Absence of influence of polymorphisms of the MBL2 gene in oral infections by HSV-1 in individuals with HIV

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Abstract

Polymorphisms in the structural gene MBL-2 (mannose-binding lectin-2) may result in low MBL serum concentration, associated with greater susceptibility to infection. The study evaluated the effects of MBL-2 polymorphisms with the oral manifestations of the HSV in human immunodeficiency virus (HIV)-infected patients. An observational case-control study was carried out, with the sample comprising 64 HIV+ and 65 healthy individuals. The signs and symptoms of HSV oral infection were evaluated, and oral mucosa buccal smears were collected. Polymorphisms of the MBL-2 gene and HSV-1 DNA were amplified through real-time PCR. The data revealed that of 64 HIV+, 29.6% presented signs and symptoms of HSV oral infection. Of these, the HSV-1 DNA was detected through real-time PCR in 21% of cases, and in 13.3% of asymptomatic individuals. There was no statistically significant difference between the symptomatic ($p = 1$) and the asymptomatic ($p = 0.52$) individuals, HIV+ and HIV-. Different genotypes (AA, A0, or 00) did not contribute to the oral manifestation of HSV in the HIV+ patients ($p = 0.81$) or HIV- ($p = 0.45$). There was no statistically significant difference in either group ($p = 0.52$). No significant association was identified between the MBL-2 gene polymorphisms in the oral manifestation of HSV infection. However, further studies are recommended with larger population groups before discarding this interrelationship.

Keywords HIV infection · HSV-1 · Mannose-binding lectin · Gene polymorphisms · Oral manifestation

Introduction

Human immunodeficiency virus (HIV) infection is associated with an increased risk of co-infection by the herpes simplex virus (HSV) [1], a viral pathogen that causes significant morbidity, especially in immunosuppressed individuals [2]. HIV causes the depletion of the immune system, making individuals more vulnerable to several infections; symptomatic reactivations and mucocutaneous

shedding of HSV are more frequent and occur in greater numbers in HIV-infected persons [3].

The reactivation of the virus is followed by clinical signs but for laboratory verification, the detection of HSV reactivation depends on the selected population and the technique used for diagnosis. Before the advent of polymerase chain reaction (PCR), the presence of HSV was determined by non-specific tests without detection of virus DNA. Real-time PCR offers advantages over conventional PCR when detecting HSV DNA being more efficient at detecting these viruses [4–6].

Some studies have proven that the reactivation of HSV occurs independently of its clinical manifestations and that while 60% of the world's adult population carries this virus, many have never developed any related disorders [7, 8]. The first defense of the organism against microorganisms such as viruses is the innate immune response. It is known that viruses cross a physico-chemical barrier and activate pattern-recognizing molecules independently of the production of specific antibodies.

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These molecules facilitate the phagocytosis and lysis processes of the invading microorganisms, effectively interconnecting with adaptive immunity (specific immunoglobulins). Mannose-binding lectin (MBL) is one of the most widely researched collectins, and is part of this innate immune system [9]. Mannose-binding lectin captures a great variety of bacteria, fungi, viruses, and parasites via recognition of the repeated patterns of sugars, such as those that occur in the capsules of these pathogens [10].

A number of polymorphic alterations of the *MBL-2* gene, which affect few individuals, cause low serum MBL concentrations, resulting in defects in opsonization [11, 12]. This occurs when one of three substitutions is present in the nucleotides in exon 1 of the *MBL-2* gene: one in codon 54 results in the replacement of glycine by aspartic acid (allele B), one in codon 57 changes glycine to glutamic acid (allele C), and one in codon 52 replaces arginine with cysteine (allele D) [13]. The normal MBL allele is A, while the other three variant alleles are referred to as “0” in literature. When present, even in heterozygotic individuals (i.e., A0), they make the structural subunits of MBL more vulnerable to degradation, reducing the functional capacity of this collectin by a factor of 5–10 times [14].

These genetic variants are also more common in adult patients with recurrent infections than in controls [15]. The prevalence of each *MBL-2* mutation varies in different populations, and it is still possible that a deficiency in the MBL protein confers a selective advantage against certain diseases [9].

The clinical implications of MBL deficiency have been studied in detail, and there has been speculation about the possibility of using it as a therapeutic aid in diseases probably influenced by its deficiency [12, 16].

Harandi et al. [17] suggested that the activation of the complement against HSV is mediated primarily through natural antibodies that recognize specific glycoproteins on the surface of the HSV-1 and HSV-2 envelopes. Nonetheless, what leads an individual to develop clinical signs of HSV infection as vesicles and ulcerations, when the virus is activated, remains unknown.

Low production of MBL is associated with greater susceptibility to and increased severity of various infections, particularly in individuals with a damaged or immature adaptive immune response [9]. The present study evaluated whether there was a relationship between the polymorphism of exon 1 of the *MBL-2* gene and the oral manifestations of HSV in patients HIV+/HIV–.

Materials and methods

This observational case-control study was approved by Research Ethics Committee from Federal University of Pernambuco under protocol no. 194/08. The participants in the study signed an informed consent statement.

A non-probabilistic sample consisted of 129 patients, of whom 64 were HIV+ and 65 HIV–, from the Infectious and Parasitic Diseases Clinic of the Hospital das Clínicas (HC) at the Federal University of Pernambuco (UFPE).

The inclusion criteria were adults patients aged 18–50 years, positive ELISA test for HIV being treated for HIV disease who had not been treated for HSV in the previous six months, and patients seronegative for HIV (negative ELISA test for HIV) and without immunosuppressive systemic disease or have undergone organ transplantation, who had not been treated for HSV in the previous six months.

The patients were initially given information about HSV and its oral manifestations, transmission, and treatment. One researcher recorded the history of the patients to obtain information about the presence or absence of oral herpes based on the information of the clinical characteristics with vesicle presence, ulcerations, pain, and subsequent cicatrization, whether in the present or the past, as well as its location and duration. Patients were submitted to physical examination of the mouth and the presence of vesicles and ulcerations due to HSV infection was observed. Patients who presented lesions clinically compatible with HSV infection were considered symptomatic and when patients declared themselves symptomatic before intraoral clinical examination. The subjects were then divided into four subgroups: HIV+ symptomatic for HSV (19 patients); HIV+ asymptomatic for HSV (45 patients); HIV– symptomatic for HSV (16 patients); and HIV– asymptomatic for HSV (49 patients).

Sample collection

Oral smears were obtained bilaterally by a researcher, including from the tongue apex and the lips in duplicate, with a disposable sterile gynecological cytobrush (Kolplast Comercial e Industrial, Brazil), packed in a microcentrifuge tube with 1 mL of saline solution, and transported to the virology sector of the Immunopathology Keizo Asami Laboratory (LIKA) at UFPE, where they were stored frozen at –20 °C for later analysis.

DNA extraction

The DNA was extracted using a GENECLEAN® DNA purification kit (BIO 101; La Jolla, CA, USA). Briefly, samples were centrifuged at 5.000 rpm for 5 min, the supernatant was discarded, and the pellet was resuspended in 400 µL of NaI. To extract the DNA, 10 µL of *Glass Milk* (an aqueous suspension of fine silica powder) was added and left at room temperature for 5 min with sporadic shaking.

Next samples were centrifuged (1.400 rpm, 5 min) and the supernatant was discarded. Then, 500 µL of *New Wash* (a solution of sodium chloride and EDTA) was added and centrifuged (1.400 rpm, 5 min). This procedure was repeated

twice. The final supernatant was discarded and the remaining pellet was incubated at 55 °C for 5 min in a Cool Block. Nuclease-free water (Promega®; Madison, WI, USA) was then added to homogenize the precipitate. Samples were centrifuged (1,400 rpm, 5 min) and the supernatant containing the extracted DNA was carefully pipetted and transferred to a new microcentrifuge tube and stored at –20 °C until the PCR procedure.

MBL2 polymorphism detection

Exon 1 of *MBL-2* was amplified in a final volume of 25 µL with SYBR Green PCR Master Mix 1× (Applied Biosystems), with 0.5 pmol of the specific forward (5'-AGG CAT CAA CGG CTP CCC A-3') and reverse (5'-AGA ACA GCC CAA CAC GTA CCT-3') primers and approximately 2 µL of genomic template DNA. The reaction was started with denaturation at 95 °C for 2 min followed by 35 cycles of 95 °C for 15 s and 60 °C for 1 min. After the amplification of exon 1, the product melting curve was determined using Rotor Gene™ RG3000 software (Uniscience-Cobert Research).

Single nucleotide polymorphisms (SNPs) at positions –52, –54, and –57 of exon 1 of the *MBL-2* gene were analyzed in real time in the same PCR product using the dissociation curve and grouped as “0” when a mutation occurred in the codon. This protocol began with slow heating from 60 to 95 °C, and the calculations refer to the variation in the emission of fluorophores automatically determined using the Rotor Gene™ RG3000 software (Uniscience-Cobert Research) to generate the dissociation curves to detect the SNPs.

HSV-1 detection

HSV-1 DNA was detected in a final volume of 25 µL with SYBR Green PCR Master Mix 1× (Applied Biosystems) and 1.5 µL each of the specific HSV-1 forward (5'-TGC TGG AGG ATC ACG AGT TTG-3') and reverse (5'-CAT CGT CTT TGT TGG GAA CTT-3') primers, together with 2 µL of DNA. It was then subjected to the same PCR process described above. The presence of viral DNA was determined automatically with the Rotor Gene™ RG3000 software (ver. 6.0).

Data analysis

Data were registered in the SPSS database (Statistical Package for Social Sciences) for Windows, version 20.0. Data were analyzed to evaluate the correlation between MBL2 and oral infection by HSV using Fisher's exact test. The *p* value was 5% and 95% confidence intervals included.

Results

With respect to anamnesis, it was observed that of the 64 HIV-positive patients, 59 (92%) regularly used antiretroviral drugs and 19 (29.7%) reported that they had experienced herpes in the oral cavity. In the HIV-negative group, 16 patients (24.6%) confirmed they had experienced herpes in the oral cavity (Table 1).

After PCR analysis in real time to detect the HSV-1 DNA, it was observed that of the HIV-positive patients symptomatic for HSV, 4/19 (21%) had HSV-1 detected through the analysis of the quantification and the melting curves. Of the asymptomatic patients, this figure was 6/45 (13.3%). Among the negative-HIV patients, such detection occurred in 4/16 (25%) of symptomatic cases and 11/49 (22.4%) of asymptomatic cases (Table 1).

No correlation between the detection of HSV-1 and whether the patient was symptomatic or not was found in either the HIV+ (*p* = 0.66) or HIV– (*p* = 1) groups. No significant statistical difference was observed between the symptomatic (*p* = 1) and the asymptomatic (*p* = 0.52) patients in either group.

From the amplification of the *MBL-2* gene through real-time PCR, it was detected that 10/19 (52.6%) patients who are the HIV positive and symptomatic for herpes group presented the A/A genotype (normal wild), 5/19 (26.3%) the A/0 genotype (heterozygote), and 4/19 (21%) the 0/0 genotype (recessive homozygote). Among the asymptomatic patients from this group, these genotypes occurred in 22/45 (48.8%), 16/45 (35.5%), and 7/45 (15.5%) of cases, respectively. The incidence of the A/A, A/0, and 0/0 genotypes in the group of HIV-negative patients was 33/49 (67.3%), 12/49 (24.4%), and 4/49 (8.1%), respectively, among asymptomatic patients and 8/16 (50%), 6/16 (37.5%), and 2/16 (13%) among symptomatic patients (Table 2).

The different genotypes AA, A0, or 00 found in the *MBL-2* gene were not factors that contributed to the manifestations of herpes in the oral cavity, either in the HIV+ patients (*p* = 0.81) or HIV– patients (*p* = 0.45) groups, with no significant statistical differences between them (*p* = 0.19) (Table 2). In addition, the study revealed that there was no relationship between polymorphisms in the *MBL-2* gene and whether they had HIV+ or HIV– (*p* = 0.29).

In relation to the duration of the herpes manifestations, of the total of 41 patients symptomatic for HSV, 30/41 (73.2%) were able to provide the mean duration in days of such manifestations. Of these, 17/30 (56.6%) reported a cycle of 1 or 2 weeks (of whom 8 were HIV+), and only 5 (16.6%) mentioned a longer cycle than 15 days (HIV+). No association between duration and polymorphic alterations in the *MBL-2* gene (*p* = 0.93) was found. With respect to the HIV+ patients, it was observed that the length of time a patient was HIV positive did not interfere with whether they had already manifested HSV (*p* = 0.3) or not.

Table 1 Distribution of the clinical manifestations of HSV among HIV-positive and HIV-negative patients, and the presence of HSV-1 DNA detected by PCR

	HIV positive (<i>n</i> = 64)		HIV negative (<i>n</i> = 65)	
	Asymptomatic for HSV ^a	Symptomatic for HSV ^b	Asymptomatic for HSV ^a	Symptomatic for HSV ^b
PCR (+) HSV	45 (70.3%) 6/45 (13.3%)	19 (29.6%) 4/19 (21%)	49 (75.3%) 11/49 (22.4%)	16 (24.6%) 4/16 (25%)
PCR (–) HSV	39/45 (86.6%)	15/19 (78.9%)	38/49 (77.5%)	12/16 (75%)

HIV positive: a x b ($p = 0.66$) / HIV negative: a x b ($p = 1$) / a x a ($p = 0.52$) / b x b ($p = 1$)

Discussion

Herpes viruses are usually acquired in childhood or young adulthood, establish a state of asymptomatic latency, and can eventually reactivate, resulting in clinical disease later in life or following an induced decline in cell-mediated immune control. HIV infection is associated with an increased risk for human HSV and related diseases [18].

Mbopi-Keou et al. [19] reported that seropositivity to HSV in people infected by HIV is always greater in individuals who are HIV negative, while Miller et al. [1] added that by using PCR methodology, the viruses belonging to the *Herpesviridae* family are significantly more prevalent in the saliva of HIV-positive than HIV-negative individuals. In contrast, in the present study, the virus was detected in only 15.6% of HIV-positive patients and 23.1% of HIV-negative patients [2].

It should be pointed out, however, that this percentage can vary on a daily basis as the virus is only detected by PCR if the patient undergoes a reactivation at the moment of collection [20]. The variation in the detection of herpes viruses in the oral cavity of different groups could be due to the viral diagnostic methods used, the clinical status of these patients, and the geographical occurrence of these pathogens, as well as climatic conditions, sexual profile, psychological stress, immunosuppression and infectious diseases [21].

One of the factors that might also contribute to the lower incidence of HSV-1 in HIV-positive patients is the fact that 92.2% of these individuals reported the regular use of antiretroviral drugs, while the other patients did not present a clinical indication for the specific treatment. In other words, most

of the HIV-positive patients in this study were at a stage where their infection was probably controlled by medication.

It is worth emphasizing that the present study does not aim to prove the existence or otherwise of infection caused by HSV, but to correlate polymorphic MBL-2 gene alterations in individuals with HIV with clinical manifestations of HSV.

Although the present study involved only one collection of the cells of the buccal mucosa for analysis, it was possible to detect the HSV-1 DNA in 35.7% of patients who had never manifested HSV in the oral cavity (asymptomatic patients of both groups).

Low levels of MBL have been related to its susceptibility in acquiring and manifesting viral infections such as those caused by the influenza A virus [22] and HIV-1 and HIV-2 [9], as well as more severe manifestations of these pathologies [23, 24] and/or bacterial infections [25, 26]. Nonetheless, other studies have stated that the presence of mutating alleles in the MBL-2 gene may offer a selective advantage against intracellular pathogens, such as *Mycobacterium tuberculosis* [27]. The interrelation between deficiency in MBL and a higher incidence of outbreaks of certain infections occurs mainly when the immune adaptive response of these individuals is also damaged or immature, as is the case with newborn babies and very young children, as well as patients who have undergone chemotherapy or transplants [9, 28] or who are immunocompromised for other reasons [29].

Although no significant relationship between the symptomatology of HSV and the presence of polymorphisms of the MBL-2 gene was found, the distribution of the types of the genotypes A/A, A/0, and 0/0 (55%, 31.2%, 13.2%,

Table 2 Distribution of alterations of the MBL-2 gene according to clinical manifestation of HSV

MBL-2 Gene	HIV positive		HIV negative	
	Asymptomatic for HSV (<i>n</i> = 45)	Symptomatic for HSV (<i>n</i> = 19)	Asymptomatic for HSV (<i>n</i> = 49)	Symptomatic for HSV (<i>n</i> = 16)
AA	22 (48.8%)	10 (52.6%)	33 (67.3%)	8 (50%)
A0	16 (35.5%)	5 (26.3%)	12 (24.4%)	6 (37.5%)
00	7 (15.5%)	4 (21%)	4 (8.1%)	2 (13%)

respectively) among the 129 patients studied were similar to a study in Australia with 236 volunteers, where the following prevalences were observed: 57.6% A/A, 34.8% A/O, and 7.6% O/O [30].

There are few articles in the literature that address the relationship between MBL and HSV. According to Christensen et al., [31] there is a possible relationship between HSV and MBL. These authors evaluated the relationship between multiple sclerosis with positive serology for HSV, VZV (varicella zoster virus), and EBV (Epstein Bar virus), and three components which participated in the activation of the lectin pathway of the complement system: MBL, MASP-2, and MASP-3, serine proteases which connect with the MBL protein, causing its activation. The study did not find a significant difference between the presence of anti-HSV, anti-VZV, anti-EBV antibodies and MBL levels, either with patients with multiple sclerosis or in the control group. This study also did not find an interrelation between HSV and the alteration of the MBL-2 gene; however, different study groups were used with the analysis of the gene, rather than the MBL serum levels.

It is understood that the limitations of the sample may have generated this result, preventing it from being conclusive. The present study therefore described an observation from a selected population of cases and a control group and did not find a significant statistical relationship between polymorphic alterations in exon-1 of the MBL-2 gene and the oral manifestations of HSV in HIV-positive patients. However, further studies are recommended with larger population groups before discarding this interrelationship.

Compliance with ethical standards

This observational case-control study was approved by Research Ethics Committee from Federal University of Pernambuco under protocol no. 194/08.

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