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Differential Criteria to Evaluate Parasite (Plasmodium) and Bacteria (Salmonella) for Diagnosis of Malaria and Typhoid Fever.

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ABSTRACT

Background: In the investigation of fever, Malaria and Typhoid are major health problems in tropical and subtropical countries. Both cause significant morbidity, mortality and economic loss. The aim of study is to evaluate the prevalence of Malaria and Typhoid. **Methods:** Five hundred three samples were collected from patients suspected for malaria and typhoid fever. Peripheral blood smears (thick and thin) were used for microscopic examination and also malaria card test used for malaria parasite. For Typhoid, Widal agglutination test and Typhi dot IgG/IgM conducted for the identification of antibodies. **Result:** The study indicated that out of 503 patients, in which 158 male (69%) followed by 71 female (31%) were found positive. The prevalence rate of infection was 45.52%. **Conclusion:** The prevalence rate of malaria and typhoid fever was high. Salmonella typhi appears to be the most prevalent species infecting people. In other studies prevalence rate were quite low. Most of the studies should be done on the other determinants of plasmodium, salmonella and their co-infection in different format of diagnosis and case study.

Keywords: Co-infection, Malaria, Plasmodium, Salmonella, Typhoid.

INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoan's (a group of single- celled microorganism) belonging to the genus Plasmodium.[1] The disease is transmitted by an infected female Anopheles mosquito. The mosquito bite introduces the parasite from the mosquito's saliva into a person's blood.The disease is widespread in tropical and sub-tropical regions that are present in a broad band around the equator.[2] According to WHO Malaria is endemic in 108 countries including India and while parasite based diagnosis is increasing, most suspected cases of malaria are still not properly identified, resulting in poor disease monitoring and overuse of anti-malarial drugs.[3] Whereas Typhoid fever is caused by Salmonella typhi, a Gram- negative bacterium. A very similar but often less severe disease is caused by Salmonella serotype paratyphi A.

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Cases are more likely to be seen in areas like India, South and Central America and Africa with rapid population growth.[4] Typhoid and paratyphoid fever are usually transmitted via the fecal - oral route, either directly from person to person or by ingestion of food or water contaminated with feces or urine.[5,6] Historically, in the pre-antibiotic era, the case fatality rate of typhoid fever was 10-20%. Today, with prompt treatment, it is less than 1%.[7] However, about 3-5% of individuals who are infected will develop a chronic infection in the gall bladder.[8] Since S.typhi is human-restricted, these chronic carriers become the crucial reservoir, which can persist for decades for further spread of the disease, further complicating the identification and treatment of the disease.[9] Lately the study of typhi associated with a large out break and a carrier at the genome level provides new insights into the pathogenesis of the pathogen.[10,11] Treatment of the disease with antibiotics reduces the case-fatality to about 1%.[12] When untreated, typhoid fever persists for three weeks to a month. Death occur in 10% to 30% of untreated cases.[13] Malaria and typhoid fever are among the most endemic disease in the tropics including India. Most cases of malaria and typhoid co-infections are based on clinical suspicion alone, so this study was carried out to determine the actual rate of co-infection of Malaria and typhoid fever and also to access the reliability of Widal test in the diagnosis

of Salmonella typhi. Millions of individuals residing in these endemic cases contact these diseases either concurrently or as acute infection superimposed on a chronic one. There are more typhoid cases in areas of drug resistant malaria and a cross reaction between malaria parasites and salmonella antigens may cause false positive Widal agglutination test.[14] Definitive laboratory-based diagnosis is thus required to differentiate the two infections as well as detect co-infection.[15] The objective of this study was to evaluatethe prevalence of malaria and typhoid.

MATERIALS AND METHODS

This study was prospective cross sectional study. During the period from February 2012 to January 2013.During this period we have performed various tests for the diagnosis of disease in which, Five hundred three samples were collected from patients who were suffering from fever and was suspected to infect from malaria or typhoid.

Specimen Collection: Samples were collected by venipuncture from each person were tested for malaria parasite and salmonella typhi O (somatic) and H (flagellar) in EDTA vials.

Blood smears and staining: A drop of blood is placed at one edge of the slide, another slide is placed at an angle of 45 degree at the spot where the blood is placed, with a swift movement a tongue shapeblood film is produced and stained with Leishman stain. Films were examined microscopically for the presence of malaria parasite within red blood cells in thin films. For thick films, the ring forms, trophozoites and gametocytes were looked for. A smear was considered negative for malaria parasite if no parasites were seen after examining at least 100 microscopic fields.[16]

Widal test: The widal agglutination test performed on blood samples by slide agglutination method to determine the antibody titers of the sera against Salmonella O (somatic) and H (flagellar) antigens. Commercially prepared antigen suspension was used. The serological testing was done in accordance with manufactures guidelines.

Typhoid IgG/IgM: This test was performed using rapid test card. Presence of IgG and IgM antibodies was visually detected by formation of separate colored band. Formation of IgG band or IgM band or both were considered Typhi Dot positive. Test performed according to manufacture instructions.

Malaria card test: Malaria card test was performed and both antigen and antibody were detected for each sample. These tests were performed according to manufacturer's instructions. The antigen detection card detects HRP-II (Histidine rich protein II) specific to P.falciparum and PLDH (Plasmodium lactate dehydrogenase) pan specific to Plasmodium species in human blood samples.[17] Malaria antibody detecting card test detected all isotypes of antibody against the same antigens.

Patients found to be positive by any of the tests i.e. widal or typho DOT or any malaria card test positive (antigen or antibody) tests were considered suffering co-infection and were further tested for confirmation of malaria a peripheral blood smear stained by Leishman's stain was prepared.

RESULT

A total of 503 samples were collected from the patients, out of which 229 (45.52%) were positive in which 158 male (69%) followed by 71 female (31%) were found. It was observed that the ratio of male patients as compared to female patients is much higher. Total number of male patient operated (n=356) and female patient (n=147) [Table.1].

Maximum numbers of male patients from normal were from the age group of (21-30) were 39yrs while maximum number of infected male patient from age group of (41-50) was 53yrs, on the other hand maximum number of normal female patient from age group of (51-60) were 19yrs while maximum number of infected female patients from the age group of (31-40) were 27yrs [Table. 2].

Table 1: Gender wise distribution of infected patients.

Sex of patients	Patients Operated	Infected	Percentage (%)
Male	356	158	69
Female	147	71	31
Total	503	229	100

Table 2: Age wise distribution of infected male and female patients

Age	Male		Female	
	Normal	Infected	Normal	Infected
0-10	17	14	6	7
11-20	24	21	8	6
21-30	39	16	7	5
31-40	28	25	11	27
41-50	22	53	6	4
51-60	17	13	19	4
61-70	31	18	10	8
71-80	20	11	9	10
Total	198	158	76	71

DISCUSSION

Malaria and Typhoid infections have increased worldwide, contributing considerably to morbidity of the hospitalized patients. This can prolonging hospital stays, which can add significantly to the economic burden to manage the underlying disease. Poverty mal-nutrition, poor sanitary status, poor personal hygiene, poor health facilities, poor social service and low level of education are among the factors that make tropical areas disease laden. Mal-nutrition gives room to susceptibility to infection.[17] Most people are ignorant of the causative agents, means of transmission, spread and acquisition of some diseases. Typhoid fever is acquired from contaminated water, food, ice creams but the presence of a symptomatic carriers worsen the situation as unusual prolonged outbreak of typhoid fever from 1988 to 1994 in Terrassa (Barcelona, Spain) was caused by a causal food handler who was a carrier.[18] In our study, the result revealed that the prevalence of malaria and typhoid infection was found to be 45%. In tropical countries like India which are endemic for both typhoid and malaria, both disease can co- exist and difficult to differentiate on clinical suspicion alone due to overlapping clinical sign and symptoms as well as antigenic cross reactivity. Despite the fact that malaria and typhoid fever are indistinguishable regarding their clinical signs and symptoms and there are some overlaps in their pathology, Plasmodium and Salmonella are not of same phylum, can't share their antigens nor have same method of transmission, this association only be a co- incidentals. Unlike the diagnosis of malaria, typhoid fever presents a greater diagnostic challenge. Typhoid fever diagnosis is still based on clinical presentation and on diagnostic tests that are associated with numerous limitations. In our study the prevalence of bacterial and parasite infection was 45% which is quite higher. These results are consistent with those of previous studies conducted in tropical and subtropical areas. The prevalence found in present study is comparable to the prevalenceof 36.5%, 37.6% and 39% in eastern parts of Sudan, Akoko State, Nigeria and other study in Imo State, Nigeria.[19] Some of the studies have reported the higher prevalence of Sierra Leone, West Gojam, Ethopia, and Ibadan, Nigeria, prevalence of 62.3%, 62% and 48%.In most of the studies, higher prevalence rate of infection due to Typhoid was 30.41%. The high prevalence of typhoid fever serologically determined as strongly associated with malaria should be unreliable since Widal test is sensitive but non- specific and salmonellae share antigens. Therefore, there may have been cross reaction. Widal test is capable of detecting salmonella antibodies in vaccinated or previously exposed individuals. Widal test have been found to be positive in acute malaria patients.[20] 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. A high index of suspicion is necessary to diagnose a co-infection as most clinicians are used to linking every sign and symptoms to a single pathology. The high rate of typhoid and malaria co-infection using Widal test may be responsible of for the frequent treatment of mixed infections. It is therefore suggested that regular screening of for malaria and typhoid fever be carried out in rural communities so as to improve diagnosis and treatment of malaria and typhoid cases. Control of typhoid and fever and malaria could be achieved through source reduction of breeding sites for mosquito vectors, prevention of contaminated food and water source with salmonella through proper environmental sanitation and sewage disposal and also treatment of infected patients.

CONCLUSION

The highest prevalence rate of malaria and typhoid infection is due to poor hygienic conditions, lack of

proper antibiotics selection and routines checkup. Communities should also help themselves by keeping their streams clean and free from contamination with faeces and urine, boiling of household drinking water, environmental sanitation and personal hygiene. Other factors that may be considered as risk factors are presence of mixed infection, increased stay in hospitals, proper timing of antibiotics administration etc.

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