

# ORIGINAL ARTICLES

## Presence of Herpes Simplex Virus on the Oral Mucosa in Patients Undergoing Chemotherapy

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### ABSTRACT

#### Background

The aim of this study was to confirm the presence of herpes simplex virus type 1 and 2 on the oral mucosa, in patients undergoing chemotherapy, by means of polymerase chain reaction (PCR).

#### Methods

The research was carried out on 40 patients receiving chemotherapy as treatment for different malignancies. The status of oral mucosa and viral presence were assessed in all patients at the initial examination (prior to chemotherapy), and at the control examination (two weeks after the initiation of the chemotherapeutic cycle).

#### Results

The presence of HSV-1 was detected in 28 patients (70%) prior to chemotherapy, of whom 7 (25%) manifested oral complications. The control examination showed the presence of HSV-1 in 35 patients (87.5%), of whom 23 (65.7%) presented oral mucosa changes. HSV-2 has not been detected in any of the patients.

### Introduction

Patients receiving chemotherapy commonly develop pathological changes in the oral mucosa. Oral mucositis can occur as a result of direct cytotoxic effect of cytostatics on the oral tissue, but also as a result of underlying immunodeficiency. This immune dysfunction is an enhancing factor for opportunistic infections localised in the oral cavity, the agents being bacteria, fungi or viruses. Most frequently, oral mucosa damage consists of exanthemas, vesicles, erosions and ulcerations, usually followed by a subjective stinging and burning sensation and pain.<sup>1,2</sup> What is more important is that the mucosal damage is the port of entry for a great number of pathogens.<sup>3,4</sup> Infections caused by herpes simplex virus are very frequent in immunocompromised patients. However, due to a usually atypical clinical picture, they remain mostly unrecognised or misdiagnosed. Due to diminished resistance, herpes simplex virus can easily cause lesions of the oral mucosa, or worsen the already existing damages caused by the stomatotoxic effect of cancer therapy. There is also a possibility of its systemic dissemination followed by numerous changes in the visceral organs.

Therefore, sensitive laboratory methods aimed at detecting the presence of the virus in various biological specimens within the shortest possible time should be introduced as routine practice.<sup>5,6</sup>

### Objective

The objective of this study was to confirm the presence of herpes simplex virus, type 1 and 2 (HSV-1, -2) by means of polymerase chain reaction (PCR), on the oral mucosa of patients suffering from various forms of malignancies and receiving chemotherapy.

### Methods

The study consisted of 40 patients of both sexes and different age, all undergoing chemotherapy. (Table I).

**Table I Classification of patients according to age, sex and type of malignancy**

Age	Type of malignancy										Σ
	Colorectal CA		Acute leukemia		Chronic leukemia		Breast CA		Head and neck CA		
	m	f	m	f	m	f	m	f	m	f	
19-29			3								3
30-39			2	5					1	1	9
40-49	1		3	3			3				10
50-59	2	1				2			1		6
60-69	5	2		3		1					11
70-79	1										1
Σ	9	3	8	11		3		3	2	1	40

### Clinical study

The initial clinical examination was done prior to chemotherapy, while the control examination was conducted two weeks after the initiation of the therapy cycle. Clinical tests were done at the Department of Haematology, Institute of Internal Diseases, Clinical Centre in Novi Sad, and at the Institute of Oncology in Sremska Kamenica, Serbia. The oral mucosa status was determined according to the mucositis severity criteria of WHO, which is as follows:

grade 0= None  
 grade 1= Soreness +/- erythema, no ulceration  
 grade 2= Ulcers, patient can swallow solid diet  
 grade 3= Ulcers, extensive erythema, patient cannot swallow solid diet  
 grade 4= oral mucositis to the extent that the patient cannot swallow.<sup>7</sup>

#### Laboratory analysis

This part of the research was carried out at the Department of Microbiology and Immunology of the School of Medicine in Belgrade, as well as in the Laboratory for Molecular Biology at the School of Dentistry in Belgrade. In order to establish the presence of HSV-1 and 2, swab samples of all patients were taken from soft tissues of the oral cavity, as well as from lesioned sites. The swab samples were sown in test tubes containing transportation medium (MEM), then each sample was placed in a centrifuge vial, homogenised by vigorous vortex mixing and centrifuged for five minutes at 1200 rpm; the supernatant was poured into sterile test tubes and held at -70°C until processing.

The extraction of potentially present viral DNA was performed by boiling the collected material at 100°C for ten minutes, after which PCR was applied aiming to confirm the presence of specific viral segments. Primers for HSV-1 (**forward 5' -ATA CCG ACG ATA TGC GAC CT** and **reverse 5' - TTA TTG CCG TCA TAG CGC GG**) are specific for the region which encodes a type-specific thymidine kinase. The size of the PCR product is 110-bp. HSV-2 specific primer pair for glycoprotein G (**forward 5' - TCA GCC CAT CCT CCT TCG GCA GTA** and **reverse 5' - GAT CTG GTA CTC GAA TGT CTC CG**) generated a 183-bp PCR product.<sup>8</sup> The reaction mixture in a total volume of 25 µl comprised: 0.2 mM "up-stream" and "down-stream" primers, 10xPCR buffer, 0.2mM deoxyribonucleotide triphosphate mix, 1 unit of *Taq* polymerase (Fermentas), 3 µl of sample. The number of amplification cycles was 35, performed in a thermal cycler (PCR Express, Hybaid). The PCR consisted of an initial denaturation of 3 minutes at 94°C followed by 35 cycles (denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and extension for 3 min at 72°C) and a final extension of 7 minutes at 72°C.

PCR products were run on an 8% polyacrilamide gel, stained with ethidium bromide and visualized on a UV transilluminator (Power Station 300 plus, Labnet International, Inc.).

MANOVA and Roy's *t* test were used for statistical analysis.

#### Results

At the initial examination, 9 out of 40 patients (22.5%) revealed changes in the oral mucosa. Six were suffering from acute leukaemia. The severity of mucositis in 3 patients was marked as grade I, in two patients as grade 2, and in one patient as grade 3. In one patient suffering from chronic leukaemia, pathological conditions of the oral mucosa were observed, and marked as grade 3. At the initial examination, oral mucositis marked as grade I was also observed in a female patient suffering from breast cancer, as well as in one patient suffering from head and neck cancer, graded 2 (Table II). The changes on the oral mucosa presented as paleness, petechial haemorrhage, vesicles and erosions.

**Table II Mucositis severity at the initial clinical examination according to WHO criteria**

	Mucositis severity					Σ
	0	I	II	III	IV	
Acute leukemia	13	3	2	1		19
Chronic leukemia	2			1		3
Colorectal CA	12					12
Brcast CA	2	1				3
Head and neck CA	2		1			3

However, contrary to the relatively infrequent manifestation of pathological changes in the oral mucosa, the presence of the HSV-1 genome in samples obtained from the oral cavity was revealed in a much greater number of patients. HSV-1 was detected in 28 patients (70%), versus 7 (17.5%) patients only, presenting oral complications (Table III).

**Table III Presence of viral genome in samples obtained at the initial examination**

	PCR +	PCR -	Σ
Patients with oral complications	7	2	9
Patients without oral complications	21	10	31
Σ	28	12	40

At control examination, the changes localised on the oral mucosa appeared to be more frequent. Their presence was detected in 26 patients (65%). The occurrence of oral mucositis was mostly found in patients suffering from acute and chronic leukaemia. 17 out of 22 leukaemia patients (77%) had oral mucosa damage at the control examination. The pathological conditions seen on the oral mucosa of these patients were not only more frequent, but they were also more severe than in patients with other malignancies. At the control examination, exanthema, vesicles and erosion of the oral mucosa, as well as ulcerations were observed, while one patient presented

necrosis of the oral mucosa. Oral mucositis marked as grade 2 or 3 was detected in 4 patients with colorectal cancer. In 2 female patients suffering from breast cancer, oral mucositis was scored as grade 1 and grade 2. Changes of grade 1 or 2 in the oral mucosa also appeared in 3 patients with head and neck cancer (Table IV).

**Table IV Mucositis severity at control examination according to WHO criteria**

	Mucositis severity					Σ
	0	I	II	III	IV	
Acute leukemia	5	5	5	3	1	19
Chronic leukemia			1	2		3
Colorectal CA	8	2	2			12
Breast CA	1	1	1			3
Head and neck CA		2	1			3

At control examination the presence of HSV-1 was detected in 35 cases (87.5%), 23 showing oral complications (57.5%). Five patients (12.5%) did not reveal the presence of virus (Table V). None of the samples was positive to HSV-2.

**Table V Presence of viral genome in samples obtained at control examination**

	PCR +	PCR -	Σ
Patients with oral complications	23	3	26
Patients without oral complications	12	2	14
Σ	35	5	40

The differences between the presence of viral genome on initial and control examination were not statistically significant.

## Discussion

Until recently, no attention has been paid to viral infections in patients with malignancies because lesions of viral aetiology present on the oral mucosa of these patients often have an atypical clinical picture, and are consequently misdiagnosed.<sup>5,9,10</sup> Thanks to new, sensitive and specific tests, it has been shown that oral infections of viral origin are frequent in patients suffering from malignancies, especially in patients with haematological malignancies.<sup>6,11,12</sup> Numerous studies point out to frequent infections of viral aetiology (incidence ranging between 50% and 90%), mostly with Herpesviridae, localised in the oropharyngeal region. The studies have demonstrated that oral infections caused by these viruses can have a severe clinical picture.<sup>10,13</sup> The data obtained from available sources are in accordance with the results obtained in our study, which revealed a high incidence of cases positive to HSV-1, both on initial (70.0%) and control examination (87.5%). HSV-1 infection was found in patients with oral lesions but also without any change

on the oral mucosa suggesting that asymptomatic viral shedding is very common among patients with malignancies.

It should be also emphasised that certain malignancies are more prone to viral infections than others. Some studies point to the fact that viral infections localised in the oral cavity are more frequent in patients with acute leukaemia than in patients suffering from other forms of malignancies. Barrett et al estimate that 40% of patients with acute leukaemia have a recurrent herpetic infection in the oral cavity during chemotherapy.<sup>14</sup> Epstein et al presented in their study an even higher percentage of herpetic infections.<sup>15</sup> With almost 80% of HSV-1 positive cases among patients suffering from leukaemia, the results of our research are in agreement with the data from other studies dealing with haematological malignancies. A high rate of HSV-1 detection in oral changes can be attributed to the application of sensitive PCR techniques.

The majority of authors agree on the fact that the greatest number of infections occur owing to reactivation of the latent virus in the host's body.<sup>6,16</sup> Primary herpetic infections in patients with malignancies are extremely rare, since they occur during the early years of life, regardless of the immune status.

## Conclusion

Oral infections caused by herpes simplex viruses in patients undergoing chemotherapy have a much higher occurrence than previously estimated. Immune dysfunction, as a product of primary malignancy and application of chemotherapy, represents an enhancing factor for the reactivation of HSV infection. It can lead to a manifested herpetic infection that affects oral mucosa previously damaged by the cytotoxic action of chemotherapy, but it can also lead to asymptomatic shedding. Individuals with immunodeficiency, together with patients suffering from malignancies, are often at risk of virus dissemination in the body, which in some cases leads to lethal outcome. For that reason, the choice of sensitive, specific and rapid laboratory methods for virus detection is a crucial prerequisite for an adequate therapy administration. By using PCR methodology, minute quantities of viral genome can be confirmed in various types of biological specimens within a very short time, which can occasionally be of crucial importance to the course of the disease and survival of the patient.

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