Iron metabolism markers and haptoglobin phenotypes in susceptibility to HSV-1 or/and HSV-2 lesion relapses

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Different haptoglobin (Hp) phenotypes play a role in several pathologic processes including infectious diseases. In order to evaluate the role of iron storage and metabolism in susceptibility to herpetic manifestations, we studied the frequency of the Hp phenotypes and iron metabolism in patients affected by H. Simplex virus 1 or 2 (HSV-1 or HSV-2), compared with controls. Hp phenotype and iron metabolism were determined in 100 patients with recurrent HSV-1 or HSV-2 manifestations during the relapses, and in 110 healthy subjects. The frequencies of the three Hp phenotypes in the patient group compared to the control group were 18% versus 14.5% for Hp 2.1, 25% versus 40% for Hp 2.2 and 57% versus 45.5% for Hp 2.3. All iron metabolism parameters tested showed significant differences between patients and controls; haemoglobin (Hb), ferritin, and serum iron were lower, while transferrin was higher in the patients than in controls. Reductions in iron availability may be a risk factor for relapsing lesions of HSV-1 or HSV-2. Hp 2.2 phenotype may offer some protection against the recurrence of Herpes labialis or genitalis manifestations. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS—haptoglobin phenotypes; HSV-1; HSV-2; iron

INTRODUCTION

During primary Herpes simplex (HSV) infection (HSV-1 or HSV-2), the virions are transported through the neurons in a retrograde manner to the dorsal or cervical neural ganglia, where, after a replicative cycle, latency can be established. 1 Relapsing HSV-1 or HSV-2 infections is due to the reactivation of latent HSV-1 or HSV-2 in previously infected individuals. 1–3 The efficient replication of a DNA virus is strictly dependent on iron since this metal plays a crucial role in the catalytic centre of viral ribonucleotide reductase (RR). Consequently, iron metabolism is an important area for virus/host interaction and some evidences suggest that viral infection is potentially influenced by the iron status of the host. 4,5 It is also interesting to note that during conditions of limited iron availability, HSV-1 or HSV-2 RR is more efficient than the human RR. 6 Human RR is an enzyme present in all cells which catalyses the reduction of ribonucleotides into their corresponding deoxyribonucleotides; this enzyme plays a key role in the synthesis of DNA precursors in all living cells. Some prokaryotes, including Escherichia coli, most eukaryotes and some DNA viruses such as Herpes simplex virus (HSV) possess iron-dependent RR and the HSV RR has a lower Michaelis–Menten Kinetics (Km) for iron compared to human RR. 6,7 Several studies have suggested an association between host iron metabolism alterations and Herpes labialis (HSV-1) and Herpes genitalis (HSV-2) recurrences. In fact, a reduction of serum ferritin and iron levels has been reported in patients during relapsing herpetic lesions. 8,9 Furthermore, an interesting study demonstrated that iron supplementation in patients with history of recrudescent Herpes labialis is able to reduce their recurrence frequencies. 10 In vitro studies suggested that intracellular iron chelation could reduce the efficient...
replication of HSVs. There are many lines of evidence illustrating that iron plays a pivotal role in modulating the battle for survival between mammalian hosts and their pathogens. Each of which displays considerable genetic investment in a wide range of mechanisms for acquiring and maintaining iron. These competitive mechanisms are highly complex, existing within an interacting matrix of absorption, transport, storage and detoxification systems, that are iron-responsive and thus able to adapt to the different phases of infection. Considerable genetic polymorphism in some of these systems, with signals of geographic selection in the hosts, and niche selection in the pathogens, indicates that they are critical for species survival. Only about 5% of daily iron turnover comes from intestinal absorption, mostly comes from Hb turnover, that requires three proteins: haemopexin, Hp, haeme oxygenase. Recently, several new proteins have been described that play critical roles in iron regulation including the master regulator of iron metabolism (hepcidin). Haptoglobin is a plasma protein responsible for the removal of free Hb from the circulation. Hp plasma concentration increases several-fold in response to infection or injury. Following haemolysis, stable Hp–Hb complexes are formed which are delivered to the hepatic parenchymal cells by receptor-mediated endocytosis. In this way, Hp reduces the loss of Hb through the glomerulus, protects against peroxidative kidney injury, and allows the recycling of haeme iron. In humans, Hp is characterized by a genetic polymorphism with three structurally different phenotypes (Hp 1–1, Hp 2–1 and Hp 2–2) which are the result of the expression of two different alleles (Hp1 and Hp2) of the Hp gene located on chromosome 16q22. Also Hp 0 allele, which is an allelic deletion in the Hp gene cluster, has been identified. Although Hp is found in serum of all mammals, this polymorphism exists only in humans. The Hp2 allele contains an intragenic DNA duplication originating from an unequal crossing-over that occurred during evolution after the divergence of man, resulting in a variant α2 polypeptide with two free Cys residues. The additional Cys residue in α2 leads to the formation of polymers in individuals carrying the Hp 2–1 and 2–2 phenotypes. Human Hp phenotypes show an important molecular heterogeneity, Hp 1–1 being a small molecule (86 kDa) of well-defined structure, whereas Hp 2v1 is characterized by heteropolymers (86–300 kDa), and Hp 2–2 forms large macromolecular complexes (170–1000 kDa). The geographic distribution of Hp phenotypes has been under a strong genetic pressure, with the lowest Hp1 allele frequency (0.10) in South-East Asia and the greatest Hp1 frequency (0.80) in indigenous populations of South America. The phenotypic distribution in European populations shows that approximately 15% individuals are Hp 1–1, 50% Hp 2–1, and 35% Hp 2–2, corresponding with a Hp1 allele frequency of approximately 0.40. We focused our attention on Hp because it has a well known polymorphism. Several studies have demonstrated significant correlations between different Hp phenotypes and various infectious diseases, including viral hepatitis and HIV infection. In spite of the large amount of literature on the topic no study were performed to investigate a possible difference between the incidence of Hp phenotypes in patient group with relapses of herpetic manifestations and the incidence of Hp phenotypes in the whole population. On the basis of the above evidence, we evaluated if iron metabolism and a genetic markers of iron recovery and disposal (Hp genotypes) could play a role in relapsing HSV-1 or HSV-2 lesions.

MATERIALS AND METHODS

Patients

One hundred HSV-1 and HSV-2 symptomatic patients (52 females mean age 40 ± 11 years, and 48 males, mean age 39 ± 13 years) during relapsing herpetic labialis or genitalis signs were enrolled in the study. Fifty HSV-1 symptomatic patients (26 females mean age 40 ± 11 years, and 24 males, mean age 39 ± 13 years) during relapsing herpetic labialis signs were enrolled in the study. Fifty HSV-2 symptomatic patients (26 females mean age 40 ± 11 years, and 24 males, mean age 39 ± 13 years) during relapsing herpetic genitalis signs were enrolled in the study. The diagnosis was made by isolation of either HSV-1 or HSV-2 from vesicular fluid or active lesions using primary human fibroblast cultures. All vesicular fluid samples of patient enrolled were heads on primary cultures of fibroblasts. In HSV-1 or HSV-2 positive cultures, fibroblasts showed the cytopathic effects virus-correlated at 3–5 day. The supernatant of cultures with cytopathic effects was collected and the fibroblast HSV-1 or HSV-2 productive virions were confirmed using ELISA (Merck Diagnostics Co., Darmstadt, Germany). HSV-1 or HSV-2 typizations were found using specific monoclonal antibodies against HSV-1 or HSV-2 antigens. All patients enrolled were positive either for HSV-1 than HSV-2 antigens after vesicular fluid samples analysed, regardless of the body district (lip, genitals or other) with herpetic vesicular lesions.

Controls

The control group consisted of 110 asymptomatic subjects (54 females mean age 42 ± 9 years, and 56 males, mean age 41 ± 10 years) without occurrences of HSV-1 or HSV-2 lesion relapses (anamnestically recalled).

Patients and controls

All the subjects tested were seronegative for HIV, HBV and HCV.

Informed consents were obtained from all subjects enrolled in this study. All of the enrolled subjects were Italian and did not have haemoglobinopathies.

Haptoglobin determination

Blood samples from patients with relapsing herpetic lesions were collected during relapses, immediately centrifuged, and serum stored at −70°C. Five millilitres of plasma diluted 1:50 were mixed with 2 ml of 1 M phosphate buffer pH 7.0
and 10 ml of 9 M urea and immediately frozen at –70 °C for 2 h. Before electrophoresis, they were mixed with bromophenol blue and 10 ml of sample buffer 5 × (4 SDS 10% and 2.5 ml Tris-HCl 0.5 M, pH 6.8 and 2 ml glycerol) and, finally, boiled for 5 min.10 Samples were run on an 8% acrylamide/bis-acrylamide gel (30% w/v GIBCO Life Technologies, Gaithersburg, MD, USA), using the buffer system of Laemmli for 45 min at 200 V, then transferred onto nitrocellulose membranes (Hybond ECL, Amersham, Cleveland, Ohio, USA) for 80 min at 50 V, according to the Western blotting procedure.40,61

After overnight saturation in PBS–BSA 10% (phosphate buffered saline–bovine serum albumin), the membranes were washed with PBS–Tween 0.1% and incubated with the primary antibody (mouse anti-human haptoglobin Abs, Sigma–Aldrich, Milano, Italy), diluted 1:4000, for 90 min. Before being exposed to a 1:1000 PBS–Tween dilution of peroxidase conjugated anti-mouse IgG from sheep (Amersham, Cleveland, Ohio, USA), the strips were washed for 30 min with four changes of PBS–TWEEN buffer. The Hp bands were visualized with a chemiluminescence method using ECL reagents (Amersham). The maximum light emission was at 428 nm, detected by a short exposure to blue-light sensitive autoradiography film (Hyperfilm ECL, Amersham).

On SDS–PAGE electrophoretic runs type 1.1 has a single band, the farthest from the origin, and it is estimated to have a molecular weight of 86–100 kDa. Haptoglobin types 2.1 (200 kDa) and 2.2 (400 kDa) appear as a series of bands nearer to the origin (Figure 1). The protein concentrations were determined with BCA reagents (Pierce, Rockford, Illinois, USA), using bovine serum albumin as the standard.

Iron metabolism markers

Blood samples from patients with HSV-1 or HSV-2 lesions were collected during relapses. Serum levels of iron metabolism markers, including Hb, serum iron, ferritin and transferrin were evaluated according to standard laboratory methods.

Statistical analysis

The statistics were performed using SPSS 12.0 for Windows and in particular Hp genotypes in patients and controls were compared by contingency tables (Fisher’s exact test). Serum levels of iron metabolism markers were compared between patients and controls by the ANOVA one way with post hoc Bonferroni correction to look for possible differences among the Hp phenotypes. p-values were considered significant if equal or lower than 0.05.

RESULTS

Frequency of Hp phenotypes

The frequency of Hp 1.1 was 18% in the patient group and 14.5% in the control group (not statistically significant); the frequency of Hp 2.2 was 25% in the patient group and 40% in the control group (p = 0.03); the frequency of Hp 2.1 was 57% in the patient group and 45.5% in the control group (not statistically significant) (Figure 2).

There were no patients or controls with Hp 0.0.

Evaluation of iron metabolism in the patients with relapsing HSV-1 or HSV-2 lesions and in the control subjects

In order to study the possible alterations of martial metabolism, in patients with relapsing HSV-1 or HSV-2 lesions, we determined Hb, serum iron, transferrin, and ferritin levels in the patient group and in the control group. Blood samples of patient group were collected only during HSV-1 or HSV-2 lesion relapses. Data reported in Table 1 showed significantly reduced levels of Hb, serum iron, and ferritin in HSV-1 or HSV-2 patients during the relapsing lesions compared to the controls (normal range values). In contrast to the reduction of the Hb, serum iron, and ferritin concentrations, a significant increase in serum transferrin levels was found in the patients with relapsing HSV-1 or/and HSV-2 signs compared to the asymptomatic controls.

Hp phenotypes and iron metabolism

We analysed ferritin, transferrin, serum iron, and Hb values in the subjects with different Hp phenotypes (Hp 1.1, 2.1, and 2.2), both in the patient group during HSV-1 or/and HSV-2 manifestations and in the asymptomatic control group (Table 2). There were significant alterations in iron metabolism in the patients with HSV-1 or/and HSV-2 relapsing signs, and during the same fallen back one, compared to the control asymptomatic group; a significant decrease in Hb, serum iron, and ferritin levels was found in all of the Hp phenotypes in the HSV infected patients compared to the control group. Levels of Hb were markedly decreased in those patients with Hp 1.1 (14%) and Hp 2.1 (15%) phenotypes. Additionally, decreased ferritin (78% in both the cases) along with increased transferrin was observed in patients with Hp 1.1 (19%) and Hp 2.1 (26%) phenotypes.

DISCUSSION

The initial host–virus interaction in primary HSV infections can lead to the establishment of latency.31,32 After latency is established, a stimulus that can produce viral reactivation...
Haemoglobin 12.0 g/dl
Ferritin 41.5 ng/ml
Transferrin 249 mg/dl
Serum iron 71.5 mg/dl

Haemoglobin 12.6 g/dl
Ferritin 45.0 ng/ml
Transferrin 252 mg/dl
Serum iron 18 ng/ml

Fifty HSV-1 symptomatic patients (26 females mean age 39 ± 13 years) during relapsing herpetic disease due to Herpes labialis or genitalis were enrolled in the study. Fifty HSV-2 symptomatic patients (26 females mean age 40 ± 11 years, and 24 males, mean age 39 ± 13 years) during relapsing herpetic genitalis signs were enrolled in the study. P-values resulted from the one-way ANOVA.

Table 1. Serum levels of iron metabolism markers among patients with relapsing herpetic disease due to Herpes labialis or genitalis compared to controls (100 patients and 110 controls)

<table>
<thead>
<tr>
<th>Markers</th>
<th>Patients (n = 100)</th>
<th>Controls (n = 110)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>12.6 ± 1.0 g/dl</td>
<td>14.4 ± 1.45 g/dl</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum iron</td>
<td>69.5 ± 27 μg/dl</td>
<td>80.75 ± 18 μg/dl</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transferrin</td>
<td>250 ± 44.5 mg/dl</td>
<td>214 ± 40.5 mg/dl</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ferritin</td>
<td>43.25 ± 19 ng/ml</td>
<td>135 ± 47.5 ng/ml</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2. Serum levels of iron metabolism markers in patients with relapsing herpetic infections with HSV1 and HSV2 and controls for each of haptoglobin (HP) phenotypes

<table>
<thead>
<tr>
<th>HP 1.1</th>
<th>Patients</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl⁻¹)</td>
<td>12.3 ± 1.1</td>
<td>14.3 ± 2.3</td>
<td>0.025</td>
</tr>
<tr>
<td>Serum iron (μg/dl⁻¹)</td>
<td>70.0 ± 23.0</td>
<td>83.0 ± 22.0</td>
<td>NS</td>
</tr>
<tr>
<td>Transferrin (mg/dl⁻¹)</td>
<td>310.0 ± 38.0</td>
<td>260.0 ± 40.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Ferritin (ng/ml⁻¹)</td>
<td>23.0 ± 11.0</td>
<td>105.0 ± 50.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HP 2.1</th>
<th>Patients</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl⁻¹)</td>
<td>12.1 ± 1.0</td>
<td>14.3 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum iron (μg/dl⁻¹)</td>
<td>68.0 ± 29.0</td>
<td>80.0 ± 15.0</td>
<td>0.003</td>
</tr>
<tr>
<td>Transferrin (mg/dl⁻¹)</td>
<td>250.0 ± 51.0</td>
<td>198.0 ± 41.0</td>
<td>0.007</td>
</tr>
<tr>
<td>Ferritin (ng/ml⁻¹)</td>
<td>30.0 ± 15.0</td>
<td>135.0 ± 50.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HP 2.2</th>
<th>Patients</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl⁻¹)</td>
<td>13.4 ± 1.1</td>
<td>14.8 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum iron (μg/dl⁻¹)</td>
<td>70.0 ± 28.0</td>
<td>78.0 ± 20.0</td>
<td>0.003</td>
</tr>
<tr>
<td>Transferrin (mg/dl⁻¹)</td>
<td>220.0 ± 32.0</td>
<td>200.0 ± 40.0</td>
<td>0.007</td>
</tr>
<tr>
<td>Ferritin (ng/ml⁻¹)</td>
<td>60.0 ± 25.0</td>
<td>165.0 ± 40.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Statistical analysis was done using the one-way ANOVA with post hoc Bonferroni correction Student’s t-test, comparing the values of corresponding HP phenotypes, in infected and control subjects.

Figure 2. Haptoglobin (Hp) phenotype frequencies as distributed in patients with relapsing herpetic infections and controls. **p-value = 0.03

(e.g. transitory immunosuppression, ultraviolet (UV) rays exposure, stress, hormonal factors, infectious diseases, chromatin structures alterations, etc.) may cause the reactivation of the virus with the appearance of characteristic skin vesicles and mucosal lesions like of the original and primary infection, that we can define like ‘relapsing of HSV-1 or/and HSV-2 lesions or signs’.

The frequency of HSV-1 or/and HSV-2 recurrences varies among individuals. The severity of the primary infection appears to correlate with the frequency of herpetic-1 or/and herpetic-2 manifestation recurrences have more limited numbers of vesicles on either the skin or genital mucosa than primary infections. Some findings suggest that efficient inhibition of MHC class I by HSV is a key factor in its ability to generally occurs in the oral mucosa while HSV-2 infection has a genital and perineal and labial localization. The frequency of HSV-1 or/and HSV-2 recurrences varies among individuals. The severity of the primary infection appears to correlate with the frequency of herpetic-1 or/and herpetic-2 manifestation recurrences have more limited numbers of vesicles on either the skin or genital mucosa than primary infections. Some findings suggest that efficient inhibition of MHC class I by HSV is a key factor in its ability to generally occurs in the oral mucosa while HSV-2 infection has a genital and perineal and labial localization. The frequency of HSV-1 or/and HSV-2 recurrences varies among individuals. The severity of the primary infection appears to correlate with the frequency of herpetic-1 or/and herpetic-2 manifestation recurrences have more limited numbers of vesicles on either the skin or genital mucosa than primary infections. Some findings suggest that efficient inhibition of MHC class I by HSV is a key factor in its ability to
reactivate in humans. HSV-specific CD8+ T cells can block HSV-1 reactivation from latency in ex vivo trigeminal ganglia cultures through the production of interferon gamma (IFN-γ). Pathogens and their hosts share an ambiguous relationship with iron. Viruses have not evolved mechanisms for actively scavenging host iron. Several studies have shown a relationship between martial metabolism and recurrent HSV-1 or/and HSV-2 manifestations. As previously reported, several studies have demonstrated that Hp phenotypes play a role in sparing Hb. This protein expresses three phenotypes with different affinities to bind to Hb. Hp 1.1 has a higher Hb-binding ability than the 2.2 and 2.1 phenotypes and limits Hb elimination by the liver. Differences in Hp phenotypes have been reported in several pathologic conditions. The different affinities of the three Hp phenotypes for Hb are relevant cofactors along with iron deficiency to explain differences in the susceptibility to Herpes virus. Moreover, it has been demonstrated that low levels of iron can decrease immune responses and favour reactivation of HSV. A variety of immunomodulatory effects have been described to Hp. It has been studied more closely for presumed anti-inflammatory activities. Besides its inhibiting effect on cathepsin B, it was shown to decrease neutrophil metabolism and the chemotactic response of monocytes and to inhibit proliferation of stimulated peripheral blood mononuclear cell at supraphysiologic concentrations. There is some indication that heterologous Hp might modulate B cell proliferation and decrease antibody production. Hp has further been shown to bind to cells through CD11b/CD18 and CD22 suggesting that Hp exerts its immunomodulatory effects through receptor-mediated signaling. CD22 mediates B cell interactions with erythrocytes, T lymphocytes, monocytes, neutrophils and endothelial cells by specific binding to glycoproteins with terminal α2-6-linked sialic acid residues. In human blood plasma, many glycoproteins with α2-6-linked sialic acids are present, but only IgM and Hp can selectively bind CD22. Hp is present, albeit not synthesized, in fully differentiated neutrophils, leading to the hypothesis that neutrophil-derived Hp is endocytosed from plasma. It has been demonstrated that Hp binding to activated neutrophils inhibits calcium influx and subsequent generation of reactive oxygen species demonstrating that neutrophil respiratory burst activity can be inhibited by Hp. In addition, it has been reported that Hp suppresses macrophage functions such as lipopolysaccharide-induced production of tumour necrosis factor alpha (TNF-α), proliferation and cytokine production by T cells, and proliferation of B cells. Flow-cytometric analysis has shown that Hp types 1–1, 2–1 and 2–2 bind the cell surface of human B lymphocytes with equal affinity. However, the saturation of CD22 molecules depends on Hp type because of differences in molar Hp concentrations required. Recent observations show that Hp is concentrated within granulocytes and monocytes and is exocytosed after neutrophil activation, suggesting that Hp concentrations may be enhanced locally at sites of inflammation to modulate granulocyte activity. Collectively, these findings indicate that Hp contributes to the re-establishment of homeostasis after local or systemic infection by propagating various anti-inflammatory activities. Recently, Delanghe et al. investigated the role of Hp phenotypes in oxidative stress during HIV infection. HIV infected patients carrying the Hp 2.2 phenotype have a more rapid rate of viral replication and a worse prognosis. They had lower antioxidative activity and higher iron availability in the peripheral tissues compared to patients with phenotypes 2.1 and 1.1. The less favourable prognosis of patients with the 2.2 Hp phenotypes suggests that iron availability may be important during HIV-1 infection. In general, patients with the Hp 2.2 phenotypes have higher ferritin values compared to patients with the Hp 2.1 or 1.1 phenotypes. These differences in ferritin levels could be related to the different affinity of Hp phenotypes for Hb derived from haemolysed red blood cells. This phenomenon could also explain the different patterns of iron storage in various organs. The decreased frequency of the Hp 2.2 phenotype in patients with recurrent HSV-1 and HSV-2 signs suggests that this phenotype may be a protective factor for relapsing herpetic 1 or/and herpetic-2 signs. In our study, the patients carrying the Hp 1.1 allele with relapsing of HSV-1 or/and HSV-2 lesions had lower levels of ferritin, serum iron and Hb, in parallel with higher levels of transferrin than the patients with Hp 2.1 and 2.2 alleles. Decreased iron in patients with HSV-1 or/and HSV-2 manifestations carrying the Hp 1.1 allele may contribute to recurrences of lesions. This is different from HIV where the Hp 2.2 phenotype with the lowest antioxidant activity has the highest mortality. HIV produces a systemic infection with a particular, virus-induced, ferritin storage compartment that predicts mortality, while HSV-1 or/and HSV-2 reactivation and lesions-related occur at localized sites in the oral and genitourinary mucosa. Additionally, HSV replication enzymes are different from those of HIV. In intracellular iron deficiency, HSV primary infection or endogenous virion reactivations induce a compensatory increase in iron uptake with enhanced ferritin synthesis that is insufficient to restore intracellular iron levels. There is no doubt due to the fact that viruses produce limited metabolic machinery except for that required for the replication of their genome. A competition for iron between virus and host enzymes takes place in the cell in which viral replication occurs. On the other hand, DNA viruses are directly dependent on iron for their proliferation as a result of the essential role that iron plays in the catalytic centre of RR. It is encoded in most if not all large DNA viral genomes (e.g. pox and herpes viruses). When iron is limited in the host cells, the viral RR with a higher iron binding capacity permits replication of the Herpes viruses by the sequestration of all of the available iron. HSV-1 is a DNA virus that replicates in the nucleus. Endogenous iron-response protein (IRP1) is active as an RNA-binding...
protein when in an iron-depleted state; when iron-replete, IRP1 is equivalent to aconitase with its 4Fe-4S cluster and cannot bind to RNA. IRP1 binding to ferritin mRNA inhibits translation initiation of this transcript, whereas binding to the mRNA for the transferrin receptor stabilizes this message thereby enhancing receptor synthesis. A large body of evidence links the severity and/or progression of cellular pathogenesis to cellular iron status. However, little is known about possible underlying mechanisms of this linkage. The manipulation of the labile iron pool in mammalian cells alters the progression of one type of cellular pathogenesis.63 Evidence suggests that sideropenia, a condition resulting from a deficiency of iron in the body, is common in individuals with recrudescent \textit{Herpes labialis}. Host response to infection may also play a pathogenetic role. It is well known that human defence against viral infection is related to the development of cell-mediated immunity, specific cellular cytotoxicity and/or combined humoral and cellular immunity. A number of immunological parameters including natural killer cells, macrophages, CD4+ and CD8+ lymphocytes, interferon alpha (IFN-\textalpha) and IFN-\gamma, interleukin-2 (IL-2) and leukocyte migration inhibitory factor are all significant in protection against HSV infection. Interestingly, reduced numbers of natural killer cells and reduced specific lymphokine-activated killer cell activity have previously been shown to be linked to an increased frequency of recurrent genital herpes. Reduced levels of IL-2, IFN-\textalpha and HSV-specific natural killer cell activity have also been described at the time of HSV-1 recurrences.10 Interleukin-6 (IL-6) has been suggested to be involved in the pathogenesis of several diseases. In the hyperthermia- and UV light-induced mouse models of HSV infection, treatment with anti-IL-6 antibodies results in a significantly lower frequency of ocular reactivation compared with that in mice treated with a control immunoglobulin. HSV has been demonstrated to induce a concomitant release of IL-6, thereby disturbing immune homeostasis.66 Therefore, the host’s ability to withstand infection may be impaired by reduced levels of iron. Alternatively, HSV-1 infection may affect iron metabolism in humans.10

In summary, we demonstrate that there were significant differences in marital metabolism including Hb, serum iron, ferritin and transferrin between patients with HSV-1, HSV-2 sign relapses and healthy controls. Levels of Hb and ferritin were reduced in all of the Hp phenotypes but most markedly with the Hp 1.1 and Hp 2.1 phenotypes while transferrin was increased. Reductions in iron availability may be a risk factor for relapsing herpetic lesions regardless to HSV type (1 or 2). Our study also supports the hypothesis that the Hp 2.2 phenotype may offer some protection against the recurrence of \textit{Herpes labialis} or \textit{genitallis} manifestations.

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