1(1.0%) and malaria/HIV/HBV 1(1.0%), followed by (C)

Girls Secondary School, Mmaku, 1(1.0%) and (A) Boys Secondary School, Mgbowo, 1(1.0%) to malaria/HBV, respectively and it differed significantly at P <0.05 (Table 5).

The distribution of the co-infections according to Secondary Schools in Aninri LGA revealed that (D) Community Secondary School Oduma and (E) Community Secondary School, Mpu were the only infected Secondary Schools in Aninri LGA to malaria/HBV 1(1.0%), respectively and it differed significantly at p<0.05, other values are shown in table 6.

The distribution of the co-infections according to Secondary Schools in Ezeagu LGA revealed that (I) Comprehensive Secondary School, Iwollo was the most infected to malaria/HBV 3(3.0%) in females, followed by (G) Community Secondary School, Akama Oghe 2(2.0%), while the least was (H) Community Secondary School, Owa, 0(0.0%) and it differed significantly at p<0.05, other values are shown in table 7.

The distribution of the co-infections according to Secondary Schools in Oji-river LGA revealed that ((J) Model Secondary School Inyi and (K) Urban Secondary School Oji river were the only respectively infected schools to malaria/HBV 3(3.0%) in females and 1(1.0%) in males, which differed significantly at p<0.05, other values are shown in table 8.

The distribution of the co-infections according to Secondary Schools in Udi LGA revealed that (O) Community Secondary School, Amaokwe, Udi was the only school infected to malaria/HBV 2(2.0%) in males and 1(1.0%) in females, which differed significantly at p<0.05, other values are shown in table 9.

Table 1: Gender distribution of the infections among study population

Gender	Total No of subjects screened	MP+ (%)	HIV+ (%)	HBV+ (%)	Total no infected to all the infections (%)
Males	518	22(4.3)	0(0)	14(2.7)	36(7.0)
Females	982	41(4.2)	3(0.3)	22(2.2)	66(6.7)
Total	1500	63(4.2)	3(0.2)	36(2.4)	102(6.8)

p <0.05, was considered statistically significant.

Key: MP = Malaria Parasite, HIV = Human Immunodeficiency Virus, HBV = Hepatitis B Virus, + = Positive, - = Negative,

Table 2: Gender distribution of the co-infections among the study population

Gender	Total no of subjects screened	MP+/HIV+ (%)	MP+/HBV+ (%)	HIV+/HBV+ (%)	MP+/HIV+/HBV+ (%)
Males	518	0(0.0)	10(1.9)	0(0.0)	0(0.0)
Females	982	2(0.1)	18(1.8)	1(0.1)	1(0.1)
Total	1500	2(0.1)	28(1.7)	1(0.1)	1(0.1)

p<0.05, was considered statistically significant Key: HBV = Hepatitis B Virus, HIV = Human Immunodeficiency Virus, MP = Malaria Parasite, + = Positive.

Table 3: Age distribution of co-infections among the study population

Age group (yr)	Total No of subjects screened	MP+/HIV+ (%)	MP+/HBV+ (%)	HIV+/HBV+ (%)	MP+/HIV+/HBV+ (%)
11-15	246	0(0.0)	1(0.4)	0(0.0)	0(0.0)
16-20	1221	0(0.0)	25(2.1)	0(0.0)	0(0.0)
21-25	33	2(6.1)	2(6.1)	1(3.0)	1(3.0)
Total	1500	2(0.1)	28(1.9)	1(0.1)	1(0.1)

P <0.05, was considered statistically significant Key: HBV = Hepatitis B Virus, HIV = Human Immunodeficiency Virus, MP = Malaria Parasite, + = Positive.

Table 4: Gender distribution of the co-infections according to LGA in Enugu West Senatorial Zone

LGA	Total no of subjects screened	MALES				FEMALES			
		MP+/HIV+ (%)	MP+/HBV+ (%)	HIV+/HBV+ (%)	MP+/HIV+/HBV+ (%)	MP+/HIV+ (%)	MP+/HBV+ (%)	HIV+/HBV+ (%)	MP+/HIV+/HBV+ (%)
Agwu	300	0(0.0)	2(0.7)	0(0.0)	0(0.0)	2(0.7)	4(1.3)	1(0.3)	1(0.3)
Aninri	300	0(0.0)	2(0.7)	0(0.0)	0(0.0)	0(0.0)	2(0.7)	0(0.0)	0(0.0)
Ezeagu	300	0(0.0)	2(0.7)	0(0.0)	0(0.0)	0(0.0)	5(1.7)	0(0.0)	0(0.0)
Ojiriver	300	0(0.0)	2(0.7)	0(0.0)	0(0.0)	0(0.0)	6(2.0)	0(0.0)	0(0.0)
Udi	300	0(0.0)	2(0.7)	0(0.0)	0(0.0)	0(0.0)	1(0.3)	0(0.0)	0(0.0)
Total	1500	0(0.0)	10(0.7)	0(0.0)	0(0.0)	2(0.1)	18(1.2)	1(0.1)	1(0.1)

p<0.05, was considered statistically significant Key: HBV = Hepatitis B Virus, HIV = Human Immunodeficiency Virus, MP = Malaria Parasite, + = Positive.

Table 5: Distribution of the co-infections according to Secondary Schools in Awgu LGA

		MALES				FEMALES			
Secondary Schools	**Total no of subjects screened**	**MP+/HIV+ (%)**	**MP+/HBV+ (%)**	**HIV+/HBV+ (%)**	**MP+/HIV+/HBV+ (%)**	**MP+/HIV+ (%)**	**MP+/HBV+ (%)**	**HIV+/HBV+ (%)**	**MP+/HIV+/HBV+ (%)**
A	100	0(0.0)	1(1.0)	0(0.0)	0(0.0)	ND	ND	ND	ND
B	100	0(0.0)	1(1.0)	0(0.0)	0(0.0)	2(2.0)	3(3.0)	1(1.0)	1(1.0)
C	100	ND	ND	ND	ND	0(0.0)	1(1.0)	0(0.0)	0(0.0)
Total	300	0(0.0)	2(0.7)	0(0.0)	0(0.0)	2(0.7)	4(1.3)	1(0.3)	1(0.3)

P <0.05, was considered statistically significant.

Key: A= Boys Secondary School, Mgbowo; B= Alpha Secondary School, Agwu; C= Girls Secondary School, Mmaku; HBV = Hepatitis B Virus, MP = Malaria Parasite, + = Positive ND= Not Determined

Table 6: Distribution of the co-infections according to Secondary Schools in Aninri LGA

		MALES				FEMALES			
Secondary Schools	**Total no of subjects screened**	**MP+/HIV+ (%)**	**MP+/HBV+ (%)**	**HIV+/HBV+ (%)**	**MP+/HIV+/HBV+ (%)**	**MP+/HIV+ (%)**	**MP+/HBV+ (%)**	**HIV+/HBV+ (%)**	**MP+/HIV+/HBV+ (%)**
D	100	0(0.0)	1(1.0)	0(0.0)	0(0.0)	0(0.0)	1(1.0)	0(0.0)	0(0.0)
E	100	0(0.0)	1(1.0)	0(0.0)	0(0.0)	0(0.0)	1(1.0)	0(0.0)	0(0.0)
F	100	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	300	0(0.0)	2(0.7)	0(0.0)	0(0.0)	0(0.0)	2(0.7)	0(0.0)	0(0.0)

p<0.05, was considered statistically significant.

Key: D= Community Secondary School Oduma; E= Community Secondary School, Mpu; F= Comprehensive Secondary School, Ndeabor; HBV = Hepatitis B Virus, MP = Malaria Parasite, + = Positive.

Table 7: Distribution of the co-infections according to Secondary Schools in Ezeagu LGA

		MALES				FEMALES			
Secondary Schools	**Total no of subjects screened**	**MP+/HIV+ (%)**	**MP+/HBV+ (%)**	**HIV+/HBV+ (%)**	**MP+/HIV+/HBV+ (%)**	**MP+/HIV+ (%)**	**MP+/HBV+ (%)**	**HIV+/HBV+ (%)**	**MP+/HIV+/HBV+ (%)**
G	100	0(0.0)	1(1.0)	0(0.0)	0(0.0)	0(0.0)	2(2.0)	0(0.0)	0(0.0)
H	100	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
I	100	0(0.0)	1(1.0)	0(0.0)	0(0.0)	0(0.0)	3(3.0)	0(0.0)	0(0.0)
Total	300	0(0.0)	2(0.7)	0(0.0)	0(0.0)	0(0.0)	5(1.7)	0(0.0)	0(0.0)

P <0.05, was considered statistically significant.

Key: G= Community Secondary School Akama Oghe; H= Community Secondary School, Owa; I= Comprehensive Secondary School, Iwollo; HBV = Hepatitis B Virus, MP = Malaria Parasite, + = Positive.

Table 8: Distribution of the co-infections according to Secondary Schools in Oji-river LGA

Secondary Schools	Total no of subjects screened	MALES				FEMALES			
		MP+/HIV+ (%)	MP+/HBV+ (%)	HIV+/HBV+ (%)	MP+/HIV+ /HBV+ (%)	MP+/HIV+ (%)	MP+/HBV+ (%)	HIV+/HBV+ (%)	MP+/HIV+/ HBV+ (%)
J	100	0(0.0)	1(1.0)	0(0.0)	0(0.0)	0(0.0)	3(3.0)	0(0.0)	0(0.0)
K	100	0(0.0)	1(1.0)	0(0.0)	0(0.0)	0(0.0)	3(3.0)	0(0.0)	0(0.0)
L	100	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	300	0(0.0)	2(0.7)	0(0.0)	0(0.0)	0(0.0)	6(2.0)	0(0.0)	0(0.0)

P <0.05, was considered statistically significant.

Key: J= Model Secondary School Inyi; K= Urban Secondary School Oji river; L= Anglican Grammer School, Achi; HBV = Hepatitis B Virus, MP = Malaria Parasite, + = Positive.

Table 9: Distribution of the co-infections according to Secondary Schools in Udi LGA

Secondary Schools	Total no of subjects screened	MALES				FEMALES			
		MP+/HIV+ (%)	MP+/HBV+ (%)	HIV+/HBV+ (%)	MP+/HIV+ /HBV+ (%)	MP+/HIV+ (%)	MP+/HBV+ (%)	HIV+/HBV+ (%)	MP+/HIV+ /HBV+ (%)
M	100	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
N	100	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
O	100	0(0.0)	2(2.0)	0(0.0)	0(0.0)	0(0.0)	1(1.0)	0(0.0)	0(0.0)
Total	300	0(0.0)	2(0.7)	0(0.0)	0(0.0)	0(0.0)	1(0.3)	0(0.0)	0(0.0)

P <0.05, was considered statistically significant.

Key: M= St. Paul Secondary School Eke; N= Community Secondary School, Ngwo; O= Community Secondary School, Amaokwe, Udi; HBV = Hepatitis B Virus, MP = Malaria Parasite, + = Positive.

DISCUSSION AND CONCLUSION

In the developing world, concomitant infections are the rule rather than the exception, and the complete clinical picture involves several microorganisms that influence each other as well as the host (Lundqvist *et al*., 2010). Malaria, HIV/AIDS and hepatitis B virus (HBV) are among the most devastating diseases in many low-income countries, particularly in sub-Saharan Africa.

Omalu *et al*. (2012) reported that malaria, HIV and HBV are the three most important infectious diseases and have similar global distributions, with the majority of those infected individuals lived in countries with constrained resources like sub-Saharan Africa, the Indian subcontinent, and Southeast Asia. However, this study was done to evaluate the concomitant infections of malaria, human immunodeficiency virus and hepatitis B virus among Secondary School Students particularly in the study area.

In this study, the prevalence rate of single infection was found to be 4.2% for malaria, 0.2% for HIV and 2.4% for HBV. It is important to note that while these rates may be suggesting low endemicity, there is need to appreciate their public health implication considering the high rate of urbanization that the state is currently experiencing. This calls for public health alertness and proper screening of blood donations for medical emergency purposes including child-cum-parental closeness. The prevalence rate of hepatitis B infection 14(2.7%) for males and 22(2.2%) for females among the subjects observed in this work contrasts with the results of Ejele *et al*. (2004), who recorded 4.9% prevalent rate in Port Harcourt while Siriena *et al*. (2002) recorded 10.3% in Jos.

which is similar to the work Uko *et al*. (1998) in Calabar and Etusim *et al*. (2013), who studied the concomitant infections of Hepatitis B Virus, malaria and human immunodeficiency virus in Owerri metropolis. This study is also in accordance with Omalu *et al*. (2012), who studied the seroprevalence of malaria and hepatitis B (HBsAg) with associated risk factors among pregnant women attending antenatal clinic in General Hospital Minna, North-Central Nigeria and obtained similar results.

The gender and age related concomitant infection yielded four forms of mixed infections among the secondary school students in Enugu west. These include hepatitis B Virus / malaria, hepatitis B Virus / HIV, malaria / HIV and hepatitis B Virus / Malaria / HIV.

The age distribution of the co-infections of malaria/HIV, malaria/HBV, HIV/HBV and malaria/HIV/HBV among the study population showed that the age group of 21- 25 years was the most prevalent to the co-infections at a prevalent rate of 6.1% to malaria/HIV and malaria/HBV, respectively, while 3.0% to HIV/HBV and malaria/HIV/HBV, respectively. The least prevalent was the age group of 11-15 years which recorded 0.4% to malaria/HBV. The higher prevalence of malaria infection among the age group of 21-25yrs could be as a results of parental negligence to the children as they are now grown up or as reported by Omalu *et al*. (2012), who stated that in Nigeria, malaria is one of the most health problems top ranking in the list of common infectious diseases and three quarter of the total land mass of the country is regarded as malarious and about 68% of the total population is at risk of malaria infection. Surprisely, the prevalence of HIV infection among females in the age group of 21-25yrs could be as a result of illicit sexual interactions, lack of proper sexual awareness and lack of sex education among our Secondary Schools.

The gender occurrence of coinfection of malaria/ HBV among the study subjects showed that an overall of 28(1.7%) was infected with the mixed infection of malaria/ hepatitis B Virus. The co-infection of malaria/ HBV was most prevalent in males 10(1.9%) than in females 18(1.8%). The age distribution of malaria/ HBV co-infected subjects revealed that the age group of 21-25 years had the highest prevalence rate of 1(3.0%) both in males and females, followed by 16-20 years 8(0.7%) in males and 17(1.4%) in females, while 11-15 years 1(0.4%) and 0(0.0) in males and females, respectively. The values among the age group was significantly different at $p<0.05$.

The gender and age distribution of the concomitant occurrence of malaria/HIV/ HBV among the study subjects revealed that the females were only infected with the concomitant occurrence of malaria/HIV/HBV at a prevalent rate of 1(0.1%). The age group of 21-25 years had the highest prevalent rate of 1(3.0%) to malaria/HIV/HBV and the mixed infection was only present among this age group. At $p<0.05$, it was significantly different. The result is in line with Etusim *et al*. (2013), who studied the concomitant infections of hepatitis B Virus, malaria and human immunodeficiency virus in Owerri metropolis.

The co-infection of HIV/ HBV among the subjects showed that a total of 1(0.1%) was infected with HIV/ hepatitis B virus. The prevalence rate of 0.0% and 0.1% was recorded among the males and females, respectively. The age distribution of HIV/ HBV co-infected subjects revealed that only the age group of 21-25 years was infected with HIV/HBV 1(3.0%), 16-20 years and 11-15 years was 0(0.0%), respectively.

The prevalence of mixed infection of malaria/HIV among the Secondary School Students in Enugu West revealed that the mixed infection was only present in females 2(0.1%) and among the age group of 21-25 years 2(6.1%). An overall of 2(0.1%) was infected with Malaria and HIV.

The gender distribution of the co-infections of malaria/HIV, malaria/HBV, HIV/HBV and malaria/HIV/HBV according to LGA in Enugu West Senatorial Zone revealed that females from Oji-river were the most infected to malaria/HBV 6(2.0%), followed by Ezeagu 5(1.7%) and the least was Udi 1(0.3%), at $P<0.05$, was considered statistically significant. While Males had equal co-infection rate of 2(0.7%) to malaria/HBV across all the LGA's. Only in Agwu LGA were females infected to HIV/HBV and malaria/HIV/HBV at a prevalent rate of 1(0.3%), respectively and it differed significantly at $P<0.05$. Shankarkumar *et al*. (2011) stated, in areas of stable malaria, transmission is intense and continuous, although seasonal variations may

occur. Immunity develops early in life, and young children and pregnant women are at greatest risk of morbidity and mortality from malaria. In these areas, HIV-related immunosuppression may increase rates of malaria infection and clinical malaria disease, but does not increase the rates of severe or complicated malaria as reported by Shankarkumar *et al*. (2011).

Relative risk for parasitemia and malarial fever increase with decreasing CD4 count and increasing viral load. These findings suggest that HIV infection not only may interfere with parasite control, but also, perhaps more important, may cause the loss of antitoxic immunity, which protects persons with parasitemia from clinical disease. In regions of unstable malaria, transmission is intermittent and less predictable, and epidemics may occur. The disease burden is similar in all age groups because preexisting ant malarial immunity is limited. As a result, malarial fever rate as a direct function of parasite transmission rate. Thus, HIV co-infection has its impact on disease presentation, with an increased risk of complicated and severe malaria and death (Shankarkumar *et al*., 2011). Studies of malaria and HIV interactions in children living in areas of stable malaria epidemiology have been inconclusive. A study in rural Kwazulu-Natal, an area of unstable malaria, reported that HIV- infected children were more likely to experience severe disease, coma, and death.

Conclusion

The gender and age distribution of the concomitant occurrence of malaria/HIV/ HBV among the study subjects revealed that the females were only infected with the concomitant occurrence of malaria/HIV/HBV at a prevalent rate of 1(0.1%). The age group of 21-25 years had the highest prevalent rate of 1(3.0%) to malaria/HIV/HBV and the mixed infection was only present among this age group. This could be as a result of illicit sexual interactions, lack of proper sexual awareness and lack of sex education among our Secondary Schools. There is therefore, an urgent need to institute public health measures that will reduce disease burdens and transmission among students in Enugu West. This measures may include parental health talks, proper diagnosis, treatment and increased surveillance activities to protect the populations at risk.

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