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CO-EXISTENCE AND SEROPREVALENCE OF BRUCELLOSIS IN A MALARIA-ENDEMIC METROPOLIS OF SOUTH-EASTERN NIGERIA

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ABSTRACT

Malaria and typhoid are known major causes of febrile conditions in the South-Eastern Nigeria, hence healthcare providers usually co-administer antibiotics and anti-malaria on febrile patients without or before proper laboratory diagnosis. Despite this, fever and other symptoms of malaria sometimes persist after repeated treatment regimens. We investigated possible co-existence and prevalence of another fever-causing condition – brucellosis, with malaria and typhoid. The systematic study done between January 2015 and June 2016 involved 682 febrile patients referred to a private medical laboratory in Enugu metropolis in South-Eastern Nigeria for investigation for malaria and typhoid only. The number was made up of 295(43.3%) males and 387(56.7%) females, aged between 10 and 50 years. Identification of malaria parasites was done using thick films stained with Giemsa stain while typhoid and brucellosis were investigated serologically using Chromatest® febrile antigen kits. Our results showed prevalence of 39.1%, 66.0% and 28.6% for malaria, typhoid and brucellosis respectively in the studied population. Prevalence among male and female patients was 46.1% and 33.9% for malaria, 80.3% and 55.0% for typhoid, and 34.2% and 24.3% for brucellosis respectively. The results also showed that prevalence of malaria decreased with age while typhoid and brucellosis increased with age. We opine that 28.6% prevalence of brucellosis in a city with rare pastoral activities is high, and may be the cause of persistent fever after repeated combined treatment for malaria and typhoid. We advocate that these disease conditions should be simultaneously investigated for in all cases of febrile conditions to ensure wider investigation and treatment options, improvement on patients' recovery time and reduction in man-hour loss.

Keywords: Malaria; Typhoid; Brucellosis; Co-existence; Seroprevalence

INTRODUCTION

Brucellosis is a highly contagious zoonotic infection caused by ingestion of non-pasteurized or inadequately pasteurized milk or undercooked meat from infected animals, or close contact with their secretions (Brucella Subgroup, 2004). The causative bacterium is *Brucella* with many species – *B. abortus*, *B. canis*, *B. melitensis*, and *B. suis*. These species are small, gram-negative, non-motile, non-spore-forming, rod-shaped bacteria called *Coccobacilli*. They function as facultative intracellular parasites that cause chronic diseases. Brucellosis in humans is primarily from goats and cows, with occupational exposure of laboratory workers, veterinarians, and slaughterhouse workers. The symptoms are like those associated with many other febrile diseases (like malaria and typhoid fever), including inconstant fevers, chills, malaise, arthralgia, headache, tiredness, miscarriages, sweating, weakness, anaemia, depression, and muscular and bodily pain (Al Dahouk et al, 2003).

Prevalence in Nigeria varies widely from 7.6% in Benue State (Alausa and Awoseyi, 1976) to 24.1% in Abuja (Ofukwu et al, 2014) and 55% in a pooled study of Western States (Aworh et al, 2011). Malaria infection is caused by Plasmodium species, with *P. falciparium* and *P. vivax* of the known five species being more rampant in our environment. Between 300 and 500 million clinical cases of malaria occur each year worldwide, approximately 2 million of which are fatal, primarily in children (Artavanis-Tsakonas et al, 2003). Prevalence in

the South-Eastern Nigeria range from 43.8% to 59.9% (Chukwura et al, 2003; Nwagha et al, 2009; Ogbodo et al, 2009; Ogbodo et al, 2010). Repeated exposure to malaria infection causes development of clinical and anti-parasitic immunity. Children who have not developed or who fail to develop protective immune mechanisms are at greater risk of clinical malaria, severe disease and death than adults. These effects are mainly due to many biochemical and haematological changes occasioned by this infection.

These changes have been variously reported among malaria parasitemic immune-compromised people, especially children and pregnant women from urban and rural areas of South-Eastern Nigeria (Ogbodo et al. 2010; Nwagha et al. 2011; Ogbodo et al. 2013; Ogbodo et al. 2014). Typhoid fever, also simply known as typhoid, is a life-threatening infection caused by Salmonella serogroup typhii and paratyphi bacteria. The severities of the infections vary from mild to severe and usually begin six to thirty days after exposure (Newton and Mintz, 2014; Wain et al, 2015). Often, there is a gradual onset of a high fever over several days (Newton and Mintz, 2014) which may include weakness, abdominal pain, constipation, and headaches while diarrhea is uncommon and vomiting is not usually severe (WHO, 2008). In 2000, typhoid fever caused an estimated 21.7 million illnesses and 217,000 deaths (Crump et al, 2004; Crump and Mintz, 2010), while in 2013 death toll reduced to about 161,000 deaths (GBD Collaborators, 2014).

Documented prevalence in different parts Nigeria includes 16.7% in Oyo State, 39.4% in Nasarawa State and 68.2% in Cross River State (Igbeneghu et al, 2009; Ishaku et al, 2013; Uttah et al, 2013). In South-Eastern Nigeria, malaria and typhoid are recognized by majority of healthcare providers as major causes of fever. Hence, patients presenting with such conditions are blindly treated with anti-malaria in combination with antibiotics, preferably fluoroquinolones, before proper laboratory diagnosis (especially if the initial treatment fails). Despite these combinations, some of the patients still experience persistence of fever and other symptoms, indicating either a relapse or co-existence of another causative organism. This study was therefore taken to investigate the prevalence and possible co-existence of another fever-causing condition – brucellosis, with already known causes – malaria and typhoid.

MATERIALS AND METHODS

Subjects: This is a systematic study involving 682 febrile patients referred to a private medical laboratory – Goldlife Medical Laboratories, in Enugu metropolis for investigation for malaria and typhoid only. The number was made up of 295(43.3%) males and 387(56.7%) females, aged between 10 and 50 years. The period of the study was between January, 2015 and June, 2016.

Sample Collection and Processing: After counseling and obtaining informed consents of the patients, a total of 5.0ml of blood sample was collected from each patient. From this, a thick blood film was made on a grease-free microscope slide and air-dried. The remaining was put in a clean grease-free glass test tube, and allowed to clot and retract before centrifugation at 5000rpm for 5 minutes. The serum was stored frozen until needed for analysis.

Analytical Methods: The thick films were stained with Giemsa stain, allowed to air-dry and examined with x100 objective (oil immersion) lens for malaria parasites. Density of malaria parasites was recorded in notations of + to +++ according to the number of parasites per high power field (Dacombe, 2006). Typhoid and brucellosis were investigated serologically using Chromatest® febrile antigen kits (Linear Chemical SL, Barcelona, Spain). All procedures and instructions given by the manufacturer were strictly adhered to in order to ensure that results were reliable and reproducible.

Statistical Analysis: Patients were grouped according to sex and later according to age. Simple percentage prevalence was calculated among the sexes and then among age groups.

RESULTS

Table 1 shows the prevalence of the disease conditions as 39.1%, 66.0% and 28.6% for malaria, typhoid and brucellosis respectively in the studied population. It also shows that prevalence among male and female patients

55.0% for typhoid, and 34.2% and 24.3% for brucellosis respectively, indicating more exposure of males to the causative agents than females.

TABLE 1: Seroprevalence of the disease conditions in different genders

Disease	Male	Female	Total
Malaria	136 (46.1)	131 (33.9)	267 (39.1)
Typhoid	237 (80.3)	213 (55.0)	450 (66.0)
Brucellosis	101 (34.2)	94 (24.3)	195 (28.6)
Total	295 (43.3)	387 (56.7)	682 (100.0)

**percentage in parenthesis*

Table 2 shows the age distribution of the patients in both sexes. It shows that number of patients that seek medical attention decreases as age increases. This is particularly noticeable in males while the difference may not be significant in females, implying that adult females seek medical attention more than adult males. This is supported by the observation from the table that in all the age groups, female patients are more in number than male patients.

TABLE 2: Number and percentage age distribution of the patients

Age Range (Yrs)	Male	Female	Total
10 - 20	102 (15.0)	104 (15.2)	206 (30.2)
21 - 30	82 (12.0)	110 (16.1)	192 (28.1)
31 - 40	66 (9.7)	101 (14.8)	167 (24.5)
41 - 50	45 (6.6)	72 (10.6)	117 (17.2)
Total	295 (43.3)	387 (56.7)	682 (100.0)

** percentage in parenthesis*

Table 3 shows the prevalence of each of the disease conditions at different age group. This indicates that prevalence of malaria decreases with age while prevalence of typhoid and brucellosis increase with age. The age range 31 – 40years has the highest number of patients who suffer from typhoid and brucellosis in both males and females.

TABLE 3: Distribution of different disease conditions in different age group

Age Range (Yrs)	Malaria	Typhoid	Brucellosis	Total
10 - 20	107 (40.1)	61 (13.6)	8 (4.1)	176
21 - 30	65 (24.3)	113 (25.1)	49 (25.1)	227
31 - 40	51 (19.1)	139 (30.9)	78 (40.0)	268
41 - 50	44 (16.5)	137 (30.4)	60 (30.8)	241
Total	267 (100.0)	450 (100.0)	195 (100.0)	

** percentage in parenthesis*

DISCUSSION

This study shows that in all the disease conditions, greater percentage of males is affected than females despite the fact that female patients are more in number (Table 1). Again, the number of patients in both male and female category decreases as age increases though this is not well pronounced among female patients (Table 2). The observation that the number of patients that seek medical attention decreases with age is typical in this part of the world where adults do not care so much about themselves except at critical stages. This may be related to socioeconomic conditions in the affected areas. Following poor economic conditions, adults (particularly adult males) are forced to be pre-occupied with providing the basic necessities of life for the young ones in particular and the family in general before giving attention to their own health. Thus, we had observed earlier that even among pregnant women, multigravidae do not register for antenatal visits until the last trimester of their pregnancies, unless they develop complications before then (Ogbodo et al, 2012).

Furthermore, this study showed that prevalence of malaria decreases with age while prevalence of typhoid and brucellosis increase with age. The inverse relation between malaria prevalence and age may be related to the development of immunity against malaria infection with age, given that the extent of effects of malaria parasites on the red blood cells of patients depend largely on the patients' immune status (Ogbodo et al, 2016a). This may also be related to the nutritional status of the patients, which is expected to be higher in adults than children. The thought of the involvement of nutrition in this is sequel to the finding that inadequate nutrition contributes significantly to malaria anaemia (Fawzi, 2007). In addition, it

has been found that the concentrations of antioxidant micronutrients are among the determinants of the virulence of malaria parasites (Ogbodo et al, 2016b). On the other hand, seemingly positive relation between the prevalence of typhoid and brucellosis and age may be ascribed to outgoing behavior of the age group that is mostly affected (31 – 40years). This implies that contact of typhoid and brucellosis has much to do with outdoor life style, especially eating outside the house, in low income restaurants (derogatorily called bucateria or mama put) where hygiene is hardly observed.

A prevalence of 28.6% for brucellosis in a city not known for pastoral activities is quite high, and could account for the persistent febrile conditions after repeated combined treatment. This figure varies from 7.6% in Benue State (Ofukwu et al, 2014) and 55% in pooled study of the Western States (Alausa and Awoseyi, 1976) but similar to 24.1% reported in Abuja (Aworh et al, 2011). This indicates that geographical location and other factors like pastoral activities may also influence prevalence. In humans, contacts are mainly from goats and cows, with occupational exposure of laboratory workers, veterinarians, and slaughterhouse workers. This means that laboratory workers are as vulnerable as those who have direct link with animal carriers in endemic areas. The exposure in the laboratories is especially during isolation from blood and other body fluids by culture method. This exposure of diagnostic laboratory personnel to *Brucella* organisms remains a problem in both endemic settings and when brucellosis is unknowingly imported by a patient (Yagupsky and Baron, 2005). Though definite diagnosis requires the isolation of *Brucellae* from blood, bone marrow or other tissues, cultural examinations are time-consuming, hazardous and often times not sensitive (Al Dahouk et al, 2003). This culture method takes two to four weeks to yield positive growth, hence serological methods are now the main test systems readily used in many settings, especially in endemic areas. Hence, though some gram-negative bacilli, including E. coli, show some cross-reactions in serological procedures, they are still preferred because they are simple and quick to meet medical emergencies. In addition, the procedure reduces contact with the organism by the laboratory workers.

On the other hand, malaria prevalence of 39.1% from this study is relatively lower than values obtained from different groups from different parts of South-eastern Nigeria (Chukwura et al, 2003; Nwagha et al. 2009; Ogbodo et al. 2009 Ogbodo et al. 2010) and Ibadan metropolis in Oyo State in the South-western part of Nigeria (Igbeneghu et al, 2009). The possible explanation for the observed low prevalence in this study is that most patients, especially adult males, use several doses of over-the-counter drugs before going to clinics or laboratories for proper diagnosis and treatment, and this practice is hardly revealed to the health worker for fear of being rebuked. This form of malaria treatment is usually in combination with antibiotics, particularly the fluoroquinolones that are generally the drugs of choice for the treatment of typhoid fever in Nigeria (Ogbodo et al, 2011). In cases of the said co-administrations or combined treatments, fluoroquinolones are usually given for about seven to fourteen days.

However, the essential element in the treatment of all forms of human brucellosis is the administration of effective antibiotics for an adequate length of time (WHO, 2006). Moreover, the drugs of choice for brucellosis are gentamicin or streptomycin in combination with any tetracycline, particularly doxycycline. Such treatment is also expected to last as long as 7 to 10 days for intramuscular injection of gentamicin or streptomycin and up to six weeks oral doxycycline for effective response/recovery (WHO, 2006). Therefore, not only that fluoroquinolones are wrong antibiotics for brucellosis but the treatment regimen is inadequate. This repeated wrong antibiotic administration has been identified as one of the major causes of antibiotic resistance (Ogbodo et al, 2011).

Furthermore, the prevalence of 66.0% for typhoid fever in this study is higher than 16.7% and 39.4% obtained in Ibadan, Oyo State and Nasarawa State respectively (Igbeneghu et al, 2009; Ishaku et al, 2013) but similar to 68.2% obtained in Cross River State (Uttah et al, 2013). This variation in prevalence may be attributed to differences in environmental and individual cleanliness. It may also be related to life style of

the inhabitants of the environment involved, some of whom depend largely on local restaurants for most of their foods. Most of these restaurants hardly observe adequate hygiene, thereby spreading these diseases among the patrons.

CONCLUSION

From the study we are of the opinion that 28.6% prevalence of brucellosis in a city not known for pastoral activity is quite high, and could be a source of persistent febrile conditions, in many cases, after repeated combined treatment. We therefore advocate that these disease conditions should be simultaneously investigated for in all cases of febrile conditions to ensure wider investigation that will ensure proper direction for our clinicians on treatment options, improvement on patients' recovery time and reduction in man-hour loss. There is also the need to frequently screen and treat people who are at risk of contacting this stubborn disease by virtue of their work, given that *Brucella* can go as far as attacking the male reproductive tract (Atluri et al, 2011). Furthermore, there is need for periodical enlightenment, screening and treatment of operators of restaurants, especially the local ones in all the urban areas of the country to reduce the spread of typhoid and brucellosis through improper cooking and unhygienic activities. There is also need to include the investigation of other disease conditions that mimic malaria including rheumatism, connective tissue diseases, metabolic disorders etc as part of general screening for first time patients. This is to avoid what appears to be over-diagnosis of malaria in our environment.

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