A Possible Correlation between the Host Genetic Background in the Epidemiology of Hepatitis B Virus in the Amazon Region of Brazil

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The Amazon region of Brazil is an area of great interest because of the large distribution of hepatitis B virus in specific Western areas. Seven urban communities and 24 Indian groups were visited in a total of 4,244 persons. Each individual was interviewed in order to obtain demographic and familial information. Whole blood was collected for serology and genetic determinations. Eleven genetic markers and three HBV markers were tested. Among the most relevant results it was possible to show that (i) there was a large variation of previous exposure to HBV in both urban and non-urban groups ranging from 0 to 59.2%; (ii) there was a different pattern of epidemiological distribution of HBV that was present even among a same linguistic Indian group, with mixed patterns of correlation between HBsAg and anti-HBs and (iii) the prevalence of HBV markers (HBsAg and anti-HBs) were significantly higher (P = 0.0001) among the Indian population (18.8%) than the urban groups (12.5%). It is possible that the host genetic background could influence and modulate the replication of the virus in order to generate HB carrier state.

Key words: hepatitis B virus - seroepidemiology - Amazon region - genetic correlation

The epidemiology of hepatitis B virus (HBV) is well defined in several geographical areas of the world (Cokburn 1981, Deinhardt & Deinhardt 1983, Zuckerman 1984, 1987, Bensabath et al. 1986, Black et al. 1986). The modes of transmission are clear and similar in both developed and developing countries. Some of the tropical areas however, harbor a higher prevalence of HBV. As a consequence, there is also a higher rate of active carriers among the apparently healthy population of such endemic areas.

The West of the Amazon region of Brazil has been long shown to be a highly endemic area for HBV (Bensabath et al. 1986, Fonseca et al. 1988), but information is still fragmentary and incomplete to define the descriptive epidemiology of the virus in the region as a whole.

The present work aimed to describe the general prevalence of HBV dissemination and the rate of HB carrier among native Indians and urban populations of the Amazon. Furthermore, the existence of a genetic profile of those populations, was used to attempt to correlate the influence of genetic markers and persistence of HBV.

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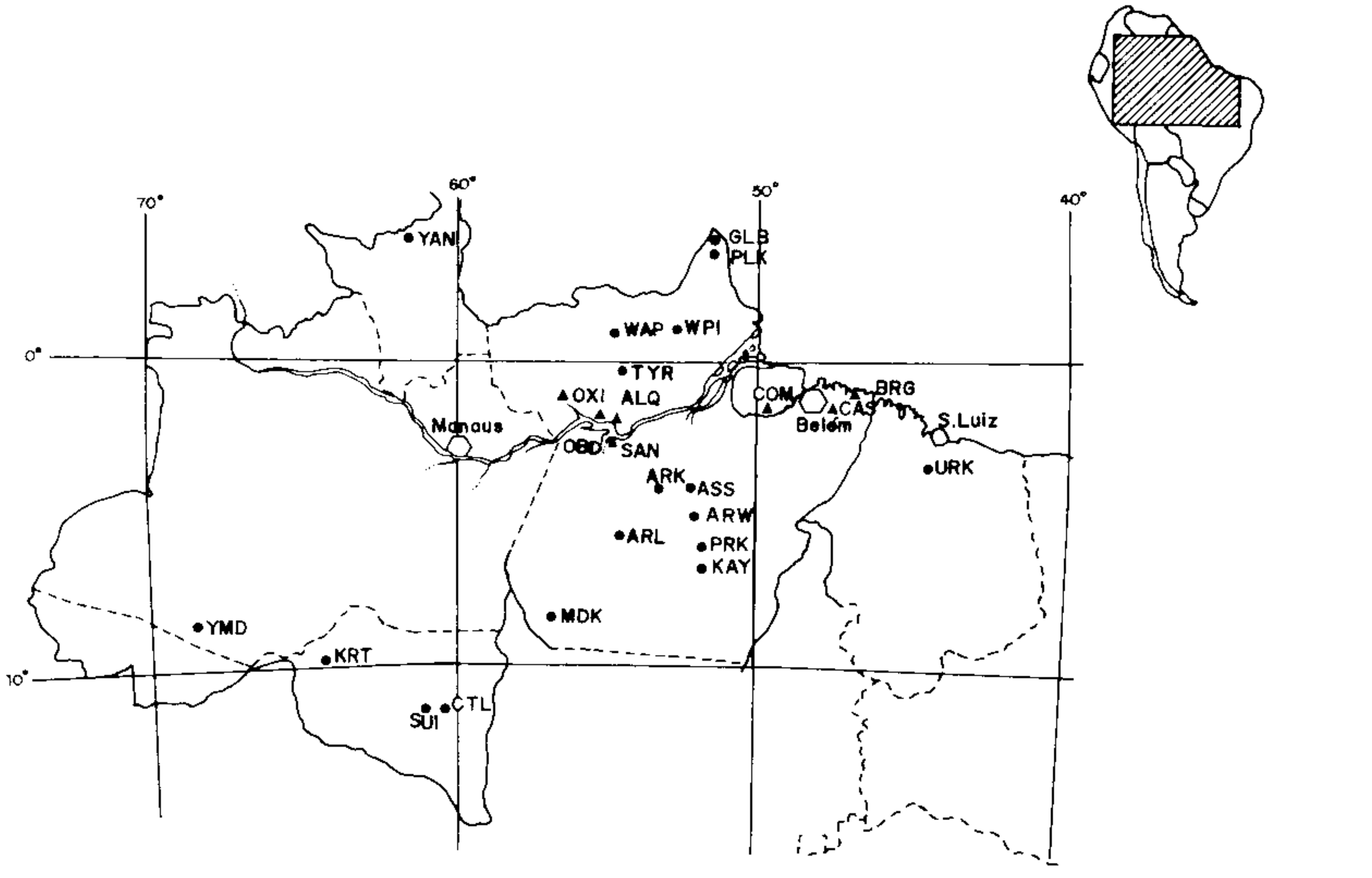
MATERIALS AND METHODS

Populations examined - Blood samples were collected during the period of 1983 to 1991 from 4,244 individuals residing in seven urban communities in the State of Pará (Santarém, Castanhal, Oriximiná, Óbidos, Alenquer, Bragança, and Combu) and 24 Indian communities residing in the States of Maranhão (Urubu-Kaapor), Amapá (Galibi, Palikur, Waiapi), Pará (Wayana-Apalai, Tyriyo, Assurini do Kuatinemo, Assurini do Trocara, Arara do Laranjal, Arara do Kurambe, Arawete, Parakana, Kararao, Aukre, Kubenkokre, Pukany, Kikretun, Kokraimoro, Munduruku), Roraima (Yanomami), Amazonas (Yamamadi), and Rondônia (Cinta Larga, Surui, Karitiana). Their geographical distribution can be seen in Fig. The samples tested represented more than 50% of the Indian population groups examined and approximately 2% to 3% of the urban communities. Serum or plasma were separated and frozen at -20°C.

Hepatitis B markers - HBsAg, anti-HBs (Biomanguinhos, FIOCRUZ) and anti-HBc IgM (Institute Pasteur) were detected through enzyme immunoassays commercially available.

Statistical methods - The association between genetic and epidemiologic markers was attempted using the Woolf (1955) method through a computer program prepared by H Krieger and P Cabello. Genetic variability was measured through



Geographical distribution of urban and Indian communities examined. Amazon region of Brazil.

KAY Kayapó (villages Kararao, Aukre, Kubenkokre, Pukany, Kikretun and Kokraimoro)
I Indian groups: URK-U.Kaapor; GLB-Galibi; PLK-Palikur; WPI-Waiapi; WAP-W.Apalai; TYR-Tyrió; ASS-Assurini; ARK-A. Kurambé; A.Laranjal; ARW-Araweté; PRK-Parakanã; KAY-Kayapó; MDK-Munduruku; YAN-Yanomama; CLT-C. Larga; SUI-Surui; KRT-Karitiana; YMD-Yamamadi
▲ Urban groups: BRG-Bragança; CST-Castanhal; CMB-Combú; ALQ-Alenquer; SNT-Santarém; OBD-Óbidos; ORX-Oriximiná

the program BIOSYS (DL Sowfford and P Selander) taking into consideration the measurement of average heterozygozity (H), the proportion of polimorphic loci (P) and the mean number of alleles per locus (N). Eleven genetic markers (ABO, RH, haptoglobin, ceruloplasmin, transferrin, albumin, butiryl cholinesterase-locus 1, cholinesterase-locus 2, hemoglobin, esterase D and acid phosphatase) were analyzed. The comparison of the values of average heterozygosity between the two population groups used the Mann-Whitney test through the SPSS program.

RESULTS

Fifty percent of the individuals investigated from urban areas (2,022) were males and their age range varied from 9 to 60 years old. The Indian community (2,222 individuals) was represented by 49% of males and ages varied from 1 to 80 years old. None of the subjects examined was submitted to previous vaccination against hepatitis B virus.

Tables I and II show the prevalence rates of anti-HBs. and HBsAg in urban and Indian communities, respectively. HBV was absent in two Indian groups (Arara do Laranjal and Parakana) but varied its prevalence from 3.4% (Arawete) to 59.2% (Assurini do Kuatinemo). All of the HBsAg positive subjects were tested for the presence of anti-HBc IgM in order to diferentiate acute infected from hepatitis B carriers. Four were positive, indicating a recent infection close to the time of blood collection.

The comparison of prevalence rates of anti-HBs and HBsAg between urban and Indian populations is shown in Table III. There was an statistically significant difference of these HBV markers prevalence (12.5% vs. 18.8%, p=0, respectively) when considering the two population groups examined.

The comparison of the prevalence rates of HBV markers (HBsAg and anti-HBs) within the urban communities, showed that the population groups located at the mouth of the Amazon river (Bragança, Castanhal and Combu) presented signifi-

TABLE I

Prevalence (%) of HBV markers (HBsAg and anti-HBs) among urban communities of the Amazon region of Brazil

Population	No. examined	anti-HBs		HBsAg		Total (%)
		No.	%	No.	%	
Bragança	256	17	6.7	0	0	6.7
Castanhal	481	54	11.2	2	0.4	11.8
Combu	132	13	9.8	0	0	9.8
Alenquer	223	29	13.0	3	1.3	14.3
Santarém	500	67	13.4	8	1.6	15.2
Óbidos	232	31	12.8	1	0.4	13.2
Oriximiná	188	26	13.8	4	2.1	15.9
Total	2022	237	11.7	18	0.9	12.6

TABLE II

Prevalence (%) of HBV markers (HBsAg and anti-HBs) among Indian communities of the Amazon region of Brazil

Population	No. examined	anti-HBs		HBsAg		Total
		No.	%	No.	%	
Urubu-Kaapor	204	13	6.4	3	1.5	7.9
Galibi	167	42	25.1	3	1.8	26.9
Palikur I	070	20	28.6	0	0	28.6
Palikur II[a]	050	11	22.0	3	6.0	28.0
Waiapi	090	4	4.4	0	0	4.4
Wayana-Apalai	190	50	26.3	27	14.2	40.5
Tyriyo	031	2	6.4	1	3.2	9.7
Assurini do Kuatinemo	049	26	53.1	3	6.1	59.2
Assurini do Trocara	098	5	5.1	3	3.1	8.2
Arara do Laranjal	052	0	0	0	0	0
Arara do Kurambe	017	1	5.9	0	0	5.9
Arawete	116	3	2.6	1	0.9	3.4
Parakana	101	0	0	0	0	0
Kararao	032	3	9.4	0	0	9.4
Aukre	016	1	6.2	0	0	6.2
Kubenkokre I	078	13	16.7	0	0	16.7
Kubenkokre II[a]	117	38	32.5	2	1.7	34.2
Pukany	053	12	22.6	1	1.9	24.5
Kikretun	018	0	0	1	5.6	5.6
Kokraimoro	102	25	24.5	2	2.0	26.5
Munduruku	168	37	22.0	1	0.6	22.6
Yanomama	106	13	12.3	8	7.5	19.8
Cinta Larga	098	6	6.1	1	1.0	7.1
Surui	062	15	24.2	7	11.3	35.5
Karitiana	098	5	5.1	0	0	5.1
Yamamadi	039	7	17.9	0	0	17.9
Total	2222	352	15.8	67	3.0	18.8

[a]: sequential visit to the same group with a minimun of two years between visits

TABLE III

Comparison of the prevalence (%) of HBV markers (HBsAg and anti-HBs) between urban and Indian communities of the Amazon region of Brazil

Population	anti-HBs		HBsAg		anti-HBs+HBsAg	
	No.	%	No.	%	No.	%
Urban	237	11.7	16	0.8	253	12.5
Indian	352	15.8	65	2.9	417	18.8
$\chi 2=$	15.04		25.75		31.15	
P=	0.00010		0			

TABLE IV

Comparison of the prevalence (%) of HBV markers (HBsAgand anti-HBs) between urban populations of the Amazon region of Brazil, Eastern and Western of the Amazon river

Population	anti-HBs		HBsAg		anti-HBs+HBsAg	
	No.	%	No.	%	No.	%
Eastern	84	9.7	1	0.1	86	9.9
Western	153	13.3	15	1.3	169	14.6
$\chi 2=$	6.22		7.43[a]		9.95	
P=	0.0126		0.0064		0.0016	

[a]:Yates corrected

TABLE V

Average heterozigosity among urban communities of the Amazon region of Brazil

Locus[a]	Bragança	Castanhal	Alenquer	Santarém	Óbidos	Oriximiná
ABO	0.375	0.372	0.331	0.388	0.320	0.366
RH	0.354	0.366	0.368	0.357	0.374	0.443
HP	0.494	0.510	0.501	0.510	0.501	0.512
CP	0.040	0.096	0.058	0.078	0.021	0.041
TF	0.021	0.054	0.013	0.017	0.057	0.041
ALB	0.013	0.006	0.000	0.004	0.000	0.021
BCHE	0.060	0.040	0.021	0.020	0.021	0.040
CHE2	0.040	0.130	0.079	0.163	0.078	0.094
HB	0.028	0.039	0.000	0.078	0.000	0.020
ESD	0.297	0.320	0.395	0.320	0.421	0.253
ACP	0.156	0.295	0.229	0.179	0.227	0.350
H[b]	0.171 (0.053)	0.203 (0.052)	0.183 (0.057)	0.192 (0.053)	0.184 (0.057)	0.198 (0.057)
N	2.360	2.450	2.090	2.450	1.910	2.360
P	90.91	90.91	81.82	81.82	81.82	100.00

[a]: ABO, RH, HP (Haptoglobin), CP (Ceruloplasmin), TF (Transferrin), ALB (Albumin), BCHE (Butiril cholinesterase-locus 1), CHE2 (Cholinesterase - locus 2), HB (Hemoglobin), ESD (Esterase D), ACP (Acid phosphatase)

[b]: H (Average Heterozigosity), N (Mean Number of Alleles per Locus), P (Percentage of Polimorphic Loci)

TABLE VI

Average heterozygosity among Indian communities of the Amazon region of Brazil

Locus[a]	URK[b]	WAP	ASK	AST	ARW	PRK	YMD	KAY	CTL	SUI	KRT
ABO	0.020	0.000	0.000	0.019	0.000	0.000	0.000	0.020	0.020	0.000	0.000
RH	0.000	0.000	0.000	0.000	0.000	0.212	0.000	0.000	0.000	0.000	0.000
HP	0.395	0.404	0.502	0.431	0.404	0.500	0.505	0.503	0.477	0.503	0.464
CP	0.039	0.094	0.225	0.492	0.000	0.000	0.000	0.062	0.183	0.191	0.035
TF	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ALB	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
BCHE	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CHE2	0.242	0.075	0.000	0.000	0.198	0.040	0.000	0.173	0.177	0.116	0.421
HB	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ESD	0.112	0.404	0.503	0.474	0.375	0.444	0.344	0.000	0.492	0.369	0.503
ACP	0.211	0.307	0.345	0.144	0.039	0.226	0.053	0.246	0.233	0.141	0.183
H[c]	0.093	0.117	0.143	0.144	0.091	0.129	0.082	0.089	0.144	0.120	0.146
	(0.040)	(0.051)	(0.064)	(0.064)	(0.047)	(0.057)	(0.052)	(0.049)	(0.057)	(0.052)	(0.146)
N	1.550	1.450	1.360	1.550	1.360	1.450	1.270	1.360	1.550	1.550	1.450
P	54.55	45.45	36.36	36.36	36.36	45.45	27.27	36.36	54.55	45.45	45.45

[a]: ABO, RH, HP (Haptoglobin), CP (Ceruloplasmin), TF (Transferrin), ALB (Albumin), BCHE (Butiril cholinesterase-locus 1), CHE2 (Cholinesterase-locus 2), HB (Hemoglobin), ESD (Esterase D), ACP (Acid Phosphatase)

[b]: URK (Urubu-Kaapor), WAP (Wayana-Apalai), ASK (Assurini do Kuatinemo), AST (Assurini do Trocará), ARW (Arawete), PRK (Parakanã), KAY (Kaiapó), CTL (Cinta Larga), SUI (Surui), KRT (Karitiana), YMW (Yamamadi)

[c]: H (Average Heterozygosity), N (Mean Number of Alleles per Locus), P (Percentage of Polimorphic Loci)

cantly lower rates (9.9% vs. 14.6%, p=0.0016) than those found in the West (Alenquer, Oriximiná and Óbidos) as seen in Table IV.

The estimates related to the genetic variability of the two population groups are listed in Tables V and VI. Among the urban communities the average heterozygozity varied from 0.171 (Bragança) to 0.203 (Castanhal) in comparison to the range of 0.082 (Yamamadi) to 0.146 (Karitiana) among the Indians. The comparison of the two population groups were significantly different (Mann-Whitney: U=0, Z(U)=-3.3187, p<0.0009). The proportion of polimorphic loci ranged from 82% to 100% and 27% to 54% among urban and Indian groups respectively.

DISCUSSION

The prevalence of HBV markers in the Amazon region of Brazil are generally higher than in the rest of the country, but also variable according to factors that include the community examined, the social economic status and the geographical isolation of the group (Gayotto et al. 1981, Alecrim et al. 1984, Carrilho & Silva 1986, Bensabath et al. 1986, 1987, Santos et al. 1991).

The rates observed in the urban communities examined were not different to what is generally seen, and increased significantly (p=0.0016) in those communities located to the west of the Amazon river. The southwest of the Amazon region is particularly interesting especially to what concerns the HB carrier state. The prevalence of HBsAg in Northern states is not higher than 7%, while in groups residing in the States of Amazonas, Acre and Rondônia, it reaches 19% (Gayotto et al. 1981, 1984, Bensabath et al. 1986, Santos et al. 1991).

The Indian groups examined showed prevalence rates that were also not different from the scaterred results described so far (Black et al. 1974, Gayotto et al. 1981, 1984, Ferraroni & Lacaz 1982, Alecrim et al. 1984, Carrilho & Silva 1986, Bensabath et al. 1986, 1987, Santos et al. 1991). The absence of markers for the virus in the Parakana and the Arara do Laranjal may reflect the time of the blood collection (1983 and 1985, respectively). Recently, HBV has been described in the Parakana (HBsAg=10%) by Vieira-Filho et al. (1990). The Parakana represents a recent evidence of introduction of pathogens into isolated communities including sexually transmitted ones (Ishak et al. 1993).

It seems that when HBV reaches an Indian community it tends to disseminate more among its members and to persist in higher levels than what is seen in general urban communities. The prevalence rates of anti-HBs and HBsAg were significantly different between the two groups.

Endemicity of HBV based on the prevalence of markers is generally classified as low (HBsAg, <2%; total markers, <30%), intermediate (HBsAg, 2%-5%, total markers, 30%-50%) and high (HBsAg, 5%-20%, total markers, 50%-90%).

Nine Indian communities did not show a sharp correlation between the rates of anti-HBs and HBsAg, as they are regularly categorized by the WHO (Zuckerman 1984). Three additional catego-

ries were seen in that (i) previous exposure to the virus (prevalence of anti-HBsAg) could be low, but carrier prevalence (HBsAg) was intermediate like in the Tiryo (6.4% / 3.2%), Assurini do Trocará (5.1% / 3.1%), and Kikretun (0 /5.6%); (ii) exposure was intermediate, but virus was maintained in low carriage levels like in the Munduruku (22% / 0.6%), and Yamamadi (17.9% / 0); and (iii) exposure was medium, but virus was kept in high levels of persistence like in the Wayana-Apalai (26.3% / 14.2%), Yanomami (12.3% / 7.5%), and Surui (24.2% / 11.3%).

There was no link whatsoever among the eight groups involved. They were not geographically close (indeed, there were two or more pattern in the same areas), there were no association to the linguistic tree or to the time they have already been approached.

Apart from the possibility of the occurrence of different subtypes of HBV, there is the fact that Indian groups in the Amazon region of Brazil result from an interaction of factors that include movements of fusion and separation between tribes or villages, contacts with other tribes, and wedding patterns that could confer them different levels of susceptibility towards an infection with HBV.

The genetic similarity among the Kayapo Indians has been already described (Salzano 1971, Salzano et al. 1972), however these Indians showed three different patterns of interaction with the virus: (i) low endemicity - Kararao and Aukre; (ii) intermediate endemicity - Kubenkokre, Pukany, Kokraimoro, and (iii) mixed (low/medium) - Kikretun. It is possible that one (or more) genetic factor is influencing and modulating the way the virus interacts with the host during its replication. This is seen with other viruses like Saint Louis encephalitis virus; when the cell substrate is changed, the biology of in vitro persistence is also changed (Randolph & Hardy 1988).

The correlation of susceptibility to infection and maintenance of the virus in the host has been suggested and pointed to some genetic markers (Hillis et al. 1977, Penner at al. 1977, Mazzilli et al. 1977, Mota et al. 1977, Sanchez et al. 1983, Xu & Ng 1983, Padma & Velli 1988, Kishimoto et al. 1990). The communities listed in the present work have been extensively studied with the aim to establish a genetic profile regarding 33 alleles (Black et al. 1988, Salzano et al. 1988, 1991, Guerreiro & Chataur - Freire - Maia 1988, Santos 1993). It was not possible to demonstrate any consistent association pointing a specific allele and the presence of HBsAg.

Indian groups have been clearly shown to have a low level of genetic variability when considering their profile of conventional genetic markers (Salzano & Callegari-Jaques 1988) and, indeed, it has been suggested that it might be the reason for their susceptibility to infectious diseases in general (Black 1992).

The comparison of the proportion of polimorphic loci (P) between the urban groups (82% to 100%) and Indians (27% to 54%) demonstrated a clear difference in their genetic variability. The host cell has an important influence in the regulation of virus replication and it is apparently different in the two population groups involved. It is possible that the higher levels of HB carriers among the native Indians of the Amazon, as well as the differences observed in the epidemiology, the dissemination and patterns of maintenance of the virus could be related to the limited genetic component of the host.

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