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Estimating the relapse risk of *Plasmodium vivax* in Iran under national chemotherapy scheme using a novel method

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Abstract

Objectives: This study aimed to estimate the relapse risk of *Plasmodium vivax* under national chemotherapy scheme using a novel method, and assessed its pattern in Kahnooj, a malaria endemic area in Iran.

Methods: The authors traced repeated episodes of malaria attack between 1994 and 2001 and then, estimated the risks of secondary attack of *P. vivax*, classified by the species in their primary attack. It is suggested that the difference between the secondary attack rate in those who were infected by *P. falciparum* and *P. vivax* in their primary attack may estimate the *P. vivax* relapse rate indirectly.

Results: This method showed that the relapse risk of *P. vivax* with in one and two years after the primary attack were 16.8 and 24.5% respectively. The risks of relapse before three or after 18 months were very low.

Interpretation & conclusion: The relapse pattern of *P. vivax* was compatible with the dominant pattern in most of the temperate areas. In addition, the relapse risk was very close to the estimated relapse risks in clinical trials on anti-relapse drugs. Therefore, we concluded that the anti-relapse therapy in the study area was effective; also, this method may estimate the relapse risk of *P. vivax* accurately.

Key words Antimalaria treatment – Cox regression – Iran – malaria – relapse of *Plasmodium vivax*

Introduction

Multiple disease episodes of malaria within a person might be attributable to treatment failure, re-infection or relapse in *Plasmodium vivax*. Differentiation of reinfection from relapse is very difficult; therefore, most papers reported a combined risk or rate of reinfection and relapse, or estimated the relapse risk based on the frequency of *P. vivax* during the non-transmission season[1,2]. Although molecular epidemiology introduced new methods to discriminate the reinfection from relapse, the methods are very expensive, and difficult to be used in the field, also in many cases they could not address the question accurately[3–5].

It should be added that there is a relapse risk of around 25 up to 55%, even with anti-relapse therapy of *P. vivax* with primaquine[6–8]. Therefore, the high incidence of secondary attack does not imply the low coverage or compliance of antirelapse therapy.

P. vivax is the dominant malaria species in many parts of middle east countries, particularly Iran, but our knowledge about its relapse pattern is limited. In re-

cent years, >80% of malaria cases in Iran were *P. vivax* infections. Manson[9] described three relapse patterns of *P. vivax:* (i) tropical pattern with short latency (1–3 months) between attacks; (ii) temperate pattern with a latent period of 6–14 months, followed by renewed parasite activity in the form of one or more relapses with short intervals between each relapse; and (iii) intermediate form[2,9]. Although, it seems that the relapse in Iran is compatible with temperate pattern, we do not have any specific evidence available right now. According to the above explanation, this study was designed to estimate the relapse risk of *P. vivax* in Iran using a new method. In addition, this study explored the relapse pattern of *P. vivax* in the study area.

Material & Methods

This research was carried out in Kahnooj which is a district of Kerman province in southern Iran with hot dry weather and an area of approx. 32,000 km^2 and around 2,50,000 population. Annually around 1,000 to 5,000 malaria cases were diagnosed in Kahnooj for last 15 years, mostly *P. vivax* (> 80%). *P. ovale* and *P. malariae* have not been reported.

The national health policy of Iran dictates that the diagnosis of malaria disease should depend on visualisation of parasites by light microscopy of Giemsa-stained blood smear in febrile cases. There are active and passive surveillance systems in this district. All febrile cases, family and neighbours of a definite malaria case and a random sample of the population are regularly screened for malaria using microscopic methods. Microscopists are experts and their accuracy is being supervised constantly. Rural health workers and microscopists are reporting their cases to the district health organisation weekly. The private sector does not have access to the diagnostic labs and also antimalaria drugs and the diagnosis and treatment is free of charge in public sectors. In addition, the private sector is forced to refer all suspected cases to public sector. Based on the local and national reports and also the results of independent studies, it seems that nearly all cases are enrolled in the surveillance forms[10]. All cases infected with *P. vivax* receive treatment based on WHO recommendations—a 3-day course of chloroquine, followed by a l4-day course of primaquine as anti-relapse treatment. Health workers actively follow the cases to receive their medications on time.

In this study, copies of the original monthly surveillance forms from rural health centres were collected. These forms had: names of patients and their parents, age, sex, type of surveillance (active or passive), type of accommodation (permanent or temporary), nationality (Iranian or Afghani), location (name of village), and the date of taking and reading blood films. For this study, case data from 21 of March 1994 (Iranian New year) to the end of 2001 were collected.

Data were double entered in the computer and the repeated episodes within subjects were identified using MS-Access programme. The cases were traced, based on their names, parents' names, date of birth and also their locations. Those records suspected for repeated episodes of a subject were reevaluated by exploring their paper forms and even their records within health centres.

According to the species in the former and latter episodes, the subjects with multiple attacks were categorised into four groups: *P. falciparum-P. falciparum, P. falciparum-P. vivax, P. vivax- P. vivax* and *P. vivax-P. falciparum.* In this analysis, only the data of the second and third groups were enrolled. We assumed that the secondary attack in *P. falciparum-P. vivax* group was due to reinfection. However, the secondary attack in the *P. vivax-P. vivax* was due to either reinfection or relapse. Therefore, we assumed that the difference of secondary attack in these two groups can estimate the relapse risk of *P. vivax*.

According to the above explanation, we estimated the rate of secondary attacks in these groups using Cox

regression method to reduce the potential confounding effect of differences in the seasonal patterns of *P. vivax* and *P. falciparum*. Then, the risks of relapse in the first and second years after the primary attack were estimated. Stata version 7.0 was used for the statistical analysis.

Results

Between March 1994 and March 2001, 18,268 malaria attacks were recorded in Kahnooj, of that 12,337 (67.5%) were infected with *P. vivax*, 5,858 (32.1%) with *P. falciparum*, and 73 (0.4%) had mixed infections. Sixty percent of attacks (10,680) were detected in passive surveillance, and 40% (7,150) were identified in active surveillance.

The 18,268 malaria attacks were recorded in 16,297 persons. About 14,799 (91%) of cases had just one, and 1,169 (7.2%) had two attacks; <2% of cases had > 2 attacks. The frequency of more than two attacks was low, nevertheless, the following results have been adjusted for within person clustering effect. Also, only 73 cases had mixed infections with *P. falciparum* and *P. vivax*, of that only 23 showed repeated attacks. These 23 mixed infected cases have been excluded from the analyses related to the species in the former and latter attacks. In total, 353 subjects had *P. falciparum-P. vivax* attacks, while 908 subjects had *P. vivax-P. vivax* repeated attacks; the gap between two attacks in these two groups was statistically different (Kruskal-Wallis χ^2 =12.7, df =1, p<0.001) (Table 1).

Fig. 1 compares the rates of secondary attack in those who had primary infection with *P. vivax* and *P. falciparum*, classified by the gap between two attacks. The patterns of changes in both the groups were comparable; they had a sharp peak around one year, which reflected the annual variation of *P. vivax*. In other words, irrespective of the previous history of *Plasmodium* spp infections, the risk of *P. vivax* episode had an annual cycle. These two lines were very close before 3 and after 18 months gap—the estimated relapse rate was trivial before 3 and after 18 months. The relapse rates were considerable between 3 and 18 months, however, they were statistically significant only between 6 and 18 months. The monthly relapse rates in 3–5, 6–7, 8–12 and 12–17 months after the initial infection were 10.9, 15.7, 31.9

Table 1. The gap between two consecutive attacks according to the species in the first and second attacks

The gap between two attacks (months)	Species in the first attack/Species in the second attack	
	P. falciparum/ P. vivax (%)	*P. vivax/ P. vivax* (%)
≤1	15 (4.3)	28 (3.1)
1–2	25 (7.1)	59 (6.5)
3–5	22 (6.2)	72 (7.9)
6–8	22 (6.2)	79 (8.7)
9–11	40 (11.3)	149 (16.4)
12–17	36 (10.2)	134 (14.8)
≥18	193 (54.7)	387 (41.6)
Total	353 (100)	908 (100)

The gap between two episodes was compared using Kruskal-Wallis test: χ^2 = 12.7; df = 1; p<0.001.

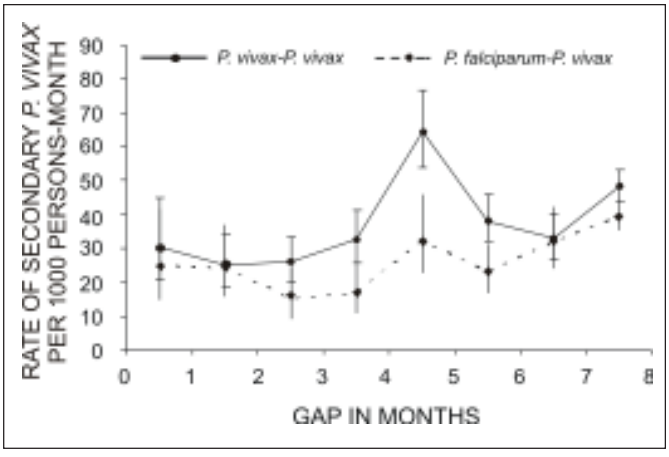

Fig. 1: Monthly secondary attack rates, classified by the gap and the species in the first and second attacks

and 15.1 per 1000 persons-month respectively on an average, the relapse rate in the first two years after the primary attack was 1.2 per 1000 persons per month. Converting the monthly rates to annual risks, the relapse risk in one and two years after the primary attack were 16.8 and 24.5% respectively.

The difference between relapse risk in males and females were comparable. The relapse risk within two years after the primary attack in males and females were 24.9 and 24.1% respectively; the difference was not statistically significant ($p = 0.21$). Having entered the age of subjects (at the time of the first attack) as a covariant in the Cox model, the relapse pattern has not changed considerably. In addition, the interaction between the pattern of relapse and age was not statistically significant ($p = 0.35$); which implies that the relapse rate in age groups was more or less comparable.

Discussion

This study showed that the relapse rate of *P. vivax* in the first three months after the primary attack and also after 18 months is very low. The peak of relapse was observed between 9 and 12 months after the primary attack. The estimated relapse risks in one and two years after the primary attack were 16.8 and 24.5%, respectively. In addition, the frequency of more than two attacks was rare in this study.

We presented a new method to estimate the risk of relapse in *P. vivax* infection. Because of limitation in the current methods in differentiation of re-infection from relapse, we suggest that this simple method can estimate the relapse risk indirectly. The logic behind this method is very simple. It compares the secondary attack rate of *P. vivax* in those who had primary infection of *P. falciparum* (group I) and *P. vivax* (group II). The secondary attack rate in the first group (*P. falciparum-P. vivax*) estimates the reinfection rates by *P. vivax*, while the secondary attack rate in the second group (*P. vivax-P. vivax*) estimates the relapse + reinfection rate. Hence, their differences may estimate the relapse rate of *P. vivax* indirectly.

However, this method has its own limitations. Firstly, it cannot differentiate the relapse from re-infection in individuals—it estimates an overall relapse risk in the population. Secondly, we assumed that immunity against *P. vivax* obtained in the primary episode is more or less equal to the cross-immunity between *P. falciparum* and *P. vivax*. In other words, we assumed that *P. falciparum* induces the same immunity as *P. vivax* does to the acquisition of new *P. vivax* infection. Based on this assumption, we crossed out the role of acquired immunity in these two groups.

It seems that this assumption does not violate our results by far. The protective effect of immune system is small in low endemic area[11,12]; and even in highly endemic area, protective immunity is generated after multiple attacks which without booster effect of repeated infection, its protective effect is short[9,13]. Therefore, in a low endemic area such as Iran, we do not expect that the acquired immunity changes our results.

Since the seasonal variations of these two species are not similar in many areas, we suggest that season or even month of either primary or secondary infection is entered in the regression models. Our results showed that Cox regression model without the covariant of month of primary infection underestimate the relapse risk, which is just because of the different seasonality pattern of *P. vivax* and *P. falciparum*. Therefore, we suggest that the results of regression models should be adjusted for the month or season of infections.

The relapse pattern of *P. vivax* in Kahnooj was compatible with its temperate pattern. The results showed that the *P. vivax* relapse rate before three or after eighteen months was very low and its maximum rate was observed around one year after the primary attack. Moreover, few cases showed more than two attacks.

These characteristics were compatible with the temperate pattern of relapse which was explained by Manson[9].

The estimated risk of *P. vivax* relapse was around 25% in two years. This risk was very close to the relapse risks which had been observed by Rowland and Durrani[8] in Pakistan in the treatment arm with 14-day primaquine regime and by Prasad *et al*[1] and Leslie *et al*[7]. Therefore, from the practical point of view, it seems that the anti-relapse treatment was effective in Kahnooj. The national protocol in Iran has recommended a 14-day regime of primaquine under supervision of health workers and unpublished reports from a few locations in Kahnooj show that around 80–90% of cases receive a 14-day regime.

The difference of secondary attack rates in subjects with *P. falciparum-P. vivax* and *P. vivax-P. vivax*, may estimate the relapse rate of *P. vivax* indirectly. Based on this method, the relapse risk of *P. vivax* in one and two years after the primary attack were 16.8 and 24.5% respectively, and its pattern was compatible with the pattern in most temperate areas.

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