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Review

Importance of malaria and typhoid co-infection due to misleading Widal results

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Rampant and unnecessary use of iron supplementation to correct possible anemia in malaria and other infections is dangerous, since excess iron may predispose to salmonellosis. Intracellular bacteria, such as *Salmonella typhimurium* has an obligate requirement for iron to support intracellular growth and survival. Complements have unique role in salmonella co-infection with malaria because complements like C1q and C4 deficiency are associated with Salmonella infection. On the other hand, misleading Widal results may keep one away from the true diagnosis because of cross reaction of antigen from other infections with *Salmonella* antibody. Therefore an erroneous interpretation of rapid diagnostic tests delays the treatment of actual infection and increases morbidity. So, further research is necessary to diagnose a true coinfection and to eliminate false interpretation of malaria rapid diagnostic tests (RDTs). Thereby true co-infections and their prevalence should be measured correctly. Effective control measures can be determined if a data of true co-infection were present. A review was carried out on the literature relating to malaria and typhoid, using various journals and published articles from internet search engines. Here we described the importance of malaria and typhoid co-infection due to misleading Widal results, associated conditions, and iron induced true co-infection and their public health importance by reviewing various scientific data.

Key word: Coinfection, malaria, typhoid, iron overload, salmonella, false positive widal, misleading widal result, true coinfection.

INTRODUCTION

Malaria is caused by obligate intracellular parasites, which live in host erythrocytes and remodel these cells to provide optimally for their own needs. It is a major public health problem in tropical areas, and it is estimated that malaria is responsible for 1 to 3 million deaths and 300 to 500 million infections annually. On the other hand, typhoid fever is widely recognized as a major public health problem in most developing tropical countries. It is a systemic infectious disease characterized by an acute illness, the first typical manifestations of which are fever, headache, abdominal pain, relative bradycardia, splenomegaly, and leucopenia (*Cecil textbook of medicine,* XIX edn., 1992; Pearson et al., 2000).

An association between malaria and typhoid fever was first described in the medical literature in the middle of the 19th century, and was named typhomalarial fever by the United States Army (Smith, 1982a). In the last 20 years, this relationship between malaria and salmonellae has been confirmed by additional studies from Africa that largely describe a higher incidence of non-typhoidal salmonella bacteraemia among patients with malarial parasitaemia (Ammah et al., 1999; Bygbjerg et al., 1982). The objective of this article is to review scientific data from studies conducted in the tropics that provided information on malaria and typhoid fever coinfection and to find the possible reason of this coinfection and its solution.

DIFFERENTIATING MALARIA AND TYPHOID BY SIGNS AND SYMPTOMS

Since antiquity, clinicians have had difficulty in differentiating typhoid fever from malaria because of some overlapping clinical features. Because the inability of physicians to clinically differentiate these two entities, they used the term ‘typhomalaria’ as a diagnosis for acute fevers without localizing signs (Cox et al., 1996). Osler (1892) clearly differentiated malaria from typhoid fever by clinical criteria alone. By recognizing and appreciating the characteristic clinical features, we were able to differentiate malaria from typhoid fever. His

observations remain valid and useful today (Osler et al., 1892; Cunha, 2004).

Osler appreciated the differences in height of fever/rapidity of onset in differentiating malaria from early typhoid fever (Cunha, 2004). Osler correctly observed that fever in malaria rises quickly and attains high levels (38.9 to 41.1ºC). Typhoid fever has a plateau fever pattern that rises slowly during the second/third week.

According to Osler, the fever curve in typhoid increases slowly stepwise over the first few days and is followed by a pulse temperature deficit as the infection progressed. Both typhoid fever and malaria are accompanied by a prominent headache. Both malaria and typhoid fever have few, if any localizing signs such as rose spots (in typhoid fever). Splenomegaly is common to both infections.

Osler also cited Malaria begins with multiple shaking chills, whereas typhoid fever begins with a single morning shaking chill. In malaria, chills are followed by spiking fevers. Except for the initial shaking chill, chills are not common with typhoid fever. In malaria, chills precede the fever followed by profuse diaphoresis and profound malaise followed by complete recovery between attacks.

Osler also appreciated the clinical features of malaria and typhoid fever using non specific laboratory tests. While the white blood count (WBC) count in malaria is usually normal/ elevated, typhoid fever is associated with a normal/slightly decreased WBC count. The platelet count in malaria is regularly decreased and thrombo-cytopenia is not a feature of early/uncomplicated typhoid fever. Mild elevations of serum transaminases may occur in both. An increased lactate dehydrogenase (LDH) clearly differentiates malaria (elevated) from typhoid fever (unelevated) (Warrell et al., 2002; Harinasuta et al., 1988; Giles, 1988; Cunha, 2005).

CONCURRENT MALARIA AND ENTERIC FEVER

Typhomalaria was first described by an army doctor, Woodward (1833 - 1884) in 1862 among young soldiers during the American Civil War who were suffering from febrile illness that seemed to be typhoid (including intestinal lesions found at postmortem) but with fever patterns also suggestive of intermittent fever. He believed that it might be a hybrid rather than a new species of disease (Bynum, 2002; Smith, 1982a, b). In the last 20 years, this relationship between malaria and salmonellae has been confirmed by additional studies from Africa that largely described a higher incidence of non-typhoidal salmonella bacteraemia among patients with malarial parasitaemia (Ammah et al., 1999; Bygbjerg et al., 1982).

High prevalence of malaria is an established fact; it is only within the last few decades that an unusually high number of illnesses have been diagnosed as malaria co-existing with typhoid fever (Ammah et al., 1999; Kanjilal et al., 2006; Sur et al., 2006; Ohanu, 2003). Both typhoid and malaria share social circumstances which are imperative to their transmission. Therefore, a person living in such an environment is at risk of contracting both these diseases, either concurrently or an acute infection superimposed on a chronic one.

Association between non-typhoidal salmonellosis and/or typhoidal salmonellosis and malaria was reported in many studies. In Lagos, Nigeria, 16 *Salmonella* spp. made up of seven each of *Salmonella typhi* and *Salmonella enteritidis*, and two of *Salmonella paratyphi* were isolated with *Plasmodium* spp. from patients with complications (Akinyemi et al., 2007). Two other studies in Nigeria that employed bacterial culture identified only typhoidal salmonellosis as responsible for the typhoid fever in co-infected cases (Ohanu et al., 2003; Smith et al., 2004). In Gambia, malarial infection was present in 11% of patients with *S. typhi* septicaemia and 42% of patients with non-typhoidal salmonellae (Mabey et al., 1987).

DIAGNOSIS AND DIAGNOSTIC DILEMMAS

Although the signs and symptoms of malaria and typhoid fever do overlap, it was observed in Pakistan that subjects with dual infection had significantly higher rates of nausea, vomiting, abdominal pain, and diarrhea, all common presenting features of enteric fever (Khan et al., 2005). Furthermore, it was noted that unlike the intermittent fever pattern generally seen with malaria, patients with dual infection tended to exhibit a continuous fever more typical of enteric fever (Khan et al., 2005). Patients with malaria and marked gastrointestinal symptoms, a continuous pattern of fever, and persistence of fever for more than 24 h after appropriate antimalarial therapy, should be investigated or empirically treated for concurrent enteric fever.

It is very common to see patients in many parts of the tropics, undergoing both typhoid and malarial treatment even if their diagnosis has not been confirmed (Mbuh et al., 2003). As far as the diagnosis is concerned:

1. For malaria: A careful examination by an expert microscopist of a well-prepared and well stained blood film remains currently the “gold standard” for detecting and identifying malaria parasites (Malaria diagnosis new perspectives: WHO 2000: 1-57). The rapid diagnostic tests for malaria which use immunochromatographic methods to detect *Plasmodium*- specific antigens in a finger prick blood sample, can be performed in approximately 15 min by individuals with minimal training, using test kits (available from several manufacturers) that require no electricity and no special equipment (Payne, 1988). The main limitations of these tests are lack of sensitivity at low levels of parasitaemia; inability to quantify parasite density; inability to differentiate between *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium*

malariae, as well as between the sexual and asexual stages of the parasite; persistently positive tests (for some antigens) in spite of parasite clearance following chemotherapy; and relatively high cost per test (Malaria diagnosis new perspectives; WHO 2000; p. 1–57; Payne,1988; Moody, 2002).

2. For typhoid: Although blood culture, which is the gold standard for diagnosis of typhoid fever, is not routinely requested by most physicians because it is expensive and final results can be obtained at the earliest, three days after specimen collection (Pearson et al., 2000). This requires laboratory equipment and technical training that are beyond the means of most primary health care facilities in the developing world. Consequently, Widal test is the only specific diagnostic investigation available in most tropical regions. The Widal test which is readily available and inexpensive was introduced as a serologic technique to aid in diagnosis of typhoid fever and has been used for more than a century. By using the aforementioned method, we cannot assess the patient that he is suffering from a true coinfection.

CAUSES OF CO-INFECTION

Misleading Widal results

Because typhoidal Salmonella antibodies are known to cross-react with other antigens including those from non-typhoidal Salmonella and malaria antigens, the use of Widal test as a diagnostic tool in patients with malaria may lead to misleading results. Cross-reactions can occur as a consequence of latent and post-infectious diseases prevalent in the tropics namely tuberculosis, pneumonia, amoebiasis, rickettsial diseases, rheumatoid arthritis and chronic active hepatitis (Koeleman, 1992). In some places, there appears to be more typhoid fever cases in areas of drug resistant malaria and a cross-reaction between malaria parasites and salmonella antigens may cause false positive Widal agglutination test (Mbuh et al., 2003; Jhaveri et al., 1995).

False positive widal reaction

1. The patient previously suffering from typhoid fever.
2. Previous immunization with *Salmonella* antigen.
3. Cross-reaction with non-typhoidal *Salmonella*.
4. Variability and poorly standardized commercial antigen preparation
5. Infection with malaria or other enterobacteriaceae
6. Other diseases such as dengue (Lateef et al., 2000)

In one study at Nigeria, there was no statistical significant difference ($p > 0.05$) between Widal titers of malaria and culture proven typhoid cases, and the study indicated that using Widal test alone, one cannot differentiate typhoid fever from malaria (Ohanu et al., 2003). In yet another study in Lagos, Nigeria, which investigated Widal agglutinin in malaria-infected individuals, it was found that 85% of patients with a negative *S. typhi* culture but positive malaria smear had Widal titers of 1:40, 12% had titers of 1:80, and 3% had titers of 1:160; in contrast, 45% of patients with both *S. typhi* cultures and malaria smears negative had Widal titers of 1:40, 15% had titers of 1:80, and 10% had titers of 1:160 (Olopoenia et al., 1996)

There are some limitations using widal test, they are as follows:

1. The inherent variabilities of the test.
2. Difficulty in establishing a steady-state baseline titer for the population.
3. Repeated exposures to *S typhi* in endemic regions.
4. Cross-reactivities with other non-*Salmonella* organisms.
5. Lack of reproducibility of the test result (Lateef et al., 2000).

It is suggestive from the aforementioned studies that presence of Widal agglutinin under conditions of positive malaria smear, negative *S. typhi* culture and negative prior typhoid immunization would suggest that malaria parasite may have some undefined antigenic determinants similar to *S. typhi* which can induce antibody production and could explain the febrile condition seen in some of the patients.

Again, a diagnosis of co-infection cannot be based on a single Widal test and RDT only. Misdiagnosis of typhoid fever leads to unnecessary expenditure and exposure of patients to the side-effects of antibiotics. But it is true, an early morbidity and mortality can be prevented by adopting these methods.

TRUE COINFECTION

Although there are so many cases of concurrent typhoid and malaria due to cross reactivity of antigen, true infection also exists. It is a well known fact that anemia due to massive hemolysis or dyserythropoesis occur in malaria. Also it has been shown that anemia due to hemolysis that leads to deposition of iron in the liver. Short-lived red blood cells might save patients from malaria; the iron they dump out could lead to death in other ways. The extra iron seems to feed the bug that causes typhoid fever (Bashyam, 2007).

However intracellular bacteria such as *S. typhimurium* also have an obligate requirement for iron to support intracellular growth and survival (Ratledge et al., 2000). Patients suffering from severe anemia show increased susceptibility to salmonellosis (Magnus et al., 1999; Wanachiwanawin, 2000). On the other hand, iron overload of the liver in malaria can support the growth of salmonella in liver.

The availability of intracellular iron must remain in a delicate balance between intracellular pathogens and their host cell. So we can conclude that sufficient iron must be available for the induction of antimicrobial mechanisms (Macrophages require sufficient intracellular iron to act as a cofactor in the induction of effective antimicrobial defense mechanisms, including the NADPH-dependent oxidative burst (Lieu et al., 2001) in host cells, while, on the other hand, the restriction of intracellular iron can prevent the growth of the bacteria e.g. *Salmonella,* within the cell. Further study is necessary to determine the actual concentration of iron to maintain a balance between two, in malaria, otherwise prescribing excess iron to correct anemia in malaria may lead to fatal co-infection.

Probably complements have unique role in salmonella co-infection with malaria. Low levels of complements like C3, C4 and C1q in the sera of children with acute falciparum malaria have been demonstrated, thus implicating the classical pathway involvement. Complement can be activated during malaria, where complement components are consumed and impair host defence (Nyakoe et al., 2009). Complement C1q-deficient mice have an enhanced susceptibility to *Salmonella* infection (Warren et al., 2002). Complement C4B-deficient children have been reported to have an increased risk of bacteremia (Bishof et al., 1990). Study should be carried out regarding the correlation of salmonella with decreased complement level in malaria.

DISCUSSION

In conclusion, a proper protocol is required to diagnose and treat the co-infection. Medical officers still too often fail to consider the possibility of a double infection during the first week of illness. Malaria and typhoid co-infection remain a threat to many people in many developing countries, like Sub Saharan Africa, India and Pakistan due to several reasons: the increasing poverty, deterioration in public health services, compounded by HIV / AIDS and the increasing resistance of malaria parasites to chloroquine (Alnwich, 2001). More so, other factors elude a true diagnosis and they are: the lack of portable water suggested towards a true co-infection, and the widespread misuse of the widal agglutination test for diagnosing typhoid fever (Nsutebu et al., 2001; Edelman et al,1986; Edet, 2002) which increased requests for widal tests as a means of making quick money by private laboratories (Nsutebu et al., 2001; Usman, 2002).

Erroneous interpretation of the test result may lead to misdiagnosis and mismanagement of the patient, resulting in major morbidity and mortality. So interpretation of Widal test results, when diagnosing concurrent malaria and typhoid fever must therefore be done with a lot of caution. On the other hand, a true infection is possible because both typhoid and malaria share social circumstances which are imperative to their transmission. Therefore, a person living in such an environment is at risk of contracting both these diseases, either concurrently or an acute infection superimposed on a chronic one. Hence further experimentation is required to detect prevalence of true coinfection in the world.

As far as the true co-infection is concerned, delicate balance in body iron must be maintained for resistance to infections. On one hand, insufficient iron levels may impair certain antimicrobial defense (e.g. NADPH oxidase) expressed by macrophages and neutrophils, including phagocytosis, cytokine production, respiratory burst, myeloperoxidase activity, and generation of oxygen radicals through the iron dependent Haber-Weiss or Fenton reactions (Patruta et al., 1999). On the other hand, excess iron (iron overload) may have a detrimental effect on host defenses, either through increased availability of nutritional iron to microbes or direct impairment of phagocyte functions (Patruta et al., 1999; Ballart et al., 1986). There are some postulations which may explain why malaria may predispose to salmonella bacteremia and sepsis. It has been demonstrated in a murine model of infection with *Salmonella murium* that hemolysis which occur in malaria may predispose to gram-negative organism as what has been seen in hemolytic disease caused by sickle cell disease and bartonellosis (Kaye and Hook, 1963).

Rampant and unnecessary use of iron supplementation to correct possible anemia in malaria or other infections is dangerous, since excess iron may predispose to salmonellosis. On the other hand, misleading Widal result may keep you away from the true diagnosis; and erroneous interpretation of rapid diagnostic tests delays the treatment of actual infection and increases morbidity. So further research is needed to diagnose a true coinfection and to eliminate false interpretation of RDTs. Thereby true co-infections and there prevalence can be measured.

Again further study is needed to establish the salmonella infection due to decreased complement level in malaria because complements like C1q and C4 deficiency are associated with Salmonella infection.

Effective control measures can be determined if data of true co-infection were present. A treatment protocol is also necessary to treat the co-infection. But according to present concept we can say for malaria, it is important to tailor control and preventive strategy to the prevailing ecological and epidemiological conditions. The strategy of mortality control involves detecting presumptive cases, determining which cases is parasite positive, and administering effective treatment. Focal interventions to minimize human-vector contact can be effected by the use of insecticide-treated mosquito nets as well as indoor spraying of insecticide (Expert committee on malaria. *WHO Tech Rep Ser* 2000; No. 892: i-v). On the other hand, improved personal hygiene, targeted vaccination campaigns and intensive community health education

have been identified as public health measures that could help to prevent and control typhoid (Sur et al., 2006). Overall, all these problems necessitate further experimentation.

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