Research Report

# Seroprevalence of Herpes Simplex virus types 1 and 2 and their association with CD4 count among HIV-positive patients

**Irna Sufiawati[1], Sunardhi Widyaputra[2],** and **Tony S. Djajakusumah[3]**
[1]Department of Oral Medicine, Faculty of Dentistry, University of Padjadjaran
[2]Department of Oral Pathology, Faculty of Dentistry, University of Padjadjaran
[3]Department of Dermatology & Venereology, Faculty of Medicine, University of Padjadjaran
Bandung - Indonesia

## ABSTRACT

*Background: Herpes simplex virus (HSV) is a common cause of viral opportunistic infections among HIV-positive patients. Frequent, more severe and prolonged episodes of recurrent HSV infection can be a source of significant morbidity and mortality among HIV-positive patients with advanced immunosuppression, reflected by low CD4 count. However, conflicting results have also been reported. Purpose: The aim of this study was to investigate the seroprevalence of HSV type 1 (HSV-1) and type 2 (HSV-2) in HIV-positive patients compare with the rate in HIV-negative patients, and to evaluate their association with CD4 count. Methods: A cross sectional study was conducted among 145 subjects consisting of 80 HIV-positive and 65 HIV-negative patients attending the top referral hospital in Bandung, West Java, Indonesia. The serum obtained was assayed for the presence of HSV-1 and HSV-2 IgG antibodies using ELISA kits. Data were analyzed using a Chi-square test, t-tests and analysis of variance (ANOVA). Results: There were no significant differences in HSV-1 seroprevalence between HIV-positive patients (71%) and HIV-negative patients (66%). HSV-2 seroprevalence was significantly higher in HIV-positive patients (30%) than HIV-negative patients (5%). The titers of HSV-1 IgG antibodies in HIV-positive patients (mean 24.63 ± 19.06 IDU) were significantly lower than those of HIV-negative patients (mean 44.62 ± 33.22 IDU). In contrast, HSV-2 IgG antibody titers in HIV-positive patients (mean 13.31 ± 20.28 IDU) were significantly higher than HIV-negative patients (mean 4.42 ± 10.99 IDU). There was no significant correlation between HSV-1 and HSV-2 seropositivity and CD4 count among HIV-positive patients. However, most of HSV-2 seropositive patients had CD4 count < 200 cells/mm³. Conclusion: Seroprevalence of HSV-1 and HSV-2 among HIV-positive patients was high with no correlation with CD4 count.*

*Key words: HSV, IgG, HIV, CD4*

## ABSTRAK

*Latar belakang: Herpes simplex virus (HSV) adalah penyebab infeksi virus oportunistik yang paling umum pada pasien HIV-positif. Infeksi HSV rekuren yang sering terjadi, lebih berat, dan episode yang berkepanjangan dapat menjadi penyebab morbiditas dan mortalitas yang signifikan pada pasien HIV-positif dengan imunosupresi lanjut, ditandai dengan jumlah CD4 yang rendah. Namun, hasil yang bertentangan juga telah dilaporkan. Tujuan: Penelitian ini bertujuan untuk mengetahui seroprevalensi HSV tipe 1 (HSV-1) dan tipe 2 (HSV-2) pada pasien HIV-positif dibandingkan dengan pasien HIV-negatif, dan untuk mengevaluasi hubungannya dengan jumlah CD4. Metode: Penelitian potong lintang ini dilakukan pada 145 subjek yang terdiri dari 80 pasien HIV-positif dan 65 pasien HIV-negatif yang berkunjung ke rumah sakit pusat rujukan di Bandung, Jawa Barat, Indonesia. Antibodi IgG HSV-1 dan HSV-2 di dalam serum diperiksa dengan menggunakan ELISA. Data dianalisis dengan uji Chi-square, t-test dan ANOVA, nilai p < 0.05 dianggap signifikan secara statistik. Hasil: Seroprevalensi antibodi IgG HSV-1 pada pasien HIV-positif (71%) tidak berbeda secara signifikan dengan pasien HIV-negatif (66%). Namun, seroprevalensi HSV-2 secara signifikan lebih tinggi pada pasien HIV-positif (30%) dibandingkan dengan pasien HIV-negatif (5%). Titer antibodi IgG HSV-1 pada pasien HIV-positif (mean 24.63 ± 19.06 IDU) secara signifikan lebih rendah dibandingkan pasien HV-negatif (mean 44.62 ± 33.22 IDU). Sedangkan, titer antibodi IgG HSV-2 pada pasien HIV-positif (mean 13.31 ± 20.28 IDU) secara signifikan lebih tinggi dibandingkan pasien HIV-negatif (mean 4.42 ± 10.99 IDU). Tidak ada hubungan yang signifikan antara seropositivitas HSV-1 dan -2 dengan jumlah CD4. Namun, sebagian besar pasien*

*seropositif HSV-2 memiliki jumlah CD4 < 200 sel/mm³.* **Kesimpulan:** *Seroprevalensi HSV-1 dan HSV-2 pada pasien HIV-positif adalah tinggi, tetapi tidak berkorelasi dengan jumlah CD4.*

**Kata kunci:** *HSV, IgG, HIV, CD4*

*Correspondence*: Irna Sufiawati, c/o: Departemen Penyakit Mulut, Fakultas Kedokteran Gigi Universitas Padjadjaran. Jl. Sekeloa Selatan No.1 Bandung 40132. E-mail: irnasufiawati@yahoo.com. Fax: +62222532805.

## INTRODUCTION

The increase in the immunocompromised populations due to HIV infection is a factor that can contribute to the change in epidemiology of herpesviruses-associated disease. The hallmark of herpesviruses infection is the ability to establish latent, life-lasting, and periodically reactivating infections in the host. The immunosuppressive state induced by HIV-1 may facilitate herpes viruses' reactivation or re-infection.[1] On the other hand, it is growing evidence that herpevirus infection may increase an pasienal's susceptibility to HIV infection,[2] and could interact with HIV to accelerate disease progression.[3,4] To date, there are eight known human herpes viruses, they are herpes simplex virus type 1 (HSV-1), HSV-2, varicella-zoster virus (VZV), and cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human herpesvirus-6, human herpesvirus-7, and HHV-8 (Kaposi's sarcoma herpes virus).[5] Among herpesviruses family members, HSV is the most common co-infection and pathogenic in HIV-1-positive patients.[6]

Herpes simplex viruses (HSV), an alpha-herpesvirus, are categorized into two types, herpes type 1 (HSV-1) and herpes type 2 (HSV-2). For each virus, the primary mode of transmission is different and there is a tendency to infect different anatomical sites, causing a wide variety of mucocutaneous infections. HSV-1 is mainly localized around the oral region and HSV-2 around the genital region, however it is quite possible to transmit the virus to either region. The prevalence of HSV-1 infection in general populations is high in most geographic areas worldwide ranges from roughly 65% to 90% and has been found to be higher than HSV-2 infection.[7,8] Studies from different parts of the world demonstrated that rates of HSV-1 infection were much higher in HIV-positive patients or with a high risk of HIV than in the general population. The prevalence rates of HSV type 1 (HSV-1) among HIV-1 infected people ranging from 90% to 100%.[9,10]

HSV-2 prevalence is highly variable and depends on many factors, including country and region of residence, population subgroup, sex, and age. Prevalence of HSV-2 infection in the general population in developing Asian countries appears to be lower (10–30%) than developed regions.[11] In the United States, HSV-2 seroprevalence was 16.2%.[12] HSV-2 seroprevalence in Central and South America are estimated at 20% to 60%.[7,11] In Europe, HSV-2 seropositivity varies by region ranging from 4.2% to 23.9%.[13] Sub-Saharan Africa has the highest HSV-2 seroprevalence in the world, reaching 80% in adult population.[7] HSV type 2 affects 50–90% of HIV-1infected people higher than in the general population.[9,11,14] HSV-2 infection reported as a major risk factor for HIV acquisition.[15] A meta-analysis of the association between HSV-2 infection and risk of HIV-1 acquisition reviewed 31 studies have demonstrated that prevalent HSV-2 is associated with a 2- to 4-fold increased risk of HIV-1 acquisition.[16,17] These epidemiological evidence indicated a strong relationship exist between HSV-2 and HIV.

Previous studies have demonstrated that HSV infections are associated with a compromised immune system in HIV-positive patients. Hoots et al.[5] reported that there was a statistically significant association between HSV seropositivity and the degree of immunosuppression, as reflected by cluster difference 4 (CD4) count. Other studies showed that in HIV-positive patients, asymptomatic HSV shedding increases with lower CD4 count.[36,37] Another study also confirmed that risk factors for increased HSV shedding among HIV-positive men were low CD4 cell count.[38] However other studies have shown conflicting results, Santos et al.[39] reported a weak and statistically non-significant association of HSV and CD4 count. Patients with HSV infection can present with severe manifestations even after their CD4 count increases to > 500 cells/mm³.[40] It has been suggested that immune reconstitution inflammatory syndrome (IRIS), usually occurs in individuals with a rapidly rising CD4 count, associated with severe HSV lesions after HAART initiation.

Due to the apparent evolving epidemiological trends of herpesviruses infection in HIV-positive people, this study was conducted to assess the seroprevalence of HSV-1 and HSV-2, and their correlation with CD4 count among HIV-positive patients in Bandung, West Java, Indonesia. Since more herpesviruses infections are asymptomatic, the seroepidemiological studies are critical in understanding the pattern and distribution of infection, which have not been previously investigated among HIV-positive patients in West Java, Indonesia.

## MATERIALS AND METHODS

Data were collected in a cross-sectional study from January until March 2012 in a referral hospital in Bandung, West Java. We recruited 80 patients who were diagnosed as HIV-positive patients. We also enrolled 65 sex and age-matched healthy volunteers as controls. Ethical clearance was obtained from the Institutional Review Board of the

Ethical Committee Hasan Sadikin Hospital and patients gave written informed consent for participation.

Samples of venous blood (5 ml) was drawn from all the enrolled subjects into EDTA blood collection tubes and immediately kept at +4° C. HIV status was confirmed by Enzyme-linked immunosorbent assay (ELISA). *CD4* testing was done using a BD FACSCount™ cytometer. The sera were obtained on the same day and stored at -20° C freezer in aliquots until tested. The presence of IgG antibodies against HSV-1 and HSV-2 were examined using ELISA kits (Indec Diagnostic, Indonesia), in accordance with the manufacturer's instructions. Positive and negative standard sera, accompanying the kit were included in each assay.

Data were entered and analyzed in SPSS 11.0 for windows. Differences in HSV-1 and -2 seropositivity rates among different groups were evaluated using the Chi-square test. Mean titer levels were compared between HIV-positive patients and HIV-negative controls using two sample t-tests. The statistical significance of correlation between HSV-1 and HSV-2 IgG titers with CD4 count was obtained using ANOVA. P-values < 0.05 were considered statistically significant. The mean, median, mode and standard deviation has also been done by using the same software.

## RESULTS

Of all 145 subjects included in the analysis, the HIV group comprised 80 HIV-positive patients (42 male and 38 female), the mean age was $30.8 + 8.3$ years (median 31.5 years, range 1-55). The control group consisted of 65 HIV-negative patients (34 male and 31 female), the mean age was $29.1 + 12.1$ years (median 28 years, range 1-56 years). There was no statistically significant different between HIV-positive patients and HIV-negative control in age (p > 0.05) and gender distributions (p > 0.05). The majority of HIV-positive patients were adequately controlled as determined by CD4 count ranging from (mean $393.4 + 210.8$ cells/mm$^3$) (Table 1).

The results showed that seroprevalence of HSV-1 IgG was found slightly higher in HIV-positive patients (71%) than in HIV-negative patients (63%), however the different was no statistically significant (p > 0.05). While, we found that HSV-2 seroprevalence was significantly higher in HIV-positive than HIV-negative patients (30% vs. 5%, respectively; p < 0.05). *HSV*-1 and-2 IgG antibodies were not found in 34% HIV-negative patients and in 20% HIV-positive patients. Further, we identified that of all subjects there were a number of subjects gave a positive test for both *HSV*-1 and-2 IgG antibodies. Out of 80 HIV-positive patients, 21% of them have both *HSV*-1 and-2 IgG antibodies, significantly higher (p < 0.05) than those of HIV-negative patients (3%) (Figure 1).

Immunoglobulin G antibodies against to HSV-1 are more frequently found in HIV-positive patients with CD4 count > 500 cells/mm$^3$. In contrast, many HIV-positive patients who were HSV-2 seropositive had CD4 count < 200 cells/mm$^3$. In detail, HSV-1 IgG antibodies were found in 13% of HIV-positive patients with CD4 count < 200 cells/mm$^3$, 22% of them have CD4 count ranging from 200 to 349 cells/mm$^3$, 28% of them have CD4 count 350-499 cells/mm$^3$, and many of them (37%) have CD4 count more than 500 cells/mm$^3$. In contrast, only 17% of HIV-positive patients with CD4 count > 500 cells/mm$^3$ had HSV-2 IgG antibodies, 28% of them have CD4 count
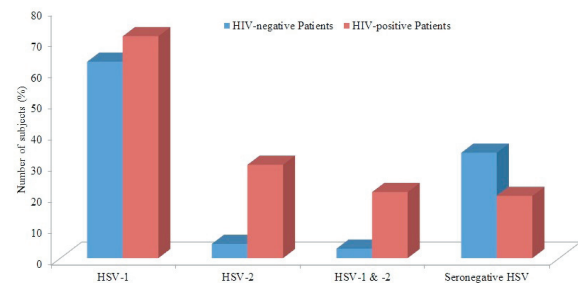


**Figure 1.** Seroprevalence of IgG antibody against HSV-1 and HSV-2 among HIV-positive and HIV-negative patients.

**Table 1.** Characteristics of study participants

| Characteristics | HIV-positive patients (n = 80) | HIV-negative patients (n = 65) | p |
|---|---|---|---|
| Age (yeard old) | | | |
|    Mean ± SD (range) | 29 ± 13 (1-58) | 31 ± 8 (1-55) | 0.24 |
|    Median | 28 | 32 | |
| Gender (%) | | | |
|    Female | 38 | 31 | 0.37 |
|    Male | 42 | 34 | 0.42 |
| CD4 Counts (cell/mm$^3$) | | | |
|    Mean ± SD (range) | 394 ± 209 | ND | – |
|    Median | 393 | | |

Note: n: number of subjects; ND: not determined; *p < 0.05, statistically significant

< 200 cells/mm$^3$, 22% of them have CD4 count 200-349 cells/mm$^3$, and 33% of them have CD4 count 350-499 cells/mm$^3$ (Figure 2). There were no significant correlation between HSV-1 and HSV-2 seropositivity and CD4 count (p > 0.05).

The mean titer of IgG antibodies against both two herpes simplex viruses were statistically *significantly* different compared to HIV-negative control group. The titer of HSV-2 IgG antibodies were detected significantly higher (p < 0.05) in HIV-positive patients compared with HIV-negative patients. In contrast, the titers of HSV-1 IgG antibodies in HIV-positive patients were significantly lower (p < 0.05) than in HIV-negative patients. In addition, Table 2 *showed that* there were no significant correlation between the titer of both HSV-1 and HSV-2 IgG antibodies and CD4 count (p > 0.05). However, when we used post hoc analysis (2-tail p-values for pairwise independent groups t-tests), we found a significant different in the titer of HSV-2 between HIV-positive patients with CD4 T-cell count < 200 cells/mm$^3$ and those patients with CD4 count > 500 cells/mm$^3$ (p = 0.0233).

**DISCUSSION**

To our knowledge, this is the first study to compare the seroprevalence of HSV type 1 and 2 antibodies in HIV-positive and HIV-negative patients in a large public referral hospital serving the urban and surrounding rural area in Bandung, West-Java, the province with the highest burden of HIV in Indonesia. We were particularly interested in determining the seroprevalence of these herpes viruses in HIV-positive patients and their associations with immune
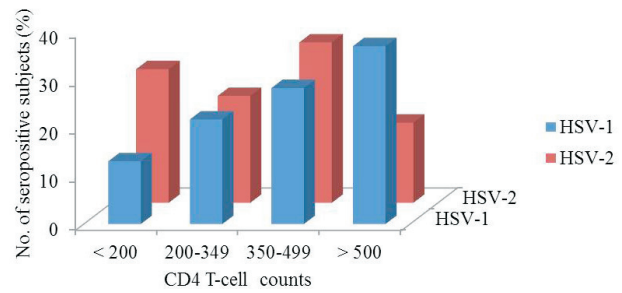


**Figure 2.** Distribution of HSV-1 and HSV-2 seropositive patients by the rate of CD4 count.

status as measured by CD4 count, since there is little known about this. Our study demonstrated that overall IgG antibodies againts HSV-1 and HSV-2 were more prevalent in both HIV-positive than HIV-negative patients (Tabel 1). However, result from statistical analysis showed that only HSV-2 seroprevalence rates were significantly higher in HIV-positive than in HIV negative patients.

In this study, seroprevalence of HSV-1 IgG was found slightly higher in HIV-positive than in HIV negative patients. This results were not much different with other studies that showed the prevalence of HSV-1 infection in general populations worldwide is high ranges from roughly 65% to 90%.[7,8] The high prevalence of HSV-1 clearly shows that most people are infected with some type of infection at least once in their lifetime. Most of them are asymptomatic during the initial stage, they remain undiagnosed for long periods of time or even throughout the life. However, the seroprevalence rates of HSV-1 in HIV-positive patients in our study lower than in the other

**Table 2.** The titers of IgG antibody against HSV-1 and HSV-2 and their correlation with CD4 count

| Virus Type | Virus Titers (IDU) | | | | | p |
|---|---|---|---|---|---|---|
| | HIV-Positive Patients | | | | HIV-Negative Patients | |
| HSV-1 | | | | | | |
| Mean ± SD | 24.63 ± 19.06 | | | | 44.62 ± 33.22 | 0.0000118 |
| Median | 21.8 | | | | 52.6 | |
| HSV-2 | | | | | | |
| Mean ± SD | 13.31 ± 20.28 | | | | 4.42 ± 10.99 | 0.0019 |
| Median | 2.1 | | | | 2 | |
| | CD4 T-cell counts (cells/mm$^3$) | | | | | p |
| | < 200 | 200 – 349 | 350 – 499 | ≥ 500 | | |
| HSV-1 | | | | | | |
| Mean ± SD | 23.14 ± 22.789 | 20.38 ± 17.734 | 21.09 ± 17.734 | 25.69 ± 17.774 | | 0.8013 |
| Median | 13.85 | 17.45 | 22 | 24.1 | | |
| HSV-2 | | | | | | |
| Mean ± SD | 22.76 ± 27.392 | 11.18 ± 18.647 | 13.43 ± 21.351 | 6.12 ± 11.675 | | 0.1665 |
| Median | 7.25 | 1.5 | 2 | 1.8 | | |

Note: SD: standard deviation; ID U (Indec Units); *p < 0.05, statistically significant

epidemiological studies carried out in various countries within the range of 90–100%.[9,10,18] The high prevalence of HSV-1 IgG antibody in HIV-positive patients than in general population is possibly because many of them were acquired HSV-1 through sexual activities. It is well known that HSV-1 is usually acquired orally during childhood via non-sexual contacts, however the virus can be transmitted also through sexual contacts. HSV-2 is the main causative agent of genital herpes worldwide.[19] However, recent data reported that HSV-1 has also emerged as a major causative agent of genital herpes in some developed countries,[20,21] and the increase in the frequency of genital herpes caused by HSV-1 compared with genital HSV-2 infection.[22,23]

The results of this study also showed that HSV-1 IgG seroprevalence was higher than HSV-2 in both groups which is in agreement with the reported results from different regions of the world where the prevalence of HSV-1 prevalence is almost always greater than HSV-2 prevalence.[7] A recent study also reported a higher prevalence rate of IgG antibody against HSV-1 than HSV-2 in both groups.[24] HSV-1 infections usually occur earlier in life. It has been suggested that a high percentage of HSV-2 antibodies because of prior HSV-1 protected from subsequent HSV-2 infection. In developed countries, while childhood acquisition of HSV-1 has decreased, HSV-2 seroprevalence has increased, suggesting the possible protective effect of HSV-1 against HSV-2 infection.[24] A prior infection with HSV-1 has an acquired immune response that confers moderate protection against getting HSV-2, and reduces its severity.[25] A study reported that previous HSV type 1 infection appeared to reduce the risk for acquisition of HSV type 2 infection by 40%.[26] It is not known whether previous genital HSV-1 infection modifies the risk of HSV-2 acquisition more substantially than previous oral HSV-1 infection.[27] However, HSV-1 infections are still at risk of HSV-2 acquisition. There are conflicting results from studies on the risk of HSV-1 positive patients of acquiring HSV-2 that are reported that previous HSV-1 infection does not reduce susceptibility to subsequent genital HSV-2.[28]

Furthermore, our results indicate statistically significant a higher prevalence of HSV-2 IgG in HIV-positive patients when compared to 5% HIV-negative patients. This finding is consistent with most previous studies in other countries. HSV-2 infection is present in 30 to 70% of those in Europe and 50 to 90% of those in Africa among patients with HIV infection.[16] A study reported that the HIV-positive men shed HSV-2 orally more frequently than did the HIV-negative men.[29] However, other studies have identified that genital shedding of HSV-2 is higher in HIV-positive patients.[30] The higher rate of HSV-2 seroprevalence of HSV-2 among HIV-AIDS patients because HSV-2 is transmitted via sexual contact with an HIV-positiveperson. Several studies demonstrated that behavioral and sexually transmitted infection (STIs) as predictors of HSV-2 acquisition.[16,17,31] Primary genital HSV-2 occurring in an HIV-1-infected person is a marker for unsafe sexual practices. Genital ulcer

caused by HSV-2 provides a site for HIV entry on HIV negative patients and the associated inflammation increases the number of activated cells that can be targeted by HIV. In contrast, many HIV-1-infected patients are already infected with HSV-2 at the time of HIV-1 acquisition and having herpes doubled the risk of subsequently catching HIV. Epidemiological studies found that at least a 2- to 4-fold increased risk of acquiring HIV among patients infected with HSV-2,[16,32] and may account for 40–60% of new HIV infections in high HSV-2 prevalence populations.[2,31-33] Susceptibility to HIV is higher among patients who have recently acquired HSV-2. Nonetheless, it also found an increased risk of HIV infection even when herpes infection appeared dormant or was causing no symptoms.[34] The high prevalence and incidence of HSV-2 infection among HIV-positive patients compared with the general population suggests a critical need for screening and preventive programs among this targeted group. This would be of help in prevention of HIV infection.

It has been stated that more frequently virus infections are associated with a compromised immune system in HIV-positive patients. Prior study confirmed that immune-suppressed patients are more vulnerable to common virus infections.[4] Our findings showed many HIV-positive patients who were HSV-1 seropositive had CD4 count more than 500 cells/mm$^3$. In contrast to HSV-1, many HIV-positive patients who were HSV-2 seropositive had CD4 count < 200 cells/mm$^3$. Some studies also reported that there were an association between HSV-2 seropositivity and CD4 count,[35] but others have shown conflicting results.[39] Interestingly, when we analysis of a comparison of IgG antibody titers, the results showed significantly higher titers of IgG antibody against both HSV-1 and HSV-2 in HIV-positive patients compared to HIV-negative patients. We also found a significant different in the titer of HSV-2 between HIV-positive patients with CD4 cell count < 200 cells/mm$^3$ and those patients with CD4 count > 500 cells/mm$^3$. However, we did not find significant correlation between the titer of both herpes viruses and CD4 count. There are some possibilities could explain this finding. First, HSV infection did not modulate the relationship of HIV-1 to CD4$^+$ T cell count suggests that the effect of HSV-2 infection on CD4+ T cell count manifests prior to acquisition of HIV-1.[41] Second, it is suggested that CD8+ T-cells are a critical component of the response to HSV infection but not CD4$^+$ T-cells. The level of anti-HSV antibody did not have any impact on the percentage or absolute number of late-differentiated CD4$^+$ T-cells.[42] In contrast, *HSV*-1 infection reduced the number of infiltrating CD8+ T cells.[43] A study also confirmed that among HIV-positive patients, the frequency of HSV-2 -specific CD-8 T cells is inversely related to HSV-2 severity.[44]

In conclusion, seroprevalence of HSV-1 and HSV-2 among HIV-positive patients was high with no correlation with CD4 count. This study may increase the understanding about the spread of herpes simplex virus and may be valuable for guiding prevention efforts of recurrent herpes

simplex virus disease among HIV-positive patients. In addition, the detection of IgG antibodies against herpes simplex virus may help seropositive people identify symptoms and protect their partners from acquiring HIV, or vice versa, protect HIV-positive patients from acquiring the most common viral opportunistic infection.

## ACKNOWLEDGEMENT

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