ASSESSING THE INCIDENCE OF INFECTION WITH *PLASMODIUM FALCIPARUM* AMONG INTERNATIONAL TRAVELERS

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Abstract. Circumsporozoite (CS) antibodies, indicating plasmodial infection but not necessarily development of disease, have been shown to be reliable indicators of transmission in endemic areas. To estimate the actual rate of plasmodial infection, the prevalence of CS antibodies was investigated by an ELISA test system in a selected population of 2,131 travelers returning from areas endemic for malaria who presented to an outpatient clinic without any apparent symptom or clinical sign of malaria. Serum specimens from 104 of the investigated 2,131 patients (4.9%) were found to be positive (titer ≈ 6.25 international ELISA units [IEU]). The geometric mean titer of antibody concentrations (IEU) in seropositive patients was 18.64 IEU (95% confidence interval [CI] = 13.15–24.13), while it was 2.1 IEU (95% CI = 1.8–2.4) in seronegative patients. A significantly above average risk for plasmodial infection could be found among travelers to East Africa (risk ratio [RR] = 4.5, \( P < 0.001 \)), West Africa (RR = 4.5, \( P < 0.001 \)), and Southern Africa (RR = 3.2, \( P = 0.015 \)), while areas with a comparatively low risk included Central America (RR = 0.86, \( P < 0.001 \)), the Indian subcontinent (RR = 0.45, \( P = 0.015 \)), South America (RR = 0.49, \( P = 0.091 \)), East Asia (RR = 0.68, \( P = 0.441 \)), West Asia (RR = 0.24, \( P = 0.099 \)), and Southeast Asia (RR = 0.69, \( P = 0.094 \)). The results of this study emphasize the importance of adequate malaria chemoprophylaxis in nonimmune travelers to endemic areas. By use of the described method, estimates of the true infection rate of malaria in travelers can be derived for certain areas and the value of prophylactic measures can be demonstrated.

The first antigens that are presented to the immune system of a host who is infected with malaria parasites are the surface antigens of plasmodia sporozoite stages expressed shortly after inoculation by the anopheline vector. For a few hours, the immune system of the infected host may produce protective antibodies before the parasites invade liver cells and transform to merozoite stages. Antibodies to sporozoites of *Plasmodium* spp. are directed against a major surface antigen, the circumsporozoite (CS) protein. Therefore, the detection of significant titers of antibodies to CS protein in a person indicates previous inoculation with sporozoites, not necessarily the development of disease. The immunodominant epitope of the *P. falciparum* CS protein consists of highly conserved tandem repeats of amino acids (Asn-Ala-Asn-Pro = NANP). Several NANP repeats of variable length have been synthesized using either chemical or recombinant DNA techniques, and a variety of immunoassays have been tested to detect humoral immunity to *P. falciparum* sporozoites. An ELISA kit using a chemically synthesized (NANP)_30 peptide is available.

In individuals living in endemic areas, the prevalence and level of sporozoite antibodies have been shown to correlate with the entomologic inoculation rate assessed at the same time for the same area, and the development of detectable titers in a given population has been used as reliable indicator of transmission in endemic areas. Seroconversion rates of 60% to *P. vivax*-specific CS antibodies have been found in patients with *P. vivax* malaria for the first time. Sensitivity and specificity of an available ELISA system (Sclavo Diagnostici, Sienna, Italy) have been evaluated in nonimmune patients after one episode of malaria infection. While the sensitivity was found to be rather low (55.8%) during a period of 8–90 days after onset of symptoms, its specificity was 100%. Therefore, although lacking high sensitivity, this test appears to be a useful and specific tool for assessment of the risk of malaria infection in endemic areas; at the very least, it can provide an estimate of the lower limit of risk for malaria infection.

There are limited data concerning the incidence of malaria infection among international travelers to malarious areas since most individuals are advised to practice appropriate prophylactic measures. Therefore, risk estimates for malaria infection among travelers are usually derived from transmission rates in semi-immune populations and semiquantitative reports of infections in unprotected tourists. On the basis of such data, the risk for malaria infection for unprotected travelers to West and East Africa has been estimated to be approximately 1.2% per month. The recommendations for prophylactic measures against malaria infection for travel to malarious areas are based in part on such estimates, depending on the anticipated infection rate and drug resistance in *P. falciparum*. Generally, precautions to avoid mosquito bites and strict intake of antimalarials assumed to be effective against most endemic strains of *P. falciparum* are emphasized. To assess the actual infection rate among international tourists to malarious areas, we investigated the prevalence of CS antibodies in a selected population of travelers who presented to our outpatient clinic without any apparent sign of malaria.

**PATIENTS, MATERIALS, AND METHODS**

A total of 2,131 patients who presented to our outpatient clinic from January 1994 to September 1995 were selected by the following criteria: they were Germans or residents of Germany for more than 10 years; they had traveled to an area endemic for malaria during a period of at least 14 days and not exceeding four months; they had not had malaria previously; they took sufficient malaria chemoprophylaxis during and four weeks after their journey; they presented to our outpatient clinic with symptoms that did not include fever no later than 90 days after their return to Germany; and they had malaria excluded by thin and thick blood films and a negative immunofluorescence antibody test (IFAT) result for merozoite antibodies. Serum specimens were drawn from patients meeting all inclusion criteria and stored at
-70°C for later use. A group of 342 sera from 342 volunteers who had never been to malarious areas served as negative controls. The study was approved by the Ethical Committee of the University of Munich and informed consent was obtained from all patients.

The (NANP)$_{40}$-ELISA with precoated ELISA plates (available as an investigator’s kit from Sclavo Diagnostici) for the detection of IgG antibodies was carried out as previously described. All specimens were screened at a 200-fold dilution in duplicate wells. Two negative and positive controls, three calibration sera, and two sera each from parasitologically negative and positive patient were included on each plate as quality controls. Results were obtained with a microplate reader (SLT-Labinstruments, Crailsheim, Germany) with a wavelength capability of 405 nm. A calibration curve was derived by plotting the absorbance values (optical densities) of the calibration sera on log-log paper versus defined unit values (international ELISA units [IEU]). The antibody levels of the specimen (expressed as IEU) were determined by interpolation of the absorbance mean of each serum on the calibration curve. Statistical analysis was performed using EPI-Info version 6.0 (Centers for Disease Control and Prevention, Atlanta, GA and World Health Organization, Geneva, Switzerland). The risk ratio for developing seropositivity at a given destination was calculated as the risk to travel to a certain destination divided by the risk to travel to a certain destination.

Table 1: Antibody reactions to circumsporozoite (CS) antigen of Plasmodium falciparum among international travelers: distribution and geometric mean titers

<table>
<thead>
<tr>
<th>Geographic area</th>
<th>All travelers (n = 2,131)</th>
<th>Positive* travelers (n = 104)</th>
<th>Proportion of seropositive travelers of all travelers to that destination (%)</th>
<th>Risk ratio$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central America</td>
<td>311</td>
<td>13</td>
<td>4.2</td>
<td>0.86</td>
</tr>
<tr>
<td>South America</td>
<td>290</td>
<td>7</td>
<td>2.4</td>
<td>0.49</td>
</tr>
<tr>
<td>East Asia</td>
<td>120</td>
<td>4</td>
<td>3.3</td>
<td>0.68</td>
</tr>
<tr>
<td>Indian Subcontinent</td>
<td>412</td>
<td>9</td>
<td>2.2</td>
<td>0.45</td>
</tr>
<tr>
<td>West Asia</td>
<td>90</td>
<td>1</td>
<td>1.1</td>
<td>0.24</td>
</tr>
<tr>
<td>Southeast Asia</td>
<td>686</td>
<td>23</td>
<td>3.4</td>
<td>0.69</td>
</tr>
<tr>
<td>East Africa</td>
<td>124</td>
<td>27</td>
<td>21.8</td>
<td>4.5</td>
</tr>
<tr>
<td>West Africa</td>
<td>72</td>
<td>16</td>
<td>22.2</td>
<td>4.5</td>
</tr>
<tr>
<td>Southern Africa</td>
<td>26</td>
<td>4</td>
<td>15.4</td>
<td>3.2</td>
</tr>
</tbody>
</table>

* Positive is defined as a CS antibody titer $>6.25$ international ELISA units.

$^a$ Risk ratio (RR) calculated as the risk of becoming seropositive at a certain location over the risk for the total population (RR = 1.0: average risk for seropositivity for all travelers).

Results

Of 2,131 investigated travelers, 1,056 (49.6%) were male and 1,075 (50.4%) were female. The average age was 36 years (range = 1–81 years). The average duration of travel was 32 days (median = 21 days) with an range from 14 to 120 days, as defined by the inclusion criteria. Most patients had been to Southeast Asia previously: 686 (32.2%) of 2,131 had traveled there (Table 1). The next most popular destinations were the Indian subcontinent (19.3%), and Central and South America (14.6% and 13.6%, respectively). Diarrhea was the main symptom in 1,511 (70.9%) of the 2,131 investigated patients, followed by skin problems in 313 (14.7%), and other nonfebrile symptoms in 307 patients (14.4%).

The cut-off value for measurement of CS antibodies was defined as 6.25 IEU with calibration sera provided with the test kit. Samples from 104 of the 2,131 investigated patients (4.9%) were found to be antibody positive, and 2,027 (95.1%) samples showed no significant antibody concentrations against CS antigen. The geometric mean titer of antibody concentrations (IEU) was 18.64 IEU (95% confidence interval [CI] = 13.15–24.13) in seropositive patients and 2.1 IEU (95% CI = 1.8–2.4) in seronegative patients. The geographic distribution of positive samples are shown in Table 1. A significantly above average risk for the acquisition of malaria infection could be found among travelers to East Africa (risk ratio [RR] = 4.5, $P < 0.001$, by the Mantel-Haenszel test), West Africa (RR = 4.5, $P < 0.001$), and Southern Africa (RR = 3.2, $P = 0.015$). Areas with a comparatively low risk of malaria infection were Central America (RR = 0.86, $P < 0.001$) and the Indian subcontinent (RR = 0.45, $P = 0.015$). Areas associated with a low risk of malaria infection but without significant differences between positive and negative travelers included South America (RR = 0.49, $P = 0.091$), East Asia (RR = 0.68, $P = 0.441$), West Asia (RR = 0.24, $P = 0.099$), and Southeast Asia (RR = 0.69, $P = 0.094$). Travelers with positive and negative reactions in the CS ELISA did not differ significantly by sex ($P = 0.665$), age ($P = 0.934$), or their symptoms after return ($P = 0.081$). All 342 patients who had never been to malaria-endemic areas reacted negatively in the CS ELISA (mean IEU = 1.2) as well as in the IFAT for merozoite antibodies (mean antibody titer $< 1:16$).

Discussion

Of the 2,131 travelers who were recruited into this study, 4.9% had significant antibody titers against CS antigen of P. falciparum after their journey to an area endemic for malaria. This figure seems surprisingly high compared with the previously estimated malaria rate of 1.2% per month for typical travelers to West and East Africa, two areas generally assumed to harbor the highest risk for malaria infection. None of the travelers with significant titers of CS antibodies in this study developed clinically apparent malaria or merozoite antibodies as a sign of a manifestation of the infection. Yet, most, if not all, of these travelers probably would have developed symptomatic malaria without appropriate prophylactic measures against malaria, most notably the intake of sufficient doses of antimalariais. These data emphasize the importance of adequate prophylactic measures when traveling to areas with a high incidence of malaria. It must be kept in mind that the results of these investigations are derived from patients who presented themselves to an outpatient clinic of the Department for Infectious Diseases and Tropical Medicine. Therefore, the data are probably biased towards disease and are unlikely to be representative for average German travelers to malarious areas. However, considering the previously demonstrated low sensitivity of the ELISA used in this investigation, at least one-third of all infected persons appear to remain undetected by this test. No specimen derived from the malaria-negative control group of 342 volunteers was positive for CS antibodies. Therefore, a high
specificity concerning the diagnosis of plasmodial infection can be safely assumed since similar results were demonstrated previously.\(^3\)\(^,\)\(^10\)\(^,\)\(^13\) Consequently, the actual infection rate is possibly considerably higher in the group of tourists investigated in this study than the rate of positive antibody responses to CS antigen that could be measured. Therefore, the probable bias towards disease that was introduced due to the selection criteria might be counterbalanced by the underreporting of malaria infection, resulting from the relatively low sensitivity of the ELISA used in this study.

A significantly above-average risk for malaria infection was detected among travelers to sub-Saharan Africa (East Africa [RR = 4.5, \(P < 0.001\)], West Africa [RR = 4.5, \(P < 0.001\)], and Southern Africa [RR = 3.2, \(P = 0.015\)]). Notwithstanding the destination, the percentage of patients with a positive antibody response to CS antigen tended to be considerably higher than previous risk estimates (Table 1).\(^1\) Areas with a comparatively low risk of malaria infection, but not necessarily significant differences between positive and negative travelers were Central America (RR = 0.86, \(P < 0.001\)), the Indian subcontinent (RR = 0.45, \(P = 0.015\)), South America (RR = 0.49, \(P = 0.091\)), East Asia (RR = 0.68, \(P = 0.441\)), West Asia (RR = 0.24, \(P = 0.099\)), and Southeast Asia (RR = 0.69, \(P = 0.094\)). No significant differences regarding sex or age between patients testing positive or negative for CS antibodies were detectable. Although there was no correlation between CS antibodies and symptoms of malaria (\(P = 0.081\)), patients with CS antibodies did present more frequently with diarrhea than travelers without CS antibodies. This finding suggests a greater exposure to infections in general in this group, possibly through behavioral differences that were not registered at admission.

Testing for CS antibodies in non-immune travelers can detect a clinically inapparent infection with \(P.\) \textit{falciparum} with high specificity. Regardless of its limited sensitivity, it might become a method for the determination of the efficacy of malaria prevention measures. This method represents a tool that enables an approximate measurement of the efficacy of malaria chemoprophylaxis in travelers. By use of this tool, screening of symptomatic and asymptomatic travelers to malarious areas could produce data useful for estimating the risk of malaria infection in tourists and, therefore, the necessity of malaria chemoprophylaxis.

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REFERENCES