Prevalence of Hepatitis B and C Virus Markers among Malaria-exposed Gold Miners in Brazilian Amazon

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Hepatitis B and C virus markers were assessed during a survey on malaria in gold mine camps in southern Brazilian Amazon in order to identify risk factors associated to these viral diseases. The study comprised 520 subjects, most of them were gold miners. Missing subjects totaled 49 (8.6%). Among these 520, 82.9% had HBV markers and 7.1% were HBsAg positive. Previous hospitalization, surgery, sexually transmitted diseases and incarceration were quite common among surveyed people, but there is no association between total HBV markers and these factors. On other hand, HBsAg was independently associated to history of sexually transmitted diseases and history of surgery after adjustment. The most frequent HBsAg subtypes identified, adw2 (59%), predominates in populations of Northeast Brazil. The most surveyed people were immigrants coming from that area suggesting that immigrants carried HBV themselves to the study area. Immunoblot (RIBA) confirmed-anti-HCV were found in 2.1%. The only variable associated to anti-HCV in multivariate analysis was illicit intravenous drug. Lack of HCV infection in subjects with such a high HBV markers prevalence reinforces the opinion that HCV is transmitted by restricted routes when compared to HBV. Furthermore, gold miners in Amazon may be considered as a risk group for HBV infection, but not for HCV.

Key words: hepatitis B virus - hepatitis C virus - emigrations and immigration - epidemiology - Brazilian Amazon

Malaria falciparum and hepatitis B virus (HBV) infections are quite common in Amazon Basin. Recent reports have shown that this situation also takes place in the southernmost part of the Brazilian Amazon, corresponding to the State of Mato Grosso (Andrade et al. 1995, Souto et al. 1998a, b). This vast sub-region became the target of a large influx of people from other Brazilian regions during the 70s and 80s. The economic activities were formerly agriculture and cattle-raising, but gold-mining has become an attractive trade to this low income population. Gold miners living and working in Amazon are exposed to body injuries, promiscuous sexual activity and tropical diseases. They frequently move to new gold prospecting places inside Amazon in as much as gold become scarce in prior areas (Santos et al. 1995). As a consequence gold miners are considered to facilitate malaria spread throughout the region (Andrade et al. 1995). Besides, a HBV markers prevalence study performed in a county of Mato Grosso suggested that gold miners may have taken a role in HBV spread as well (Souto et al. 1998b). However the routes involved in HBV transmission in this group remains unknown.

There are few reports on hepatitis C virus (HCV) prevalence in Amazon region. The prevalence of recombinant immunoblot (RIBA) confirmed-HCV antibodies ranges between 2.1% and 2.4% in studies from northernmost part of Mato Grosso (Souto et al. 1996, 1999). The only factor associated to HCV antibodies was previous use of parenteral illicit drugs (Souto et al. 1999).

A sero-epidemiologic study comprising inhabitants from mine camps located in Garimpo Satelite, county of Apiacas, north of the State of Mato Grosso, was performed in order to determine the hepatitis B and C markers prevalence and to identify factors associated to viral spread. Another aim was to study the HBV subtypes that are circulating among these subjects.

MATERIALS AND METHODS

This study was based on data collected during a survey on malaria carried out in Garimpo Satélite, a large area comprising 16 gold mine camps in...
Apiacas county. Before starting the field work, the Garimpo Satelite was censored by the researcher team in order to estimate the current number of inhabitants, regarding the mobility of this workers in Amazon and the necessity to bring up to date this information. All subject living in these camps were considered eligible to the study. The camp field was performed between March and June 1996.

The ethical and methodological aspects of this study were approved by the Federal University of Mato Grosso Research Ethical Committee. The objectives of the study were explained orally to all participants and consent of each participant was obtained. At the end of the study, individual blood tests results were given to each participant. Subjects identified as HBV or HCV carriers were directed to Hepatitis Care Program of Hospital Júlio Muller, Federal University of Mato Grosso, Cuiabá.

Each eligible individual was interviewed about demographic characteristics, previous malaria episodes and known risk factors associated with hepatitis transmission, including blood transfusion receipt, parenteral illicit drug use, dental treatment, number of sexual partners during lifetime, hospitalization, dental treatment, acupuncture and tattooing. Individuals born outside Mato Grosso were classified as immigrants and the time that each one lived in Amazon region was recorded.

Blood samples were drawn by venepuncture using vacuum tubes with EDTA. After preparing thin and thick blood smears, plasma were obtained and stored at -20°C until tested at the Department of Virology, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro. The samples were analyzed for HBV surface antigen and antibody (HBsAg and anti-HBs, respectively), and total antibodies against HBV core antigen (anti-HBc), using enzyme immunoassays (EIA) from Organon Teknika-Hepanostika, Boxtel, The Netherlands. HBsAg subtyping was also performed by EIA using monoclonal antibodies prepared at the Department of Virology (Niel et al. 1994). A subject was assumed to have been exposed to HBV if he or she had HBV markers (anti-HBc isolated or anti-HBc plus HBsAg or anti-HBs). IgG class antibodies against hepatitis D virus (anti-HDV) were also tested by EIA (Abbott, Chicago, IL, USA).

A third generation EIA was used to detect anti-HCV antibodies (EIA, Abbott, Chicago). Positive samples were retested for confirmation with an immunoblot assay (RIBA) using immunodominant epitopes of core, E2/NS1, NS3, NS4, and NS5 antigens (Inno-LIA HCV Ab III, Innogenetics, Ghent, Belgium). These tests were performed at Department of Virology, Instituto Oswaldo Cruz. HCV infection was considered present in individuals with RIBA-confirmed anti-HCV.

Standard epidemiological methods of analysis of cross-sectional data were applied, including Student’s t test, odds ratios, p values and 95% confidence intervals (95% CI). Statistical significance was assessed at the 0.05 probability level in all analysis. Variables that showed differences between groups of at least a p value of 0.2 in univariate analysis were included in stepwise logistic regression models (Stata 5.0, Stata Corporation, Texas, USA) in order to identify those independently associated with exposure to HBV or HCV.

**RESULTS**

Five hundred twenty individuals (91.4%) of the 569 Garimpo Satelite’s inhabitants were interviewed and bled. Most of missing eligible people were due to travel during the study period. Individuals refusing participation were only three. Among the study participants, 442 (85%) were male and age ranged from 3 to 66 (mean = 32). Most of them (79.6%) were aged between 20 and 40 years. Immigrants were the majority of the community, especially coming from the Northeast Brazil (72.8% of the sample). These low income communities lived in bad hygienic conditions, in rustic and precarious cots located around the gold mines. The mean time living in this area was 7.5 years, ranging from 1 to 18. Some behavioral characteristics of the sampled people appears in Table I.

Previous malaria episodes were admitted by 517 (99.4%) of them and *Plasmodium* sp. were detected in 106 of them by thick blood smear (*P. falciparum*, 56; *P. vivax*, 47; *P. malariae*, 3).

Among the 520 participants, 431 (82.9%; CI95%: 79.3%; 86%) people have been exposed to HBV and 37 (7.1%; CI95%: 5.1%; 9.7%) were HBsAg carriers. Only two (5.4%) out of 37 HBsAg carriers showed positivity to anti-HDV. The HBsAg subtypes were identified in 30 out of 37. Small amount of serum samples preclude to subtyping HBsAg in other seven. Adw2 subtype were present in 22 (59%), adw4 in 3 (8.1%), ayw3 in 3 (8.1%) and ayw2 in 2 (5.4%).

No association were found between previous infection by HBV and all variables in univariate and multivariate analysis (Table I). When the HBsAg positivity was the outcome of the logistic model there was independent association to sexual transmitted disease (adjusted OR = 3.9; 95% CI = 1.3; 11.7; P<0.05) and history of surgery (adjusted OR = 5.0; 95% CI = 2.0; 12.9; P<0.05) (Table II).

**The EIA anti-HCV was present in 22 (4.2%).** The anti-HCV positivity was confirmed in 11 (2.1%; CI95%: 1.1%; 3.9%) by RIBA. The only variable associated to RIBA anti-HCV positivity in multivariate analysis was illicit intravenous drug
use (adjusted OR = 8.1; 95% CI = 1.1; 38.6; P<0.05) (Table III).

Time living in Amazon was not associated to HBV or HCV markers.

**DISCUSSION**

A previous study on the prevalence of hepatitis B markers in south Amazon has suggested that gold miners represent a risk group for HBV infection and can facilitate its transmission as a consequence of their nomadic behavior (Souto et al. 1998b). That same study also showed that the prevalence of HBV markers among men aged between 20 and 40 ranged from 50% to 60%. The present study, based on a population of gold miner camp of south Amazon, basically composed by

**TABLE I**

Prevalence of some behavioural and previous medical conditions of risk to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection in whole surveyed, HBV exposed, HBV carriers and HCV exposed subjects

| Variable                        | No. (%) (HBV) | No. (%) HBV exposed | P value | No. (%) (+) HBsAg | P value | No. (%) Anti-HCV (+) | P value |
|---------------------------------|---------------|---------------------|---------|-------------------|---------|----------------------|---------|
| Hospitalization<sup>a</sup>     |               |                     |         |                   |         |                      |         |
| Yes                             | 379 (73.9)    | 314 (82.8)          | 0.79<sup>b</sup> | 24 (6.3)          | 0.76<sup>b</sup> | 10 (2.6)             | 0.07<sup>c</sup> |
| No                              | 134 (26.1)    | 113 (84.3)          |         | 11 (8.2)          |         | 0 (0)                |         |
| Past surgery<sup>a</sup>        |               |                     |         |                   |         |                      |         |
| Yes                             | 82 (16)       | 69 (84.1)           | 0.93<sup>b</sup> | 9 (11)           | 0.16<sup>b</sup> | 4 (4.9)              | 0.06<sup>c</sup> |
| No                              | 431 (84)      | 358 (83.1)          |         | 26 (6)            |         | 6 (1.4)              |         |
| Blood transfusion<sup>a</sup>   |               |                     |         |                   |         |                      |         |
| Yes                             | 74 (14.4)     | 61 (82.4)           | 0.97<sup>b</sup> | 7 (9.5)          | 0.47<sup>b</sup> | 1 (1.4)              | 1.0<sup>c</sup>  |
| No                              | 439 (85.6)    | 366 (83.4)          |         | 28 (6.4)          |         | 9 (2.1)              |         |
| STD<sup>a,d</sup>               |               |                     |         |                   |         |                      |         |
| Yes                             | 268 (59)      | 229 (85.4)          | 0.28<sup>b</sup> | 28 (10.4)        | <0.01<sup>b</sup> | 6 (2.2)              | 0.75<sup>c</sup> |
| No                              | 186 (41)      | 151 (81.2)          |         | 5 (2.7)           |         | 4 (1.7)              |         |
| Incarcerating                   |               |                     |         |                   |         |                      |         |
| Yes                             | 66 (12.9)     | 53 (80.3)           | 0.6<sup>b</sup> | 3 (4.5)           | 0.3<sup>c</sup> | 0                    | 0.37<sup>c</sup> |
| No                              | 447 (87.1)    | 374 (83.7)          |         | 32 (7.2)          |         | 10 (2.2)             |         |
| IVDU<sup>d</sup>                |               |                     |         |                   |         |                      |         |
| Yes                             | 17 (3.3)      | 16 (94.1)           | 0.37<sup>b</sup> | 3 (17.6)         | 0.1<sup>c</sup> | 2 (11.8)             | 0.04<sup>c</sup> |
| No                              | 496 (96.7)    | 411 (82.9)          |         | 32 (6.5)          |         | 8 (1.6)              |         |
| Male homosexual experience      |               |                     |         |                   |         |                      |         |
| Yes                             | 30 (7.4)      | 28 (93.3)           | 0.24<sup>b</sup> | 0               | <0.05<sup>c</sup> | 0                    | 1.0<sup>c</sup>  |
| No                              | 378 (92.6)    | 315 (83.3)          |         | 33 (8.7)          |         | 7 (1.9)              |         |

<sup>a</sup>: regarding the entire lifetime; <sup>b</sup>: P value of Yates corrected chi-square; <sup>c</sup>: P value of two-tailed Fisher’s exact test; <sup>d</sup>: STD: sexually transmitted disease; IVDU: intravenous drug users

**TABLE II**

Stepwise logistic regression model with variables associated in univariate analysis (outcome = hepatitis B surface antigen)

| Variables<sup>a</sup> | HBsAg(+)/no. examined (%) | Crude OR (95% CI) | P | Adjusted OR (95% CI) | P |
|------------------------|---------------------------|-------------------|---|----------------------|---|
| Surgery                |                           |                   |   |                      |   |
| No                     | 26/431 (6)                | 1.0 (Reference)   | 0.16 | 1.0 (Reference)    | <0.01 |
| Yes                    | 9/82 (11)                 | 1.9 (0.8; 4.5)    |       | 5.0 (2.0; 12.9)     |       |
| Parenteral illicit drug use |                       |                   |   |                      |   |
| No                     | 33/496 (6.5)              | 1.0 (Reference)   | 0.16 | 1.0 (Reference)     | 0.16 |
| Yes                    | 3/17 (17.6)               | 3.1 (0.7; 12.5)   |       | 3.4 (0.6; 18.6)     |       |
| Sexually transmitted disease |                     |                   |   |                      |   |
| No                     | 5/186 (2.7)               | 1.0 (Reference)   | <0.01 | 1.0 (Reference)     | <0.05 |
| Yes                    | 28/286 (10.4)             | 4.2 (1.5; 12.8)   |       | 3.9 (1.3; 11.7)     |       |

<sup>a</sup>: gender dropped out due to estimability; time of living in the study area and age removed due to no association with dependent variable (p>0.25); <sup>b</sup>: Fisher’s exact test
males aged between 20 and 40, showed a quite high prevalence of HBV markers (82.9%). Such a high prevalence of HBV markers likely precluded a statistical association between HBV exposure and analyzed independent variables, such as blood transfusion, surgical procedures, hospitalization, intravenous illicit drug use, history of incarceration, sexually transmitted diseases. However, when analyzed alone HBsAg positivity was associated to previous surgery and sexual transmitted diseases. Sexual activity is quite known as an important route for HBV spread. Association between HBsAg positivity and previous surgery may represent use of inadequately sterilized syringes and nosocomial treatments.

The adw2 HBV subtype has been shown to predominate in populations of Northeast Brazilian region (Gaspar & Yoshida 1988). This was original area of the most surveyed people, suggesting that immigrants from this Brazilian region carried the HBV themselves from there to the study area. However, Northeast Brazil HBV prevalence is poorly known (Souto 1999). Since there is no association between HBV markers and time living in south Amazon, we can not assert where and when these subjects kept infected by HBV.

Despite of the high prevalence of HBV-HDV coinfection in western Amazon, the number of coinfected individuals in the present study points out that the Delta agent has not been largely introduced in the southern Brazilian Amazon so far, in agreement with previous studies (Souto et al. 1998a,b).

The prevalence of HCV infection was low-to-moderate among these subjects, even though they were highly exposed to injuries, promiscuous sexual activity and surgical procedures. The only classical risk factor associated to the presence of anti-HCV in this survey was history of parenteral drug use. Such few cases of HCV infection in subjects with these characteristics reinforce the opinion that HCV is poorly transmitted by routes other than sharing syringes and needles or by blood transfusion.

In the present study, half of the anti-HCV positive by EIA were not-confirmed. Aceti et al. (1990) studying HCV infection in a Cameroon malarigenous area showed similar results, supposing that the stimulation of a polyclonal antibodies production from recurrent malaria infection could also produce false-positive anti-HCV EIA tests.

Finally, these data reinforce that gold mine workers in the Amazon may be considered as a risk group for HBV infection, but not for HCV. Ever since these workers are constantly moving inside Amazon and exposing themselves to sexual and parenteral transmitted diseases, a surveillance system on HDV superinfection should be developed, in as much as HDV can deteriorate the prognosis of HBV infection and both viruses share the same transmission routes.

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**TABLE III**

| Variables<sup>a</sup> | Anti-HCV(+)/Total (%) | Crude OR (95%CI) | P | Adjusted OR (95%CI) | P |
|------------------------|------------------------|------------------|---|---------------------|---|
| Age (years)            |                        |                  |   |                     |   |
| 3-25                   | 1/95 (1.0)             |                  | 1.0 (Reference) |                     |   |
| 26-30                  | 2/140 (1.4)            |                  | 2.2 (0.2; 28.6) |                     | 0.5 |
| 31-40                  | 5/193 (2.6)            |                  | 4.4 (0.4; 47.2) |                     | 0.2 |
| 41-66                  | 3/85 (3.5)             |                  | 0.18<sup>a</sup> | 4.6 (0.3; 69.1)     | 0.3 |
| Previous IVD use       |                        |                  |   |                     |   |
| No                     | 8/496 (1.6)            |                  | 1.0 (Reference) |                     |   |
| Yes                    | 2/17 (11.8)            |                  | 8.1 (1.1; 38.6) | <0.05               |   |

<sup>a</sup> IVD: intravenous drug use; P value of OR for trend with age less than 26 as reference; hospitalization dropped out due to estimability.
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