ISSN: 2141 – 3290
www.wojast.com

CONCOMITANT BACTERIA IN THE BLOOD OF MALARIA PATIENTS SAMPLED IN THE SOUTHERN PARTS OF NIGERIA.

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ABSTRACT: The presence of concomitant bacterial infection was investigated in the blood of 330 students made up of 225 symptomatic and 105 non symptomatic patients attending the University of Uyo Medical Center between July to December, 2010. The objective was to determine prevalence of concomitant bacterial infection in malaria patients and to evaluate the bacteria types and malaria parasite among patients who visit the medical centre for treatment. Malaria parasitaemia was established by thick and thin blood smears stained with Giemsa. Anemia was determined using haemoglobin level, pack cell volume and mean corspuscular haemoglobin concentration. Blood samples were cultured in MacConkey, chocolate and blood agar respectively using oxoid signal system after the manufacturers instructions. Out of 225 symptomatic cases, 111 (49.33%) were found infected with malaria out of which 58 (52.25%) had bacterial infection. Of the 105 apparently non-symptomatic cases 8(7.62%) were found infected with malaria parasite isolates. Of the 60 individuals with malaria parasitaemia and bactericaemia, 20(33.33%) were males and 40 (66.67%) were females. A total of 158 bacteria isolates were observed. The bacteria isolates in order of their prevalence were as follows: *Staphylococcus spp*, 43(21.72%), *Salmonella typhi* 40 (20.20%), *Streptococcus spp*, 36 (18.18%), *Escheria coli* 30(15.15%), *Bacillus spp* 18 (9.09%), *Klebsiella spp* 14(7.07%), *Pseudomanas spp* 11(5.56%) and *Micrococcus spp* 6(3.03%). The bacteria isolates occurred more in females than males. The presence of concomitant bacteria in malaria positive cases was concluded to have probably led to persistence of malaria-like symptoms after treatment with antimalaria drug and confuses diagnosis.

INTRODUCTION

Concomitant infection refers to the combine infection of two or more parasites of different species or groups within a single host. Some symptoms of bacterial infections are similar to those of malaria infection. Ukagai *et. al* (2006) Malaria is a debilitating as well as killer disease which affects both the physical and economic wellbeing of people living in endemic areas of Africa (Nebe, *et al.*, 2002). When some bacterial infection combines with malaria infection, it can lead to gravious consequences (Usip, and Opara, 2008).

Malaria has been one of the world's worst killer diseases throughout recorded human history. About 300 million people globally are infected and about 1.5 million deaths occur annually in Africa, south of sahara (Taylor *et al.*, 2006).

Malaria is a public health problem in Nigeria. Recent studies have shown the existence of concomitant bacterial infections in severe malaria patients, which complicate the manifestation of malaria, thereby confusing diagnosis and chemotherapy especially where they are relatively unknown. Gram negative bacteria have been implicated as important cause of septicemia in some cases of *P. falciparum* malaria infection(Ukagai *et al* 2006) . Septicemia present fever, chill, anxiety and rapid breathing which is similar to malaria. Kremsner *et al.* (1995) observed

bactericaemia in African children with severe malaria and emphasized on the need for inclusion of an antibiotic such as Chlindamycin to standard quinine treatment. Falciparum malaria is the most prevalent malaria in Nigeria but very little information on concomitant bacterial infection in severe malaria is known.

The objective of the study was to determine the prevalence of concomitant of bacterial infection in malaria parasitemia, to evaluate the type of bacteria and malaria parasite among patients who visit University of Uyo Medical Centre for treatment. The age and sex related prevalence of bacteria and malaria infections were also determined and implication of anaemia was investigated.

MATERIALS AND METHODS

The study area; University of Uyo Town and Annex Campus located along Ikot Ekpene Road in Uyo Urban is geographically positioned at Latitude E 007^0 55' 535 and longitude N 005^0 02' 282. It is located in the rain forest belt with an elevation of 1000mm above sea level. The area is surrounded with non-sloping gutters along Ikot Ekpene Road and Ikpa Road and the raven which serves as permanent reservoir for stagnant water which holds water throughout the year and favours the breeding of mosquitoes (Usip and Ibanga 2003). The climatic condition is warm humid, high temperature and heavy rain distribution almost all the year round. The rainy season progresses from May and reaches its peak in August and diminishes by October, while the dry season begins in November and ends in April, (Usip and Ibanga, 2003).

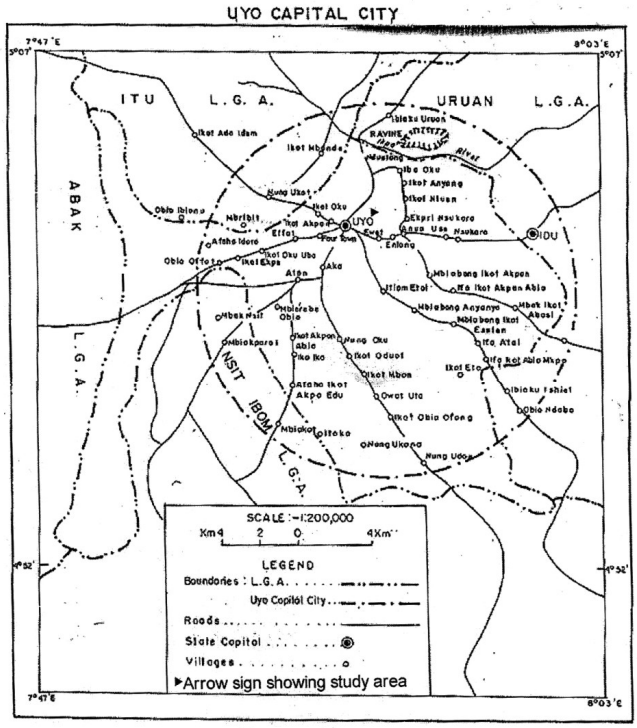

Fig. 1: Map of Uyo Metropolis showing the study area

A total of three hundred and thirty students made up of 225 symptomatic (those with symptoms of malaria) and 105 non-symptomatic (those without symptoms of malaria) students attending University of Uyo Medical Center were involved in the study. Malaria parasitaemia was established by thick and thin blood smears stained with Giemsa and was categorized into low, moderate and higher or heavy infections. Blood samples were cultured in MacCobkey, chocolate and blood agar, respectively using oxoid signal system after the manufacturer's instructions.

Various pathogenic bacteria were isolated, characterized and identified according to their colonial morphology biochemical characteristics and microscopic examination of the cells. Colonial characteristics such as colour, elevation shape, edge surface appearance, viscosity, and transparency were used for identification of the bacterial isolates found in the blood of malaria patients.

Isolates from distinct colonies from MacConkey, chocolate and blood agar plates were further subjected to bacteriological tests (Gram staining) and biochemical test (citrate, catalase, urease coagulates, methyl red, indole sugar fermentation and motility tests) for the determination of bactericaemia. Anemia was determined using Haemoglobin (Hb) level, Pack Cell Volume (PCV) Mean Corpuscular haemoglobin (MCHC).

RESULTS

The prevalence of malaria parasite in relation to age showed variation in the four age groups considered in the study. Infection cut across all age groups. The highest infections rate of 44.44% was recorded among the 19 – 28 years age group while the lowest infection rate of 16.66% was recorded among the 49 years and above age group (Table 1).

Table 1: Age and sex related prevalence of malaria infected anaemia using Hb, PVC, and MCHC.

Age Group in Years	Sex	No. of Examined	No.(%) positive mp	Nb 10.00g/dl or less	PCV 30% or less	MCHC 30gm/dl or less
19-28	M	76	31(40.78)	10(13.15)	5(7.89)	4(5.26)
	F	104	49(47.11)	15(40.78)	7(6.73)	5(4.80)
	M + F	180	80(44.44)	25(40.78)	13(7.22)	9(5.00)
29-38	M	30	10(33.33)	4(40.78)	2(6.66)	2(6.66)
	F	40	12(30.00)	6(40.78)	3(7.50)	2(5.00)
	M + F	70	22(31.42)	10(40.78)	5(7.14)	4(5.71)
39-48	M	19	5(26.31)	2(40.78)	2(10.52)	1(5.26)
	F	31	7(22.58)	3(40.78)	2(6.45)	2(6.45)
	M + F	50	11(22.00)	5(40.78)	4(6.00)	3(6.00)
49 & above	M	13	2(15.38)	0(40.78)	0(5.88)	0(0.00)
	F	17	3(17.64)	1(40.78)	1(3.33)	0(0.00)
	M + F	30	5(16.66)	16(40.78)	1(7.24)	0(0.00)
Total	M	138	48(34.78)	25(40.78)	10(6.77)	7(5.07)
	F	192	71(36.97)	41(40.78)	13(6.96)	9(4.68)
	M + F	330	119(36.06)	31(40.78)	23(0.06)	16(4.48)
% Infected	M	34.78		11.76	7.24	5
	F	36.97		13.02	6.77	4.68
	M + F	36.06		12.40	6.98	4.48

The distribution of malaria parasite showed that out of 330 students sampled, 119 (36.06%) had malaria infection. Out of the 330 students examined, 138 were male out of which 48 (34.78%) had malaria infection while 192 were female out of which 71 (36.92%) had malaria parasitaemia. A low level of anemia was recorded in all the age and there was no significant difference ($P > 0.05$) in prevalence of infection between male and female patience.

Of the 105 apparently non-symptomatic cases, 8 (7.62%) were found infected with malaria parasites in their blood, two bacterial isolate was found in the blood samples. Out of 225 symptomatic cases, 111 (49.33%) were found infected with malaria, out of which 58 (52.25%) also had bacterial infection in their blood. Of the 60 individual with malaria paracetaemia and bactericaemia, 20 (33.33%) were males and 40 (66.67%) were females.

The percentage occurrence of the bacterial isolates (Table 2) reveals that out of 198 isolates, the most abundance species was *Staphylococcus spp*, 43 (21.72%) followed by *Salmonella typhi*,

40 (20.20%), *Streptococcus spp* 36(18.18%) and *Escherichia coli* 30(15.15%) while *Micrococcus spp* were the least in occurrence 6 (3.03%).

The result of combined malaria and bacteria infections (Table 3) shows that the overall prevalence of infections were: malarial infection (36.06%), *Staphylococcus* sp. (13.03%). *Salmonella typhi* (12.12%) *Streptococcus* sp (10.91%), *Escheria* coli (9.09%) *Bacillus* sp (5.45%) *Klebsiella* sp (11.76%) *Pseudomonas* sp (3.33%) and *Micrococcus* sp 1.52%. There was no significant different ($P > 0.05$) in infections between the males and females however, the level of infection decrease with age. Although a large number of female and male were involved in the study, the bacterial isolates occurred more frequently amongst the females.

DISCUSSION

The study revealed that malaria is a serious problem among the students sampled in University of Uyo. The prevalent rate of 36.06% is low compared to the 54.4% observed among patients attending St. Luke's General Hospital, Anua in the same Uyo urban (Usip, and Opara, 2008). The reason for the low prevalence of malaria among the students in the same area could be attributed to level of education, awareness, protection and acquired immunity as well as the composition of the sample (Usip and Opara, 2008). The sample in this case comprises mostly of school children under 18 years which have little immunity.

The higher prevalence of infection in the lower age group 19 – 28 years than those with the age group 45 years and above could be as a result of lower level of both cell mediated and humoural immunity to malaria. The contributing factors to the presence of malaria among University of Uyo students could be attributed to the presence of non sloping gutters along Ikot Ekpene road and Ikpa road, as well as the ravine which is within the university environ and they holds water throughout the year thereby enhancing continuous breeding of the mosquito vector as previously reported by Usip and Ibanga (2003)

This study has shown the existence of concomitant bacterial infection in malaria patients which complicate the manifestation of malaria, thereby confusing diagnosis and chemotherapy especially where they are relatively unknown. For instance, Gram negative bacteria have been implicated as an important cause of septicaemia in some cases of *Plasmodium falciparum* malarial infection (Nduka *et al., 2006)*.

Every year, more than 10 million African children suffer from malaria, with more than 1 million dying from the diseases. Most of these malaria cases are not properly managed due mainly to the fact that therapy is targeted only to malaria parasites due to self medication and lack of proper laboratory diagnosis in Hospital. Poverty and ignorance is another contributing factor to self medication which manifest drug abuse. Similarly, high frequency of *Salmonella typhi* in malaria cases has been previously reported among patients in Calabar, Nigeria an area with drug-resistant malaria (Ukagai, *et al* 2006). Also, most of the cases diagnosed and treated as typhoid fever were, strictly speaking, malaria cases thereby suggesting the presence of a common factor in malaria and typhoid syndrome. This findings agree with Kremsmner *et al* (1995), Ejezie *et al.,* (1991) and Alaribe *et al.* (1998) on the need to include an antibiotic in management of malaria cases. There is also need for proper laboratory diagnosis of both malaria and bacterial infections before embarking on treatment. Concomitant bactericemia should not only be limited to malaria parasitiemia but both those infected and those not infected with malaria should be examined. The bacteria isolates occur more in females than males in this study. This observation is contrary to Ukagai *et al*. 2006) who observed more bacterial isolates in males than females among malaria patient in Owerri.

Table 2: Colonial morphology, characterization and prevalence of bacterial isolates from 60 blood samples

Isolate	Morphology	Gram Stain										Sugar Fermentation					Problem Organism	No.	% +ve
		Shape	Gram Reaction	Catalase	Coatage	MR	Motility	Strate	Urease	Oxidase	Indole	Mannitol	Glucose	Sucrose	Lactose	Dextrose			
1	Milky, glistering, smooth Raised, circular	Cocci in	+	+	+	+	-	+	+	ND	+	A	A	A	A	A	*Staphylococcus* spp	43	21.72
2	Irregular, creamy, shinning Translucent raised, pungent	Clust ers	+	+	-	+	+	+	-	ND	-	AG	AG	A	AG	AG	*Baccilus* spp	18	9.09
3	Circular, cream, tiny, Glistering raised, pungent	Short rod	-	-	-	+	-	+	+	ND	+	-	A	A	A	A	*Micrococcus* spp	6	3.03
4	Rough, irregular creamy filamentous flat pungent	Cocci in chain	+	-	+	+	-	-	+	ND	+	AG	AG	AG	AG	AG	*Streptococcus* spp	36	18.18
5	Smooth erose, circular milky raised pungent	rod	-	-	-	+	+	-	-	ND	+	A	A	A	A	AG	*Escherichia coli*	30	15.15
6	Rough, filamentous, creamy flat, pungent		-	-	+	+	+	+	+	ND	-	A	AG	AG	AG	A	*Salmonella typhi*	40	20.2
7	Smooth erose, circular entire, pungent	Long rod	-	+	-	+	+	+	-	ND	-	A	A	A	A	A	*Klebsiella* spp	14	7.07
8	Rough, raised, filamentous shinning, green, grace-like	rod	-	-	+	+	+	+	+	ND	-	-	A	-	-	-	*Pseudomonas* spp	11	5.56
	Total Bacteria Isolate																	198	

Keys:
+ - Positive
- - Negative
ND - Not Determined
AG – Acid and Gas
A - Acid

Table 3: Age And Sex Related Prevalence Of Malarial And Bacterial Infections

Age in Years	**Sex**	**No. of Examined**	**No (%) of infection Mp +ve**	**No. (%) *Staphylococcus* Sp +ve**	**No. (%) *Bacillus* sp +ve**	**No. (%) *Salmonella typhi* +ve**	**No (%) *Streptococcus Sp* +ve**	**No (%) *Klebsiella sp* +ve**	**No (%) *Escheria coli* +ve**	**No (%) *Micrococcus sp* +ve**	**No (%) *Pseudomonas sp.* +ve**
19 – 28	M	76	31(40.78)	8 (10.53)	3(3.95)	(7.89)	5.(6.58)	2(2.63)	4(5.26)	1(1.32)	2(2.63)
	F	104	49(47.11)	18(17.31)	45(4.81)	15(04.42)	11(10.58)	4(3.85)	7(6.73)	2(1.92)	4(3.85)
	M + F	180	80 (44.44)	26(14.44)	8(4.44)	21(11.67)	16(8.88)	6(3.33)	11(6.11)	3(1.67)	6(3.33)
29 – 38	M	30	10(33.33)	4(13.33)	1(3.33)	4(13.33)	4(13.33)	1(3.33)	4(13.33)	1(3.33)	2(6.66)
	F	40	12(30.00)	7(17.50)	4(5.17)	7(17.50)	6(15.00)	3(7.50)	6(15.00)	1(2.50)	3(7.50)
	M + F	70	22(31.42)	11(15.50)	1(5.26)	11(15.71)	10 (14.29)	4(5.11)	10(4.25)	2(2.00)	5(7.14)
39 - 48	M	19	5(26.31)	1(5.26)	3(9.68)	3(15.79)	2(10.53)	2(10.53)	2(10.53)	0(0.00)	0(0.00)
	F	31	7 (22.58)	3(9.68)	4(8.00)	4(12.90)	4(12.90)	2(6.45)	5(16.13)	1(3.23)	0(0.00)
	M + F	50	11(22.00)	4(8.00)	1(7.69)	7(14.00)	6(12.00)	4(8.00)	7(14.00)	1(2.86)	0(0.00)
49 & Above	M	13	2(15.38)	1(7.69)	1(5.88)	(0.00)	1 (7.69)	0(0.00)	1(7.69)	0(0.00)	0(0.00)
	F	17	3(17.64)	1(7.69)	2(6.66)	1(5.88)	1(5.88)	0(0.00)	1(5.88)	0(0.00)	0(0.00)
	M + F	30	5(16.60)	1(5.88)	6(4.35)	1(3.33)	2(3.33)	0(0.00)	2(3.33)	0(0.00)	0(0.00)
Total	M	138	48(34.78)	2(6.66)	12(6.25)	13(9.42)	12(8.70)	5(3.62)	11(7.97)	2(1.45)	4(2.90)
	F	192	71(36.97)	15(10.87)	18(5.45)	27(14.00)	24(12.5)	9(4.69)	19(9.90)	4(2.88)	7(3.65)
	M + F	330	119(36.06)	28(14.58)	4.35	40 (12.12)	36(10.91)	14(11.76)	30(9.09)	6(1.82)	11(3.33)
% infected	M	34.78		43(13.03)	6.25	9.42	8.70	3.62	7.97	1.45	2.90
	F	36.97		10.87	5.45	14.00	12.5	4.69	9.10	2.88	3.90
	M + F	36.06		11.58			10.91	11.76	9.09	1.82	3.33

The presence of concomitant bacteria in malaria positive cases usually lead to persistence of malaria-like symptoms after treatment with antimalarial drug and subsequently taken as resistance of parasite to the particular drug (Kremsner 1995 and Ejezie *et al* 1991)

CONCLUSION

Although malaria constitutes a major economic burden on endemic communities in Africa including Nigeria, there have been few studies to determine the implication of other disease burden in malaria infection such as the concomitant bactericemia. treatment of malaria base on clinical symptoms is sometimes misleading. this study suggests specific laboratory diagnosis of malaria for adequate treatment and differential diagnosis of other infection such as typhoid that usually present similar malaria like symptoms like fever,headache,pains, anemia, vomiting e.t.c. thus the need for proper laboratory diagnosis of both malarial and bacterial infection before embarking on treatment for effective management of malaria and other related diseases.

ACKNOWLEDGEMENT

The authors thank the HOD and the staff of the pathology department of the University of Uyo Medical Center for their assistance and valuable contributions.

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