STUDIES ON GENDER/SEX AND CO-INFECTIONS OF MALARIA, BACTERAEMIA AND INTESTINAL PARASITES OF PRIMARY SCHOOL CHILDREN IN OWERRI METROPOLIS, IMO STATE.

BY

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Abstract

Studies on gender/sex and co-infections of malaria, bacteraemia and intestinal parasites in primary school children in Owerri metropolis were carried out. Stool and urine specimen were collected with sterile containers, blood specimen were collected with syringe and EDTA bottles from 650 hospitalized and 500 school pupils (who served as control). Parasitological investigations on the stool specimen included Macroscopic Analysis, Direct Wet Method and Formol Ether Concentration Technique; On urine specimen, by centrifuging and microscopy, on Blood specimen by Thick and Thin blood smear method. Bacterial Inoculation, Isolation and Identification was done by subjecting the respective specimen to culture. Among the hospitalized pupils, 208 (32%) were infected with both *Plasmodium falciparum* and bacterial organisms out of which 101 (15.5%) were males while 107(16.4%) were females.. A total of 187 (28.8%) were infected with *Plasmodium falciparum* and intestinal parasites out of which 95(14.6%) were males while 92 (14.2%) were females. A total of 125 (19.3%) were infected with *Plasmodium falciparum,* as well as with both intestinal parasites and bacterial organisms out of which 57 (8.8%) were males while 68 (10.5%) were females. A total of 520(80.0%) hospitalized pupils had co-infections, 253 (48.6%) were males while 267(51.4%) were females. A total of 87(17.4%) school pupils had co-infections, 41(47.13%) were males while 46(52.87%) were females. Significant changes in infections among the genders were not recorded. $P < 0.005$.

Keywords: Co-Infection, Malaria, Bacteremia, Intestinal, Infection.

INTRODUCTION

Co-infection of malaria, bacteraemia and intestinal parasites, provides some public health challenges to children in tropical countries like Nigeria. This is so because there are favorable climatic, environmental and socio-cultural factors which permit transmission of these diseases for greater part of the year.

In co-infections, the burden of one or both of the infectious agents may be suppressed or one may be increased and the other suppressed. Multiple species infections may also increase susceptibility to other infections (Druihe et al,2005; Mwangi et al, 2006). An examination of age-infection profiles of different parasite species helps identify those individuals at greatest risk of concomitant infections. For most helminthes species, intensity of infection rises dramatically with age, with the age of maximum prevalence varying for each helminth species. Age-profiles of *Plasmodium spp*. suggest that blood stage infection is most prevalent in school-aged children, and it is among this age group that co-infections are most likely therefore to occur (Bundy 1995; Anderson et al, 2001).

Interactions between parasites, bacterial organisms and humans can be synergistic or antagonistic. For example, studies have demonstrated a positive association between intensity and concurrent infection of helminth species, suggesting that individuals harbouring multiple helminth species also harbour the most intense infections (Holland et al, 1989; Ferreira et al,1994; Brooker et al 2007). For instance, in malaria, an ongoing infection is thought by many not only to induce, but also to be necessary for immunity to a superimposed infection of parasites with the same or different genotype, a phenomenon called premonition (Smith 1999).

It is conceivable therefore that, co- infections may have a greater impact on morbidity than single species infections since morbidity is typically related to infection intensity for most parasite species. (Nacher 2004; Druihe et al,2005;Mwangi et al, 2006).

Differences between males and females arise because of biological, i.e. *sex* differences and as a consequence of gender-based roles and behavior. Considerable confusion surrounds the use of the words *sex* and *gender*. By and large, sex refers to biological differences between males and females

whereas gender refers to differences between males and females that are determined by societal and cultural factors. (WHO, 2007) It is worth noting, however, that although the distinction between these two concepts is important, it is not always easy to attribute differences in disease processes uniquely to either sex or gender, since sex and gender are not independent of each other.

Fundamental differences between males and females exist at every biological level, from that of the organism as a whole, to organs and organ systems, to individual cells. These biological differences are complex, and may confer advantages either to males or females depending on the infectious agent. Anatomical and hormonal differences between males and females can influence the infectious disease process. At the cell level, a major difference is that female cells have two X chromosomes, whereas male cells have one X and one Y chromosome. Although the influence of cellular differences between males and females on the infectious disease process is not fully understood, it is known that the X chromosome governs many of the immune system responses.(Institute of Medicine, 2001).

MATERIALS AND METHODS

Study Area: This study was conducted in Owerri metropolis, Imo State, South East Nigeria. The geographic position is Latitude 5 ° 45' 0'' N and Longitude 7° 7' 0'' E, located in the rain forest belt of Nigeria with an ambience temperature of about 27^{0}C. The climatic condition is warm and humid with heavy rain distribution. The rainy season starts from April, reaches its peak in August and diminishes in November. The dry season starts in December and ends in March.

Study Population:

The inclusion criterion was any sick child aged between five and twelve years treated in any of the ten selected hospitals, with infection based on any of the following features; fever ($\geq$38° C), or hypothermia ($\leq$ 35° C), cough, fast breathing, difficulty in breathing, abdominal pain, vomiting, diarrhea or clinical diagnosis of pneumonia, typhoid fever and malaria. 500 apparently healthy pupils were randomly selected from all the classes in selected primary schools and investigated for the infection of bacterial, malarial or intestinal parasites.

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Ethical considerations.

The Owerri Municipal Council Education Board granted approval for this study. The subject parents and guardians also provided written informed consent.

Collection of Samples: On reporting to hospital, stool, urine and blood specimen were collected from each sick child. Relevant clinical data such as name, age, sex and weight were also obtained. Similar specimen was also collected from 500 randomly selected seemingly healthy pupils from five selected primary schools.

Relevant permits were obtained from Owerri Municipal Council before samples were collected from primary school pupils. Prior visits were also made to the selected Primary schools to explain to their Head Mistresses the aim of the study and to solicit their approval and co-operation.

Collection of blood samples: A standard clean venepunture technique was used to collect 5mls of blood from both the sick and healthy pupils, into a dipotassium EDTA bottles and samples were analyzed within 24 hours of collection.

Thick and Thin blood films stained with 3% Giemsa were examined microscopically to establish parasitaemia.

Examination of Samples: Each stool specimen was subjected to naked eye examinations for consistency, colour and atypical components such as mucus, blood and parasites. A match-stick head of stool was emulsified in 8.5% saline on a slide. A cover slip (22mm x 22mm) was placed on the suspension and examined with the light microscope, first with x10 objective and again with x40 objective.

The same matchstick head size of stool was emulsified in lugols iodine, covered with a cover slip and examined with a light microscope. The Iodine preparation is particularly suitable for the identification of protozoan cysts. Iodine stains the nuclei and makes them quite visible. The 8.5% saline and iodine wet mount allow for the detection and identification of both protozoan and helminthic human gut parasites. The stool specimen were also examined using formol ether concentration technique (Chessbrough 1991) and were later subjected to culture in order to look for common

enteropathogens. MacConkey, deoxycholate citrate agar(DCA) and thiosulphate citrate bile sucrose(TCBS), Selenite F broth, alkaline peptone water and SS agar were used for the isolation of bacterial pathogens. The bacterial pathogens were identified by standard biochemical tests and by slide agglutination with polyvalent and monovalent sera.

The urine samples were concentrated by spinning at 2500rpm for 5 minutes. The supernatant was decanted and the sediments were placed on a slide, covered with a cover slip (22mm x 22mm) and examined with a light microscope using x40 objective. The urine specimen was later cultured to isolate bacterial pathogens.

Blood examination was done by thin and thick films. These were examined with a light microscope, using immersion oil.

Thin Film: Small quantity of the blood sample (a drop) was placed near one end of a clean microscopic slide. A spreader was inclined first at 45^0 and was then bent up to 30^0 until the blood touches the spreader. Then the spreader was moved forward such that one layer of evenly distributed blood cells was spread on the slides. The thin film method allows for the identification of the plasmodium species and filarial worms.

Thick Film: At the other end of the slide, three drops of blood were placed and with the edge of the spreader the blood was evenly spread over an area (2cm in diameter). The slide was labeled accordingly and was allowed to dry by putting it in an oven or by air dry at a dust free environment.

Staining Smear of Blood: Giemsa stain was used for the staining techniques. The stock solution prepared 24 hours prior the staining was diluted in the ratio of 1ml stock solution to 10ml of 7.2 buffer water and was then filtered before use.

The already fixed blood smears were covered with the diluted Giemsa's stain in a tray for about thirty minutes. These were rinsed thoroughly in distilled waters, then each slide was blotted dry at the edges and under surface and placed in a vertical position and allowed to air dry in a dust free environment. The pH 7.2 buffered water was prepared by mixing 1.4gm potassium phosphate plus 2gm di-sodium hydrogen phosphate plus 2 litres of distilled water.

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Blood samples were also cultured in MacConkey, chocolate and blood agar, respectively using oxoid signal system after the manufacturer's instructions. Isolates from distinct colonies from MacConkey, chocolate and blood agar plates were further subjected to bacteriological tests (gram staining) and biochemical tests (coagulase, catalase, oxidase, motility, indole, hydrogen sulphide, citrate utilization and glucose fermentation tests).

RESULTS.

The results are summarized in Table 1.

Hospitalized Pupils:In the group that had co-infections of *Plasmodium falciparum* and bacterial organisms, more males had *Plasmodium falciparum* in concomitance with *staphylococcus aureus* 3.2% , *Klebsiellae pneumoniae* 0.8% and *Psedomonas aeruginosa* 0.2% than the females 2.9%, 0.7% and 0.1% respectively. On the other hand, more females had *Plasmodium falciparum* in concomitance with *Escherichia coli* 5.5% and *Salmonella typhi* 7.2% than in males, 5.1% and 6.2%. Out of the 208(32.0%) pupils in this category, 101(15.6%) were males while 107(16.4%) were females.

Table 1. Gender Distribution Of Co- Infections In Hospitalized And Primary School Pupils.

Category	Organism/s	HOSPITALIZED PUPILS			SCHOOL PUPILS		
		Number/(%) infected	Males (%)	Females (%)	Number (%) Infected	Males (%)	Females (%)
I Co-infection of Malaria with	*Staphylococcus aureus*	40 (6.2)	3.2	2.9	17(3.4)	1.6	1.8
	Escherichia coli,	69 (10.6)	5.1	5.5	28 (5.6)	2.4	3.2
	Salmonellae typhi	87 (13.4)	6.2	7.2	9 (1.8)	1.0	0.8
	Klebsiellae pneumonia	10 (1.5)	0.8	0.7	0	0	0
	Psedomonas aeruginosa	2 (0.3)	0.2	0.1	0	0	0
	Sub-Total	208 (32.0)	15.5	16.4	54 (10.8)	5.0	5.8
II Co-infection of Malaria with	*Trichuris trichiura*	33 (5.1)	2.6	2.5	0	0	0
	Ascaris lumbricoides	53(8.2)	4.4	3.8	0	0	0
	Entamoeba histolytica	43 (6.6)	3.5	3.1	11 (2.2)	1.2	1.0
	Hookworm	34 (5.2)	2.4	2.8	22 (4.4)	2.0	2.4
	Strongyliodes Stercoralis	24 (3.7)	1.7	2.0	0	0	0
	Sub-Total	187 (28.8)	14.6	14.2	33 (6.6)	3.2	3.4
III Co-infection of Malaria with	*Ascaris lumbricoides* + *Staphylococcus aureus*	26 (4.0)	1.8	2.2	0	0	0
	Ascaris lumbricoides + *Escherichia coli,*	16 (2.5)	1.4	1.1	0	0	0
	Entamoeba histolytica + *Staphylococcus aureus*	22 (3.4)	1.4	2.0	0	0	0
	Entamoeba histolytica + *Escherichia coli*	24 (3.7)	2.0	1.7	0	0	0
	Hookworm + *Salmonellae typhi*	17 (2.6)	1.1	1.5	0	0	0
	Hookworm + *Escherichia coli*	20 (3.1)	1.1	2.0	0	0	0
	Sub-Total	125 (19.3)	8.8	10.5	0	0	0
Sub-Total of Malaria Co- infections		520(80.0)	48.6	51.4	87(17.4)	47.13	52.87
IV	*Plasmodium falciparum* only	130 (20.0)	9.8	10.2	413 (82.6)	43.4	39.2

In the group that had *Plasmodium falciparum* concomitant with intestinal parasites, more males had *Plasmodium falciparum* in concomitance with *Trichuris trichiura* 2.6%, *Ascaris lumbricoides* 4.3% and *Entamoeba histolytica* 3.5% than females 2.5%,3.8% and 3.3% respectively. However, more females had *Plasmodium falciparum* in concomitance with *hookworm* 2.8% and *Strongyloides stecoralis* 1.9% than males 2.4% and 1.7% respectively. Infection in this category of 187 pupils, was almost equally distributed among the genders, 94(14.6%) for males and 93(14.2%) for females.

In the group that had *Plasmodium falciparum*, intestinal parasites and bacterial organisms, more males had *Plasmodium falciparum* in concomitance with *Ascaris lumbricoides* and *Escherichia coli* 1.4%, *Entamoeba histolytica* and *Escherichia coli* 2.0%, compared to females with 1.1% and 1.7% respectively. Similarly, more females had *Plasmodium falciparum* in concomitance with *Ascaris lumbricoides* and *Staphylococcus aureus* 2.2%, *Entamoeba histolytica* and *Staphylococcus aureus* 2.0%, *Hookworm* and *Salmonella typhi* 1.5% as well as *Hookworm* and *Escherichia coli* 2.0%, compared to their males counterpart with 1.8%, 1.4%, 1.1% and 1.1% respectively. Overall in this category, 57(8.8%) were males while 68(10.5%) were females.

Out of 650 hospitalized pupils, 520 had concomitant infections among which 253 or 48.6% of infected pupils were males while 267 or 51.4% were females.

Generally, in the hospitalized pupils, there was no significant change in infection between the males and the females. P> 0.05.

School Pupils: In the group of 54 (10.8%) pupils that had *Plasmodium falciparum* and bacterial organisms, 17(3.4%) had *Staphylococcus aureus* out of which 1.6% were males while 1.8% were females, 28(5.6%) had *Escherichia coli* among which 2.4% were males while 3.2% were females, 9 (1.8%) had Salmonellae typhi among which 1.0% were males while 0.8% were females. Overall in this group, 5.0% were males while 5.8% were females. In the group of 33(6.6%) pupils that had *Plasmodium falciparum* and intestinal parasites, 11(2.2%) had

Entamoeba histolytica out of which 1.2% were males while 1.0% were females, 22 (4.4%) had *Hookworm* among which 2.0% were males while 2.4% were females. In this group, 3.2% were males while 3.4% were females. Generally, out of 500 school pupils, 87 (17.4%) had co-infections among which 41 or 47.13% of all infected pupils were males while 52.87% were females.

DISCUSSION.

This study portrays a lack of association between the coinfection of malaria, bacteraemia and intestinal parasites with the gender/sex of the school children. This is consistent with other studies (Holland et al,1989; Rutagwera and Tylleskar,2012). This suggests the equal predisposition of children of all genders to concomitant infections of malaria with bacterial and intestinal parasites. There are many instances where males and females differ in the susceptibility to infections. The reason for these differences in susceptibility is multifactorial. The primary cause is thought to be due to differences induced by sex hormones and their effects on gene expression as well as the immune system, but may also be due to innate physiological differences between males and females (WHO 2007). Because of the age of children involved in this study, the effects of these sex hormones are not pronounced, giving rise to the fairly equal distribution of infections between both sexes.

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