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Comparison of the Clinical Profile and Complications of Mixed Malarial Infections of *Plasmodium Falciparum* and *Plasmodium Vivax* versus *Plasmodium Falciparum* Mono-infection

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مقارنة بين الشاكلة السريرية ومضاعفات العدوى بالملاريا المختلطة – المُتَصَورَة المنجلية والمتصورة النشيطة مقابل العدوى الأحادية بالمتصورة المنجلية

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الملخص: **الهدف**: تهدف هذه الدراسة للمقارنة بين العروض والمضاعفات السريرية عند المرضى الذين يعانون من الملاريا المختلطة – المتصورة المنجلية والمتصورة النشيطة، مع أولئك المرضى الذين يعانون من مرض الملاريا نتيجة لعدوى أحادية بالمتصورة المنجلية. **الطريقة**: تم تحليل السجلات الطبية لمرضى الملاريا المرقدين في كلية طب كاستوربا، مانيبال، الهند، خلال السنوات 10–2008. وكانت معايير إدراج المرضى المصابين بكل من المتصورة المنجلية والمتصورة النشيطة من جهة، والمصابين بالمتصورة المنجلية وحدها من جهة أخرى بعد التأكد من فحص اللطاخة المحيطية. وكانت معايير الإقصاء للمرضى الذين تم تشخيص العدوى المتصورة النشيطة وحده على فحص اللطاخة المحيطية. بلغ حجم العينة عشرون مريضا تم تشخيص إصابتهم المختلطة بالمتصورة المنجلية والمتصورة النشيطة و 60 مريضا مصابا بالمتصورة المنجلية لوحدها. **النتائج**: كانت 35% من حالات العدوى المختلطة تعاني من قلة الصفيحات بالمقارنة مع 51.7% من حالات العدوى الأحادية بالمتصورة المنجلية. وعانت 5% من حالات العدوى المختلطة من الفشل الكلوي بالمقارنة مع 16.7% من الإصابات الأحادية المنجلية. وكان مستوى البيليروبين الكلي مرتفعا في 15.8% من حالات العدوى المختلطة و46.6% من المنجلية أحادية العدوى. كما كانت أنزيمات الكبد غير طبيعية في 36.8% من حالات العدوى المختلطة مقابل 66.6% من حالات العدوى الأحادية. لم يتعدَّ مؤشر الطفيلي أكثر من 2% عند المرضى المصابين بالعدوى المختلطة في حين أنها كانت موجودة في 28% من حالات العدوى الأحادية. **الخلاصة**: ظهر أن المرضى المصابين بالعدوى المختلطة يعانون أقل من المضاعفات الخطيرة مثل فقر الدم ونقص الصفيحات الدموية واضطراب عمل الكبد والكلى وتدني منسب التطفل، وبالتالي تميل الملاريا المختلطة إلى اتباع مساق حميد مقارنة بالملاريا الأحادية المنجلية.

مفتاح الكلمات: الملاريا ، المنجلية ، علم الأوبئة ، علم الطفيليات ؛ النشيطة ؛ تفاعل المضيف – الطفيلي، الهند.

ABSTRACT: ***Objectives:*** This study aimed to compare the clinical presentations and complications in patients having mixed malaria infection of *Plasmodium falciparum* and *Plasmodium vivax* with those of patients with malaria due to a *P. falciparum* mono-infection. ***Methods:*** The medical records of malaria patients admitted to Kasturba Medical College, Manipal, India, during the years 2008–10 were analysed. Inclusion criteria were patients in whom *P. falciparum* and *P. vivax* coinfection or *P. falciparum* mono-infection alone was confirmed on peripheral smear examination. Exclusion criteria were patients in whom *P. vivax* infection alone was diagnosed on peripheral smear examination. The sample size was twenty patients diagnosed with mixed infection of *P. falciparum* and *P. vivax* and 60 patients diagnosed with *P. falciparum* mono-infection. ***Results:*** 35% of mixed infections had thrombocytopenia as compared to 51.7% of *P. falciparum* mono-infections. A total of 5% of the mixed infections had renal failure as compared to 16.7% of the falciparum mono-infections. Total bilirubin was raised in 15.8% of mixed infections and in 46.6% of *falciparum* mono-infections. Abnormal liver enzymes were seen in 36.8% of mixed infections and in 66.6% of *falciparum* mono-infections. None of the mixed infections had a parasite index over 2% while it was present in 28% of the falciparum mono-infections. ***Conclusion:*** Patients with mixed infections were found to have a lower incidence of severe complications such as anaemia, thrombocytopenia, liver and renal dysfunction and a lower parasite index. Thus mixed malaria tends to have a more benign course as compared to malaria due to *P. falciparum* mono-infection.

Keywords: Malaria, Falciparum; Epidemiology; Parasitology; Vivax; Host-Parasite interactions; India

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Advances in Knowledge

1. *The literature on the clinical outcome on mixed malarial infections is scanty. It is intuitive to think that mixed infections will add to morbidity, but our study in fact shows an improvement in the outcome.*

Application to patient care

1. *Our study throws light on the potential dangers of treatment and preventive measures that selectively suppress the less virulent Plasmodium vivax infection. This can have important clinical implications.*

Malaria is a tropical disease caused by the protozoan parasite Plasmodium. It is one of leading causes of morbidity and mortality in the tropics affecting around 190–311 million people worldwide and killing 7–10 million people every year.[1] The disease in humans is caused by the direct effects of red blood cell (RBC) invasion and destruction by the parasite and the host's response. Splenic, immunologic and filtrative clearance is augmented and the removal of both parasitised and uninfected erythrocytes is accelerated. Parasitised cells escaping splenic removal are destroyed when the schizont ruptures; this in turn induces activation of macrophages and cytokines which cause fever and other pathological effects. The complications seen in malaria include severe anaemia, hypoglycaemia, coma, convulsions, renal failure, bleeding and shock.[2]

Malaria in humans is caused by *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and the predominantly simian parasite *P. knowlesi*.[3] Of these, *falciparum* and *vivax* are the two most common infecting species. *P. falciparum* causes the most severe disease and almost all the fatalities, whereas *P. vivax*, although usually considered to be benign, causes repeated debilitating relapses, sometimes life-threatening complications, and intrauterine growth retardation. As several Plasmodium species may be present in a particular area, infections with more than one species of Plasmodium transmitted by the same vector at the same time are to be expected. Malaria is not unique in this regard, mixed infections with Lyme disease, human granulocytic ehrlichiosis and babesiosis, transmitted by Ixodes ticks occur in the Northeastern USA,[4] and positive associations between geohelminths are common globally.[5] However, there are only sporadic reports on mixed malarial infections. This can be attributed to the fact that mixed infections are often difficult to diagnose on routine smear examination. This is due to a combination of observer error, difficulty in distinguishing between the ring forms of the various Plasmodium species, the undetectable presence of *P. vivax* and *P. ovale* liver hypnozoites, and low density infection below the threshold of detection by microscopy (<1 X 10^8 parasites in an adult). The relevance of interactions between the different Plasmodium species extends beyond mere academic curiosity.[6,7] Studies report variable and sometimes even contradicting results on the effect of interactions in mixed malarial infections;[8–10] therefore, we undertook this study to compare the clinical presentations and complications of mixed malarial infections of *P. falciparum* and *P. vivax* to those of malaria due to *P. falciparum* alone.

Methods

This retrospective observational study included all the malaria patients who were admitted to Kasturba Medical College, a tertiary teaching hospital in Karnataka, South India, during the period June 2008 to July 2010. Malaria is an endemic disease in this part of India. There are 150–200 annual reported cases of malaria in our College. The majority of the complicated cases are due to *P. falciparum* infection. The patients included in the study were all over 18 years of age and presented to the hospital on their own initiative with various complaints such as fever and were subsequently admitted. Only those in whom malaria was confirmed on peripheral smear examination were included in the study. On the basis of peripheral smear examination two groups were formed. Patients who showed both *P. falciparum* and *P. vivax* on their peripheral smear were allocated to Group 1, while those who showed only *P. falciparum* on peripheral smear examination were allocated to Group 2. Those patients who showed only *P. vivax* on the peripheral smear were excluded from the study. Due to the small number of mixed malaria cases (n = 20) it was decided to select all the patients from Group 1 (mixed infection of *P. falciparum* and *P. vivax*) for the study. Patients in

Table 1: Comparison of the incidence of the various study parameters among mixed malarial infection of *P. falciparum* and *P. vivax* to that of *P. falciparum* mono-infection along with their *P* values

Parameter	Mixed Infections of *P. falciparum* and *P. vivax*		*P. falciparum* mono-infection		*P* value
	Number	Percentage	Number	Percentage	
Anaemia	3	15	17	28.3	0.233
Splenomegaly	6	30	42	70	0.0015
Thrombocytopenia	7	35	31	51.4	0.196
Raised bilirubin	3	15.8	28	46.6	0.0118
Raised liver enzymes	7	36.6	32	66.6	0.155
Serum creatinine	1	5	10	16.7	0.189
Parasite index >2%	0	0	16	28	0.009

Group 2 (*P. falciparum* mono-infection) who were age and sex matched to each of the 20 cases of mixed malarial infection were taken for the study. The total number of *P. falciparum* mono-infections selected was 60, thus giving a ratio of 1:3 (i.e. three *P. falciparum* mono-infections for every mixed malarial infection). Prior to the start of the study the following terms were defined. Anaemia was defined as haemoglobin less than 10g/dl; thrombocytopenia as platelets less than 100,000; raised bilirubin as total bilirubin greater than 2 g/dl; raised liver enzymes as serum aspartate transferase (AST)/ serum alanine transferase (ALT) greater than 4 times normal range; serum creatinine was defined as raised when serum levels were greater than 1.6 g/dl. The parasite index was defined as the number of infected RBCs seen on peripheral smear examination per 1,000 RBCs expressed in percentage.

The research was undertaken in the period March to December 2010. Prior approvals from the Ethical Committee of Kasturba Medical College and from the Superintendent of the Medical Records Department at Kasturba Medical College were taken before commencement of the study. All the relevant patient records were collected from the Medical Records Department and analysed. The data of the two groups were compared using the chi square test for each study parameter using the Statistical Package for the Social Sciences (SPSS, IBM, USA, Version 18).

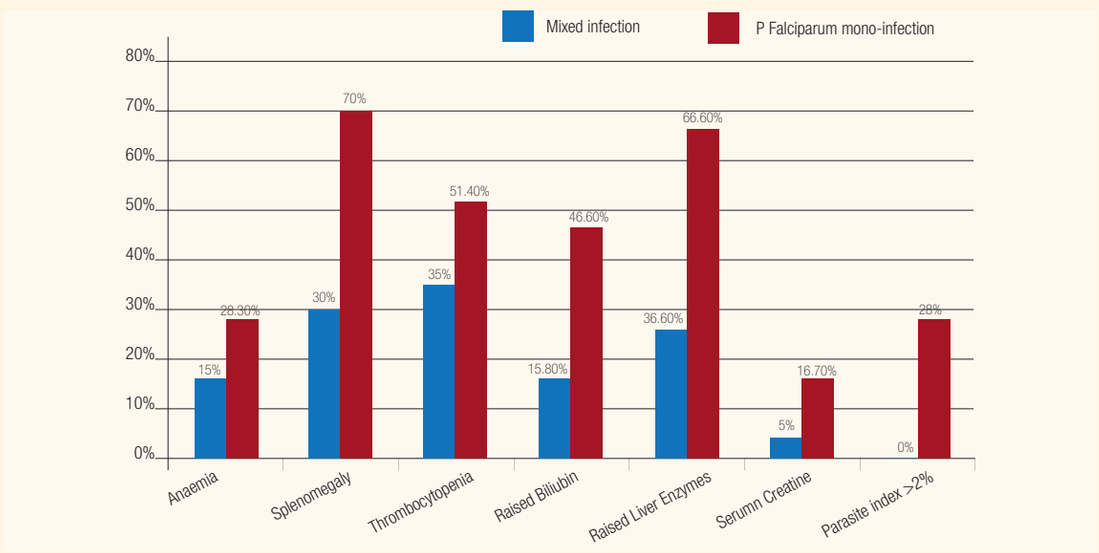

Figure 1: The incidence of complications in mixed malarial infection of *P. falciparum* and *P. vivax* compared to that of *P. falcipaum* mono-infection.

Results

The study consisted of data on 80 subjects collected over a period of 2 years. It consisted of 20 cases of mixed malarial infection of *P. falciparum* and *P. vivax* and 60 cases of *P. falciparum* mono-infection. The cases of mixed infections consisted of 14 males (70%) and 6 females (30%). The *P. falciparum* mono-infection consisted of 45 males (75%) and 15 females (25%). The mean age of both the groups was 35 years. All patients in both the groups recovered from the disease.

Table 1 shows a comparison of the results for each parameter along with their *P* values. Figure 1 gives a pictorial representation comparing the incidence of complications in mixed malarial infection of *P. falciparum* and *P. vivax* to that of *P. falciparum* mono-infection. Patients with mixed malarial infections of *P. falciparum* and *P. vivax* were found to have a lower incidence of all the study parameters as compared to *P. falciparum* mono-infection. For example splenomegaly was found in 30% of mixed malaria infections as compared to 70% of *P. falciparum* mono-infections. Similarly only 35% of mixed infections had thrombocytopenia as compared to 51.7% of *P. falciparum* mono-infections. Raised serum creatinine was seen in 5% of the mixed infections as compared to 16.7% of the falciparum mono-infections. Total bilirubin was raised in only 15.8% of mixed infections while it was raised in 46.6% of falciparum mono-infections. A parasite index of more than 2% was not seen in any mixed infections but it was seen in 28% of *P. falciparum* mono-infections.

Discussion

Mixed-malarial (falciparum and vivax) infections very often go unrecognised, or are underestimated. In our study, only 20 patients (2.9%) with mixed malaria infection, on peripheral smear examination, were identified out of a total of 689 cases admitted to Kasturba Medical College as malaria cases during the study period of 2 years. This is consistent with surveys done in Thailand, which report that mixed infections constitute less than 2% of all malaria cases. However, studies using sensitive polymerase chain reaction (PCR) methods in the same region as the survey put the incidence of mixed infections at around 30% of all malarial infections.[11] Thus mixed malarial infections are often under estimated and can have a significant impact on patient management.

In our study anaemia was seen only in 15% of mixed malarial infections of *P. falciparum* and *P. vivax* as compared to 36.6% of *P. falciparum* mono-infections. These findings correlated with studies conducted in Thailand where they found that likelihood of developing anaemia was 1.8 times less in mixed malaria as compared to falciparum malaria.[12] Similarly, in our study, 30% of patients with mixed malaria infections had splenomegaly as compared to 70% of patients with *P. falciparum* mono-infection. Thus splenomegaly is significantly ($P = 0.0015$) more common in *P. falciparum* mono-infection as compared to mixed malarial infection. A study done in Punjab, India, found that the presence of splenomegaly is three times more common in *P. falciparum* infection compared to other forms of malaria.[13] Thrombocytopenia, one of the severe complications of malaria is attributed to reduced platelet life span and splenic pooling.[14,15] Macrophage activation and hyperplasia, especially in the spleen, may also play a role. Our study noted that thrombocytopenia was more common in *falciparum* mono-infections as compared to mixed infections of both falciparum and vivax. Thrombocytopenia was seen in 51% of falciparum malaria cases as compared to 35% of mixed malarial infections; however, this finding is not statistically significant. It is interesting to notes that a study done in Kuwait showed a higher prevalence of thrombocytopenia in mixed and *P. vivax* mono-infections infections.[8]

Our study showed a higher incidence of renal failure in patients with falciparum mono-infection (17%) as compared to mixed malarial infections (5%). Studies have shown that there is a lower incidence and better prognosis for renal failure in patients with vivax malaria;[16] however, no similar studies on renal failure in patients with mixed malarial infection have been seen. The precise mechanism of renal failure in malaria is not clearly known. Cytoadherence of *P. falciparum* infected red blood cells to the vascular endothelial cells of different host organs along with rosette formation is considered as the most important mechanism of severe malaria.[16,17] Another complication of malaria is liver dysfunction which, when accompanied by other vital organ dysfunction (often renal

impairment), often carries a poor prognosis. Hepatic dysfunction contributes to the severe complications of malaria such as hypoglycaemia, lactic acidosis, and impaired drug metabolism.[2] In our study, it was seen that the signs of liver failure such as raised bilirubin levels and deranged liver enzymes were seen more commonly in *P. falciparum* mono-infection as compared to mixed infections of *P. falciparum* and *P. vivax*. The *P* value for the association of raised bilirubin with mixed and falciparum mono-infections was found to be significant ($P = 0.0118$) while the *P* value for raised liver function tests was not found to be significant ($P = 0.155$). Mixed infections of *P. falciparum* and *P. vivax* were associated a with statistically significant ($P = 0.009$) lower parasite index as compared to *P. falciparum* mono-infection. None of the mixed infections had a parasite index of more than 2%, while 28% of cases in falciparum mono-infection had a parasite index of more than 2%. Similar studies done in Tanzania arrived at the same findings.[18]

Immunity to malaria in humans is poorly understood, but it is thought to be both species and genotype specific. Our study suggests that a coinfection of *P. vivax* protects against the severe complications of *P. falciparum*; however, this protective phenomenon of mixed malaria has proved to be controversial, as some workers have suggested that mixed infections are beneficial, whereas others have suggested that they are detrimental to the host. For example, in the 1930s, the classical clinical studies of human malariotherapy for neurosyphilis demonstrated that *P. falciparum* suppressed *P. vivax* parasitaemia when both species were inoculated simultaneously.[19] In another more recent study done in Thailand, it was seen that naturally acquired mixed infections with other less severe malaria species appear to attenuate the severity of *P. falciparum* infection.[20] On the Thai-Burmese border, pregnant women whose first attack of malaria during pregnancy was caused by *P. vivax* had a significantly lower risk of developing *P. falciparum* later in the pregnancy.[9] However, there are also several conflicting reports in the literature; for example, Gopinathan and Subramanian's study in 1986 found that mixed infections with *P. falciparum* and *P. vivax* were associated with an increased incidence of complications like cerebral malaria.[10]

A major limiting factor in our study was the very small sample size of only 20 mixed malarial infections of *P. falciparum* and *P. vivax* over a period of 2 years. However, as mentioned earlier, we believe that mixed malarial infections are very often underestimated and only by using newer, more sensitive investigations such as PCR can we determine the true extent of mixed malarial infections. If further studies confirm our hypothesis that *P. vivax* co-infection indeed has a beneficial disease-modifying effect on the more severe *P. falciparum* infection, it would then be legitimate to speculate whether therapeutic suppression of the more benign *P. vivax* species in the population would cause *P. falciparum* malaria to become an even more serious public health problem. This scenario may explain why there is an increase in mortality resulting from the continued use of chloroquine as a first line treatment in Africa in the face of mounting resistance to this drug by *P. falciparum*, but not by the other parasite species.[6,7] Thus we believe that we have to ask ourselves whether two mosquito bites are better than one.

Conclusion

In this study, it was seen that patients with mixed malarial infections of *P. falciparum* and *P. vivax* were found to have a lower incidence of severe complications such as anaemia, thrombocytopenia, liver failure and renal failure that are often associated with *P. falciparum* mono-infections. It was also seen that mixed malarial infections had a lower parasite index as compared to *P. falciparum* mono-infections. From the above findings, we conclude that the *P. vivax* infection has a beneficial disease modifying effect on the more severe *P. falciparum* malaria. We believe that further studies are required to fully address this complex, but often understudied aspect of malaria.

CONFLICT OF INTEREST

The authors reported no conflict of interest.

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