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Distribution of Haptoglobin Phenotypes among Patients with HIV/AIDS, Hepatitis B, Liver Cirrhosis and Chronic Renal Failure in Sudan

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Keywords: Haptoglobin (Hp); Hardy-Weinberg equilibrium (HWE), human immunodeficiency virus (HIV), Acquired immune deficiency syndrome (AIDS), liver cirrhosis (LC), hepatitis-B (HBV), chronic renal failure (CRF); allele frequency.

Abstract

Haptoglobin (Hp) is a plasma α2-glycoprotein that binds free haemoglobin and is thought to have a role in the immune pathway of different pathologies depending on its phenotype.

A total of 497 Sudanese patients with viral infections and non-infectious diseases were typed for haptoglobin phenotypes, in this study, using the polyacrylamide non-reducing gel electrophoresis separation method to determine possible associations between haptoglobin phenotypes Hp1-1, Hp2-1 and Hp2-2. Viral infections included human immunodeficiency virus (HIV) (n= 129) and hepatitis B (HBV) (n=171). The non-infectious diseases were: liver cirrhosis (LC) (n=68) and chronic renal failure (CRF) (n= 129). Healthy controls (n= 65) were also typed. Each disease sample was compared with the control. Expected percentages of each of the three phenotypes in Sudan estimated using SPSS were between 51.38-51.49% for Hp1-1, 36.71-36.81% for Hp2-1 and 11.76-11.91% for Hp2-2. Hp1-1 was found the most significantly prevalent among patients with LC ($P \leq 0.05$). Patients with HIV/AIDS and HBV showed the same distribution of haptoglobin phenotypes as the healthy control (i.e. a majority of Hp2-1); whereas the highest percentage of patients with Hp2-2 were those with CRF (≥23%) although not significantly different from the control. Using Hardy-Weinberg equilibrium (HWE) as a null model, hp^1 allele frequency was calculated to be 0.69 and hp^2 allele frequency was 0.31 in Sudan. The healthy control, LC and CRF sampled populations were significantly deviated from HWE (P=0.05). In this study, we found that Hp1-1 has a significant association with LC. HIV/AIDS and HBV showed the same distribution of phenotypes as control and the highest percentage of patients with Hp2-2 were those with CRF. hp^1 and hp^2 allele frequencies were 0.69 and 0.31 in Sudan.

Introduction

Haptoglobin (Hp) is a plasma α2-glycoprotein that binds free haemoglobin (Hb), with a very high affinity of approximately $1x10^{-15}$ mol/l (Okazaki *et al*., 1997), and thus prevents oxidative damage. Synthesized primarily by hepatocytes (Putnam, 1975), induced by cytokines, such as interleukin-6 (IL-6), IL-1 and tumour necrosis factor (TNF) it has three major phenotypes (Hp1-1, Hp2-1 and Hp2-2); encoded by two alleles (hp^1 and hp^2) located on chromosome 16q22 (Bowman, 1993). The frequency of the hp^1 and hp^2 alleles varies worldwide depending on racial origin (e.g. Carter and Worwood, 2007). After release into the circulation, Hp has a half-life of 2–4 days (Garby and Noyes, 1959). The haptoglobin-haemoglobin

(Hp–Hb) complex is rapidly removed from the circulation by the receptor (CD163) which is found on the cell surface of monocytes and macrophages (Kristiansen *et al*., 2001).

Several reports had set conflicting outcomes concerning the association between haptoglobin phenotype and several infectious or/ and non-infectious diseases (e.g. Elagib *et al*., 1998; Carter and Worwood, 2007).

To detect the relationship, if present, between haptoglobin phenotypes and susceptibility to some viral infections and non-infectious diseases prevalent in Sudan, we phenotyped a large number of human plasma/serum samples for Hp. We classified the disease typed here into: viral infections: included infections with hepatitis B (HBV) and the human immunodeficiency virus (HIV/AIDS) and non infectious: including liver cirrhosis (LC) and chronic renal failure (CRF)

Materials and methods

Before conducting this study, an ethical clearance certificate was obtained from the Federal Ministry of Health, Sudan. Patients and healthy control agreed after explanation to participate in this study.

All samples were collected during the period from 2000-2004 in either heparinised or capillary tubes. The collected blood samples were centrifuged at 200g for 10 minutes and plasma collected into cryotubes and kept frozen at -70^{0}C or were kept vertically on benches at room temperature and serum collected and kept as previously described.

HIV/AIDS, HBV and LC samples were collected from the National Health Laboratories, Khartoum State and CRF samples were collected at Khartoum Kidney Dialysis and Treatment Centre, Khartoum State.

Healthy control samples were collected from donors: at the Faculty of Science, University of Khartoum, school children from different parts of Khartoum State and donors from some of the study areas. The polyacrylamide gel electrophoresis separation method (Davis and Ornestin, 1968) as modified by Linke, 1984 (non-reducing gel) was used for phenotyping.

The Statistical Package for Social Sciences (SPSS) was used to estimate the expected percentages of each of the three phenotypes in Sudanese patients. Chi^2-test (χ^2-test) was used for comparisons and whether the sample was in HWE or not was tested.

Results

In this study, we determined the haptoglobin phenotypes present in Sudanese patients with HIV/AIDS (n=129), HBV (n=171), LC (n= 68) and CRF (n=129) in addition to a healthy control panel of 65 individuals.

Expected percentages of each of the three phenotypes in Sudan were calculated using SPSS to be between 51.38-51.49% for Hp1-1, 36.71-36.81% for Hp2-1 and between 11.76-11.91% for Hp2-2. However, the observed frequencies were quite different (Figure 1a and b). Among the healthy control panel only 13.80% were typed as Hp1-1, 64.60% were Hp2-1 and 21.50% were Hp2-2.

Among patients with viral infections, the distribution of the three phenotypes was close to expected, with HIV/AIDS patients showing

The observed allele frequencies were calculated for each phenotype per disease and per control. The expected frequencies under Hardy-Weinberg-Equilibrium (HWE) were also calculated assuming that the two alleles' frequencies are p and q and that the frequencies of the genotypes are p^2, 2pq and q^2. The expected number of individuals with a certain phenotype was also calculated (equals the expected frequency multiplied by the number of individuals in the sample). Pearson Chi^2-test was then performed. The degree of freedom calculated

46.50% Hp1-1, 44.20% Hp2-1 and 9.30% Hp2-2 and patients with HBV showing 49.10% Hp1-1, 40.40% Hp2-1 and 10.50% Hp2-2.

Patients with liver cirrhosis phenotype distribution were: Hp1-1= 64.70%, Hp2-1 =20.60% and Hp2-2=14.70%.

On the contrary, the three phenotypes were equally distributed among patients with CRF (Hp1-1= 39.50%, Hp2-1 = 37.20% and Hp2-2 =23.30%).

When each disease population sample was tested for Hardy-Weinberg equilibrium (HWE) (Table 1), the healthy control, LC and CRF were found significantly deviated from HWE (P=0.05). The haptoglobin alleles' frequencies were calculated for each of the samples (Table 2) using HWE as a null model. A variation among the different samples was observed. Cumulatively, in Sudan, hp^1 allele frequency was 0.69 and hp^2 allele frequency was 0.31.

Discussion

Haptoglobin is an acute phase reactant protein, its plasma levels increase by 200–500% during the 7 days after the onset of inflammation and only slowly return to normal during the 2–4 weeks after the removal of the pathological insult (Gabay and Kushner, 1999).

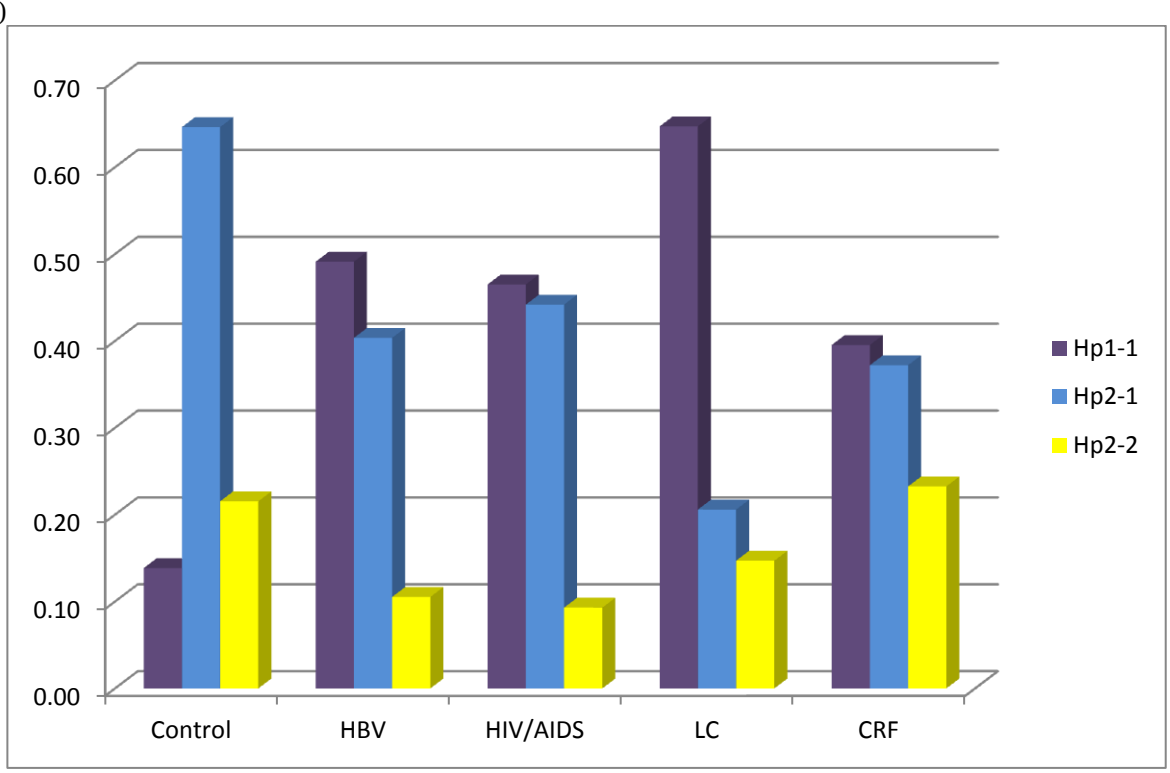
Both hp^1 and hp^2 have been linked to susceptibility to various diseases. Such associations may be explained by functional differences between the subtypes in the binding of Hb and its rate of clearance from the plasma. However, there are also corresponding negative reports for disease associations. Nonetheless, binding of the Hp–Hb complex by macrophage CD163 leads to the release of IL-10, the anti-inflammatory cytokine, and the breakdown products of haem which also have potent anti-inflammatory activity (Moestrup and Moller, 2004). Cytokine IL-10 induces CD163 synthesis and also haem oxygenase-1 via an autocrine mechanism.

In this study, we performed haptoglobin phenotyping for a large sample of Sudanese individuals with a total of 562 including healthy control and patients.

It seems that no correlation is there between the distributions of the three phenotypes among the investigated Sudanese HIV/AIDS patients and HBV. This is in accordance with Delanghe and colleagues (1998).

Haptoglobin phenotype 1-1 was the most prevalent among patients with LC. Hp1-1 has no agglutination effect (Köhler and Prokop, 1978) and is a strong prostaglandin synthesis inhibitor (Langlois and Delanghe, 1996); these functions may explain the observed association. This finding is in accordance with Blenk and Junge (1978) and Zhao and Zhang (1993).

a)



b)

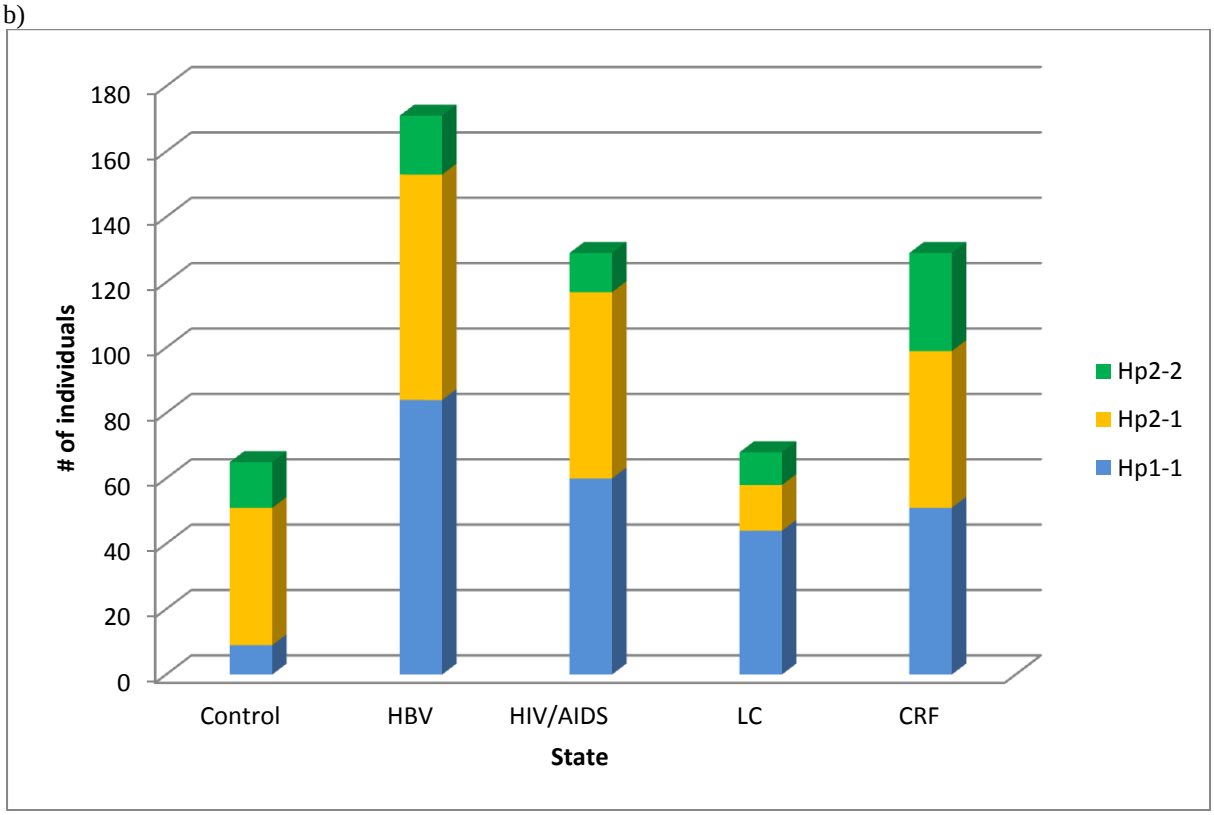


Figure 1 a) Haptoglobin phenotypes frequency among Sudanese healthy control and patients, b) number of individuals with the three phenotypes

Table 1 Testing for Hardy-Weinberg equilibrium (Pearson χ^2-test)

Disease	Sample size	Hp1-1			Hp2-1			Hp2-2			Σ
		Obs.	Exp.	χ^2	Obs.	Exp.	χ^2	Obs.	Exp.	χ^2	
Healthy Control	65	9.00	13.85	1.70	42.00	32.31	2.91	14.00	18.85	1.25	5.85[b]
HBV	171	84.00	82.12	0.04	69.00	72.76	0.19	18.00	16.12	0.22	0.46[a]
HIV/AIDS	129	60.00	60.72	0.01	57.00	55.57	0.04	12.00	12.72	0.04	0.09[a]
LC	68	44.00	38.25	0.86	14.00	25.50	5.19	10.00	4.25	7.78	13.83[b]
CRF	129	51.00	43.60	1.25	48.00	62.79	3.48	30.00	22.60	2.42	7.16[b]

Significance here is decided upon the χ^2 distribution value for df=1 and P=0.05 (χ^2=3.84) (Zar, 1996). [a] not significantly deviated from HWE. [b] significantly deviated from HWE

Abbreviations: HBV: hepatitis B, HIV/AIDS: human immunodeficiency virus/acquired immunodeficiency syndrome, LC: liver cirrhosis, CRF: chronic renal failure

Table 2 Haptoglobin allele frequencies in Sudanese healthy control and patients with infectious and non-infectious diseases

Allele	HC	HBV	HIV/AIDS	LC	CRF
hp1	0.46	0.69	0.69	0.75	0.58
hp2	0.54	0.31	0.31	0.25	0.42

HC= Healthy control, HBV= hepatitis B, LC= liver cirrhosis, CRF= chronic renal failure

Although no preference for a certain phenotype was observed among patients with CRF, Hp2-2 was the most prevalent. This phenotype has less affinity of binding free haemoglobin. Free haemoglobin can damage renal tubules. There are differences also in the binding of haptoglobin types by the CD163 receptor; Hp2-2/Hb complex for example has a 10-fold higher functional affinity for CD163 than the Hp1-1/Hb complex. This may increase the efficiency of the macrophage in clearing the Hp2-2/Hb complexes from the plasma when compared with that of the Hp1-1 (Graversen *et al*., 2002).

The finding of healthy controls, CRF and LC significantly deviated from HWE (P=0.05) raises a question about the cause for this deviation. Deviation from HWE indicates violation of one or more of the models' assumptions (Hartl and Clark, 1997); however, to identify which assumption is not an easy job. The hp^1 allele cumulative frequency in Sudan was found higher than estimated in previous studies (0.69 in this study) compared with 0.54 in Carter and Worwood (2007) whereas hp^2 allele frequency was 0.31 in this study and calculated to be 0.46 from data presented in Carter and Worwood (2007).

Conclusion

In this study, Hp1-1 correlates with LC. No preference for a certain phenotype was observed for HIV/AIDS, HBV. The three phenotypes were equally distributed among patients with CRF.

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