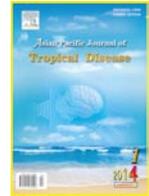




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## Ultrasonic measurement of the liver in search of *Plasmodium vivax* cases that relapse

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### PEER REVIEW

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#### Comments

This is an interesting study of which purpose was to find out whether relapse and reinfection can be differentiated in vivax patients. The ultrasonography was performed to note the enlargement of the liver. Details on Page S441

### ABSTRACT

**Objective:** To find out whether relapse can be differentiated in vivax patients.

**Methods:** Sixty three people suffering from vivax were taken and ultrasonography was done.

**Results:** Among 63 persons, previous history of vivax was obtained in 51. Out of remaining 12, previous history of falciparum was found in 5, four had previous history of uncertain malaria and in three, no previous history of malaria was recorded. The liver was within normal size ( $\leq 140$  mm) in 36 persons, and increased in size in 27 persons, of which enlarged (141–150 mm) in 14 and highly enlarged (beyond 150 mm) in 13 persons. Out of these 27 persons with enlarged livers 22 suffered from vivax previously. Altogether 18 out of 63 cases considered to be suffering from relapsed vivax cases in this study, which is alarming.

**Conclusions:** This simple, noninvasive procedure might provide a clue as to how relapse vivax cases can be determined.

### KEYWORDS

Liver, Ultrasonic, *Plasmodium vivax*, Malaria

## 1. Introduction

Relapse and reinfection are the two distinct culminative phenomena of vivax malaria. In an area where malaria is endemic, some people repeatedly suffer from vivax malaria<sup>[1]</sup>. It is important to know whether it is due to reinfection through bite of infective vector mosquito or it is relapse occurring from activated hypnozoites that have taken shelter in the liver cells of some of the victims. It is practically impossible to differentiate relapse from reinfection in an endemic zone, especially when

someone is attacked with vivax malaria several times. Can ultrasonographic measurement of the liver give some information in this regard? The enlargement of the liver can accurately be measured through ultrasonography of the upper abdomen, which is entirely a noninvasive process and very easy to perform whenever needed.

The purpose of this study is to find out whether relapse and reinfection can be distinguished in vivax patients. The study is carried out by analyzing the history of individual patients having previous attack(s) of vivax malaria and the ultrasonographic measurement of their

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livers. If a fruitful positive result is obtained, this simple procedure can then be employed as a clue or a marker to diagnose relapse cases which would facilitate entire treatment modality of vivax malaria.

## 2. Materials and methods

This study was conducted between February 2010 and August 2010 in a malaria endemic area in central Kolkata, West Bengal, India. We noted the history of patients who were sent to us by local physicians. All these patients were suffering from a short duration of fever (one week) and in their blood *Plasmodium vivax* parasites were found through examination of thin (Leishman's stained) and thick (Giemsa stained) films. The history of previous attack(s) of vivax malaria of each patient was meticulously taken, as far as practicable. Ultrasonography of the upper abdomen of all these patients was performed to note the enlargement of the liver, if any. Necessary ethical approval has been taken (Approval No. VIHC/371, dated 29.09.2009). Ultrasonography and blood examination of the patients were carried out in the Gautam Laboratories Imaging and Research Centre, 7 Kalikrishna Tagore Street, Kolkata–700 007, situated in central Kolkata.

We noted the history of 63 people suffering from vivax malaria, and measured the size of their livers ultrasonographically. According to the guideline of the WHO 1996[2], normal measurement of the liver along the right mid clavicular line is within 140 mm.

## 3. Results

In this particular experiment conducted, the size of the liver varied from 88 mm to 181 mm (mean 133.58 mm and median 134.00 mm).

Data obtained from case history and ultrasonography measurement of their livers are presented in Table 1. Out of 51 persons with history of vivax malaria, 33, 10, 3, 1 and 4 suffered previously from malaria twice, thrice, 4 times, 5 times and more than 5 times respectively.

Among 63 persons, the liver was within normal limit in 36 (57.14%), enlarged between 140 mm and 150 mm in 14 (22.22%) and beyond 150 mm in 13 (20.63%) persons.

Out of those 51 persons who had previous history of vivax malaria, the liver was found to be normal ultrasonographically in 29 persons and out of them 1, 5, 4, 14, 3 and 2 suffered from vivax malaria 1 month, 2–3 months, 4–6 months, 8–12 months, 24 months and >24 months ago respectively, and the range of the liver size in them was 108 mm, 102 mm–132 mm, 100 mm–134 mm, 88 mm–138 mm, 96 mm–128 mm and 95 mm–130 mm respectively. Among the remaining 12 persons with no previous history of vivax malaria, the livers of 7 people were within normal limit that varied from 96 mm to 140 mm with mean 119.8 mm and median 123 mm.

Out of 22 people with histories of previous attacks of vivax malaria (with enlarged livers), 17 were analyzed. Previous attack occurred 2–6 months ago in 6 people in whom enlargement of the liver varies from 141 mm–157 mm; 8–12 months ago in 9, in whom enlargement of the liver varied from 148 mm–181 mm, and 18–19 months ago in 2 persons in whom the enlargement of the liver varied from 151 mm to 159 mm. For these 17 persons the mean and median size of the liver was 154.11 mm and 153.00 mm respectively.

In 5 other persons with previous history of vivax malaria size of the liver was found to be 141 mm, 144 mm, 145 mm, 155 mm and 160 mm, having history of attacks 8 month, 4 months, 6 years, 1 month and 1 month ago, respectively.

In 12 patients out of 63, no previous history of vivax malaria was present, of whom the livers of 5 individuals were enlarged in size as 143 mm, 147 mm, 147 mm, 168 mm and 143 mm respectively, having previous history of falciparum malaria

**Table 1**

History of 63 cases presented with a short duration (about one week) of vivax malaria and their ultrasonographic measurement.

Ultrasonographic measurement	Number (%) of cases with previous history of vivax malaria	Number (%) of cases with no previous history of vivax malaria			Total
		With history of falciparum malaria	With history of uncertain malaria	No history of malaria	
Liver normal	51 (80.95%)	5 (7.93%)	4 (6.34%)	3 (4.76%)	63
Range	29 (46.03%)	3	2	2	36 (57.14%)
Mean	88–138 mm	123–140 mm	117–129 mm	96–103 mm	
Median	115.3 mm	131.3 mm	123 mm	99.5 mm	
Liver increased	22 (34.92%)	2	2	1	27 (42.85%)
Range	141–181 mm	143–147 mm	147–168 mm	143 mm	
Mean	150.81 mm	145 mm	157.5 mm		
Median	155 mm	145 mm	157.5 mm		
Relapse considered on the basis of ultrasonographic measurement	17		1		18 (28.57%)
Range	141–181 mm		147 mm		
Mean	154.11 mm				
Median	153.00 mm				

in the first two cases, some types of malaria in the third and fourth cases and no history of malaria at all in the last case.

#### 4. Discussion

In a malaria endemic area in central Kolkata, vivax and falciparum malaria co-exist<sup>[3]</sup>. Out of 63 persons presented to us with vivax malaria, previous attack(s) of vivax malaria was found in 51 (80.95%), pointing out intense vivax malaria transmission where the livers were ultrasonographically found enlarged in 27 (42.85%) persons, which is alarming indeed. It is revealed that in quite a large number of people living in a malaria endemic area, frequent attacks of vivax malaria is very common and a proportion of them are reasonably suspected to be suffering from relapsed vivax cases.

Ultrasonographic measurement of all these 63 persons has been analyzed with an assumption that in relapse vivax cases, the liver size will increase more than those persons who are suffering from reinfection(s). It is to be noted that the liver was found to be within normal limit in 36 (57.14%) persons. These were the cases, in which reinfection has occurred in all probability. Out of a total of 27 persons with enlarged livers, 22 had previous history of vivax malaria, among whom the liver was enlarged between 141 mm and 150 mm in 9 and beyond 150 mm in 13 persons. In five others with enlarged liver ranging from 143 mm–168 mm, no history of previous attack of vivax malaria was present. Some of those patients with enlarged livers were supposed to be relapsed vivax cases. Analysis of the data revealed that there was a relationship between previous attack of vivax malaria and enlargement of the liver, and 17 such cases have been identified, of whom the livers of 8 had enlarged beyond 150 mm (range 151 mm–181 mm) and in another 8 persons the size of the liver varied from 141 mm–150 mm.

In 5 other persons with enlarged liver having previous vivax attack, no strong evidence existed for relapse as the livers had very mildly enlarged in the first two cases (141 mm and 144 mm). The third case with 145 mm liver size had the history of attack 6 years ago and in the last two cases with enlarged livers measuring 155 mm and 160 mm respectively, previous attack had occurred only 1 month ago. They can therefore be regarded as nonspecific enlargement, not due to relapse.

Measurement of the liver was found to be 147 mm and 168 mm respectively in two persons who have suffered previously from some type of malaria, 1 month and 12 months before the present episode. The first case may be included in the relapse section who most probably has suffered from vivax malaria but the second case had the history of previous attack of malaria only one month ago,

and thus such enlargement of the liver had taken place due to some other causes and not due to relapse.

Measurement of the liver is 143 mm and 147 mm respectively in two persons who have suffered previously from falciparum malaria, and in one person with no previous history of malaria the size of the liver was found to be 143 mm. In these cases the enlargement of the liver seemed to be nonspecific and question of relapse did not arise.

So, altogether 18 (28.57%) cases (17 with previous history of vivax malaria and 1 with previous history of some type of malaria) out of 63 have been earmarked to be suffering from relapse.

The liver when enlarges beyond 150 mm in malaria endemic area is to be regarded as the red zone, where almost certainly the relapse is to be apprehended specially when previous attack/s of vivax malaria is present and no other liver disease is suspected. But even when the history of previous vivax attack is obtained, mild enlargement of the liver may also be taken into account. Two such cases can be cited, where ultrasonography has been performed twice in each occasion during subsequent vivax attacks. In one case the liver has enlarged from 149 mm to 156 mm (interval between two attacks is 2 months) and in other case from 105 mm to 141 mm (interval between two attacks is 3 months).

If ultrasonographic measurement of the liver is taken at the initial attack of vivax malaria and if it is compared with that of subsequent attack(s), an idea related to relapse and reinfection may also be obtained.

There are certain drawbacks associated with this procedure. Firstly it is very difficult to get proper history of each patient so far as previous attack(s) of malaria. Secondly people often go to the pharmaceutical shops and antimalarial drugs are very frequently supplied to them over the counter and they may get cured. Thirdly even when people feel feverish and go to the medical practitioners, they often prescribe antimalarials without proper investigation. So confirmed repeated attacks of malaria in such persons cannot be definitely obtained. Fourthly in an endemic area, hypnozoites infecting the persons may remain dormant for a pretty long time without producing any sign/symptom of malaria, before becoming activated, which is also difficult to ascertain<sup>[4]</sup>. Fifthly previous status of the liver (whether normal or enlarged) is not known. Again in a tropical country, the liver may enlarge for various reasons. But when a correlation exists between enlargement of the liver and previous attack of vivax malaria in patients suffering from vivax malaria, especially within 1–1.5 years, relapse can be reasonably considered.

No practical method in the endemic area exists to distinguish relapse from reinfection in patients' sufferings from repeated attacks of vivax malaria. Any

invasive procedure is not at all realistic in such cases. If reinfection case could be differentiated from relapse, unnecessary use of primaquine for 14 d, according to the recent antimalarial drug schedule in India[5], to kill the hypnozoites existing in the liver in relapse cases, can be avoided. Patients who are taking primaquine for a prolonged period are usually advised to do a costly test (G6PD) which would also not be required in reinfection cases[6].

This is merely a preliminary report, a first hand clue, in order to diagnose relapse cases by using ultrasonography; a non-invasive and cheap method which may act as a marker. Necessary ethical approval has been taken.

### Conflict of interest statement

We declare that we have no conflict of interest.

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### Comments

#### Background

In many developing countries malaria is endemic, causing acute illness and killing people. About 36% of the world population is exposed to the risk of contracting malaria and more than 75% of them are African, Asian and South American children and expectant mothers. It exist a variety of malaria diagnosis test from microscope to molecular biology techniques. However, We noted that most of these techniques are unable to separate reinfection cases from relapse cases. Differentiate reinfection from relapse in the endemic zone can improve the efficiency of recommended malaria medicine. There is a need of new strategies to improve the efficiency of malaria treatment.

#### Research frontiers

During the infection cycle, malaria parasites rapidly target hepatocytes. Then the parasites can cause hepatic damage. Studies are being conducted in objective to evaluate the hepatic damage and functional status of

hepatocytes using ultrasonography among patients. In this case a enlargement of the liver could be noted due to certain residual effect of the infection.

#### Related reports

The report of Lee SW *et al.* 2007 showed that malaria infection provoked injury of the hepatocyte without any liver disorders. This suggests probably correlation between enlargement of the liver and previous attack of the parasites. But many other tropical diseases can cause the enlargement of the liver.

#### Innovations & breakthroughs

The aim of this study is to diagnose relapse cases of malaria using ultrasonography as a marker in the order to improve the efficiency of relapse treatment. The report showed a probably correlation between enlargement of the liver and previous attack of vivax malaria among patients. This is a new methods which can be helpful in order to improve the efficiency of malaria medicine.

#### Applications

It is important in endemic area to differentiate the reinfection from the relapse in vivax. The results of the present study suggest that ultrasonography measurement of the liver can be employed as a marker to diagnose relapse cases. This method would be significant in order to change treatment modality of vivax malaria.

#### Peer review

This is an interesting study of which purpose was to find out whether relapse and reinfection can be differentiated in vivax patients. The ultrasonography was performed to note the enlargement of the liver.

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