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## Seroprevalence of Herpes viruses in a retrospective study in Southern Italy

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### Abstract

Information on age- and sex-specific prevalence of herpes viruses (HHV) is of importance to optimize control and prevention strategies. Using data from a population-based serological study in South Italy (n=1854) between 1/01/2016 and 30/06/2017, the main purpose of the present study was to assess the spread of HSV1-2, VZV and CMV seroprevalence in a random population. Results of seroprevalence in South Italy in the last year showed that CMV is the most frequent species with a significantly higher prevalence in women, and with infection rates increasing from childhood to adolescence. In our country, probability of seropositivity for HSV1-2 infections increase with age. A large proportion of teenagers and young adults remain HSV-1-2 susceptible and women were significantly more likely to be HSV1-2 seropositive. Similar to other European countries, varicella immunity in south Italy is acquired gradually, starting in early childhood and showing an increase around age 3–5 years even if the amount of susceptible adults aged over 20 years is still substantial.

**Keywords:** Seroprevalence; Herpes simplex; Varicella Zoster; Cytomegalovirus

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### Introduction

Human Herpes viruses (HHVs) are pathogens affecting 60-95% of the population worldwide with an ubiquitous distribution even if a great importance has been identified with geographic location, socioeconomic status and age to value the primary infection's acquisition. Primary infection is followed by a latency phase in the host and by possible periodical reactivated infections. During latent infections, viruses involve different tissues concerning viral species interested: sensor ganglia for HSV1, HSV2 and VZV; lymphocytes, monocytes and epithelium for EBV, CMV and HSV6-8. HHVs have been also linked to the development of chronic disorder as Alzheimer's disease, cardiovascular disease and depression.

There are two types of HSV, HSV1 and HSV2, transmitted by direct contact with infected secretions. HSV1 is transmitted by direct contact with infected secretions, localized predominantly in orolabial ulceration even if many infections remain asymptomatic and, therefore, so undiagnosed. Typically, clinical lesions occur after a primary infection. Seroprevalence rates of HSV1 varied from 50-85% or more in adults from developed country in Europe, while, epidemiological studies, in Italy,

showed a seroprevalence rates of 93% in the adult population [1]. HSV-2 is transmitted sexually and is one of the most common genital diseases, affecting adolescents and adults and facilitating HIV transmission. The circulation of HSV-2 is more limited, and is generally, associated with other sexually transmitted diseases affecting about 5% of the adult population in Italy [2-5]. Monitoring the epidemiology of HSV1-2 infections is of importance for the prevention of neonatal herpes and associated diseases and for determining antibody prevalence in the general population.

As regards primary varicella infection, this is considered a benign common illness of child with a life-long immunity that, in later

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life, could reactivate and cause herpes zoster. Although mortality is rare, varicella is more severe in old ages, pregnant women, neonates and immunocompromised individuals [6-8]. A live attenuated vaccine was developed in 1970s and an evaluation of vaccination plans in the United States reported a decrease in disease, hospitalisation and mortality but the age of infection increased with an increase of severe cases. Routine vaccination against VZV has been recently introduced in Italy recently as a national immunization programme, while was only recommended for non-immunized individual in the past. So there isn't an evaluation of its efficacy and we need epidemiological data to identify risk associated with VZV seronegativity. It's important to consider that seropositivity in adult population and high risk of infection in young children is typical in Italy such as other countries with temperate climate [9].

Human cytomegalovirus (CMV) is a highly prevalent herpes virus that infect 30-100% of population worldwide. Even if it is considered a benign infection, sometimes it causes serious diseases in immunocompromised patients and pregnant women with an active infection, but also in healthy people playing an important role in aging of the immune system. It is the most common congenital infection transmitted by maternal infection or reactivation. Early childhood infection is asymptomatic or causes a flu-like symptoms. Seroprevalence is around 40-50 % among white populations and higher about 80-100% in some non-white populations. [10,11]

To update the epidemiology of HHV infections in southern Italy, an epidemiological study was carried out to determine the seroprevalence of HSV1-2, VZV, CMV among a general population who asked for hospital care to the University Hospital Vanvitelli in the last past year.

## Materials and Methods

### Study design

A seroprevalence retrospective study from a general population was carried out using sera collected from patients of all ages (1-70 years) hospitalized at the University Hospital Vanvitelli in Naples Italy for different causes. During a period from 1/01/2016 to 30/06/2017, 1854 patients's sera were analysed for detection of type-specific HSV1-2 antibodies using Elisa (Kit Institut Virion/Serion GmbH Ref ESR 105G-105M), CMV antibodies using enzyme immunoassay Biomerieux Vidas, IgG Ref 30204-IgM REF 30205), VZV antibodies using chemiluminescence immunoassay (Gemini Combo Alifax, IgG REF ESR 04 – IgM REF ESR 104) following manufacturer's instructions.

Seropositivity was defined as follows according to the provider of the assay: VZV IgG measurement lower than 50 U/μl should be considered uninfected, while samples with measurement greater or equal than 100U/μl should be classified as infected; HSV1 IgG lower than 20 U/μl uninfected while greater or equal than 30 U/μl infected; CMV IgG lower than 0,7 U/μl uninfected, greater or equal than 0,90 U/μl infected.

### Statistical Data

Seroprevalences by age-group were tabulated with 95%

confidence interval (CI) and comparison of differences between seropositive and seronegative subjects was performed using the Chi-squared test ( $\chi^2$ ). Odds ratios (OR) were calculated with multiple logistic regression analysis of risk factors age and gender identified from the literature as potential risk factors for seroprevalence. Data were analysed using using Statistic Toolbox in the Matlab Environment.

## Results

The seroprevalence of IgG antibodies towards three of common human herpes viruses were respectively investigated. Population characteristics related to HSV1-2, VZV, CMV seroprevalence were reported in **Table 1**. Within total group of 1854 patients, 1149 were analyzed for HSV1-2, 895 for VZV and 1529 for CMV. The results for HSV1-2 antibodies detection were reported in **Table 1**. 762 patients were seropositive (66%) and 387 were seronegative for HSV1-2 with the mean age of 32.4 and 31.4 respectively. As reported in **Table 2** with the HSV1-2 seropositive men were 341 (45%) and women 421 (55%) with a mean age of 32.3 and 32.5 respectively while a mean age of seronegative men was 28.6 and 35.1 for women. Difference in mean age between seropositive and seronegative men was statistically significant at 95% level

**Table 1** Herpes virus seroprevalence.

	Virus IgG antibodies		
	HSV	VZV	CMV
Npositive/Ntotal	762/1149	668/895	1098/1529
% (Npositive/Ntotal )	66.32	74.75	71.81
95% confidence interval (Npositive/Ntotal )	66.19-66.45	74.57-74.93	71.71-71.91
Age (IgGpositive) mean ± SD	32.47 ± 23.50	32.88 ± 23.45	31.75 ± 23.47
Age (IgGnegative) mean ± SD	31.41 ± 23.01	30.64 ± 22.74	32.21 ± 23.35
p-value for difference in mean age	0.47	0.21	0.73

**Table 2** Relationship between presence of herpes virus IgG antibodies, and age and sex.

	HSV VZV CMV		
	HSV	VZV	CMV
IgG positive women n %	421 (55.2)	373 (55.7)	595 (54.1)
IgG positive men n %	341 (44.7)	295 (44.1)	503 (45.8)
Age IgGpositive M mean ± SD	32.35 ± 23.67	32.35 ± 23.87	30.51 ± 23.01
Age IgGpositive W mean ± SD	32.56 ± 23.38	33.30 ± 23.13	32.80 ± 23.82
p-value for difference in mean age	0.9	0.6	0.1
Age IgGnegative M mean ± SD	28.62 ± 22.32	30.75 ± 22.17	32.47 ± 22.97
Age IgGnegative W Mean ± SD	35.16 ± 23.46	30.50 ± 23.58	31.29 ± 23.81
p-value for difference in mean age	0.006	0.93	0.8

and we found the same results for women. As shown in **Table 3**, in our population seroprevalence for HSV1-2 increase from childhood (66%) to adolescence (69%) because children showed antibodies against HSV1-2 or for infection or for antibodies passively acquired from maternal immunity and decrease in adults  $\geq 20$  (50%). The decrease trend in seropositivity for HSV1-2, above all in adults is due to the improvement of prevention as already observed in other countries. Furthermore, as shown in **Table 4**. In table 4 the multiple logistic regression demonstrated that, for HSV1-2, gender was statistically significant while lower significance was associated with age. Collected data showed that women are more susceptible to HSV1-2 infections, predominantly as genital infection, because mucosal surfaces of external genitalia are more vulnerable even if in this study we didn't distinguish orolabial infections from genital infections.

The results for VZV antibodies detection were reported in **Table 1**. 668 were seropositive (74,7%) and 227 seronegative for VZV with a mean age of 32.8 and 30.6 respectively. Among 668 seropositive VZV patients, 295 (44%) were men and 373 (56%) were women with a mean age of 32.3 and 33.3 years respectively while between seronegative patients the mean age was in 30.7 and 30.5 respectively showing not significant differences in mean ages as reported in **Table 2**. We could notice that seropositivity is higher between women than between men even if the mean age of seropositivity is comparable. As shown in **Table 3** the vast majority acquisition of VZV antibodies was observed in children showing a seropositivity of 74.3, 72.6, and 50% respectively. In contrast with previous studies in which Nardone et al. 2007 reported that in all countries studied over 50% of children 0-5 years and 90% adolescents 10-15 were seropositive for VZV except in Italy where was reported only 38% and 78% respectively, in our study we showed an early immunization of young population maybe due to better vaccination programmes as shown well in tab. 4 in which we demonstrated with multiple logistic regression that age was important for acquiring infection whereas gender was less significant.

A larger number of patients was tested for CMV from our population. As reported in **Table 1**, between all patients analysed for CMV infection 1098 were seropositive (71%) and 431 were seronegative with a mean age of 31.8 and 32.2 years respectively. We noticed, as showed in **Table 2**, that on 1098 seropositive patients, 503 (46%) were men and 595 (54%) were women with a mean age of 30.5 and 32.8 respectively while seronegative patients men had a mean age of 32.5 and women of 31.3 years. Also in the case of CMVs seropositivity is higher between women than in men even if the mean age of seropositivity is comparable for both. As reported in **Table 3**, seroprevalence was higher in children from 1 to 9 years and adolescent between 10 and 19 years decreased in adults 72.72 and 50% respectively, showing that in our region the first infection for CMV was age-dependent. As shown in **Table 4**, the multiple logistic regression demonstrated that, for CMV, age was not statistically significant, while gender was significant for acquiring infection.

We observed, moreover, that 665/1854 patients were tested for all three viruses and 257 were seropositive for all three, 187 for two of them, 100 for only one and 100 seronegative. As shown in **Table 5a**, the multiple logistic regression demonstrated that, for them, age was statistically significant, with a  $p$ -value  $< .0001$ .

**Table 3** Seroprevalence of HSV1-2, VZV, CMV by demographic variable.  $p$ -value is  $<0.0001$  for each virus separately.

	HSV1-2	VZV	CMV
Age	% (95%CI)	% (95%CI)	% (95%CI)
Children (1-9)	65.8	74.3	72.6
	(65.3-66.4)	(73.5-75.1)	(72.2-73.1)
Adolescent (10-19)	68.5	72.6	72.4
	(67.6-69.3)	(71.5-73.6)	(71.8-73.1)
Adult ( $\geq 20$ )	50	50	50
	(49.8-50.1)	(49.8-50.2)	(49.9-50.1)

**Table 4** Multiple logistic regression of two risk factors for herpes simplex virus, varicella zoster virus and cytomegalovirus.

	HSV1-2		VZV		CMV	
	OR (95%CI)	p-value	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	1 (0.9-1)	0.8	1.1(1.06-1.1)	$<.0001$	1 (0.9-1)	0.69
Gender	1.5 (1.3-1.8)	$<0.0001$	0.8 (0.6-1.2)		0.75 (1.06-0.9)	0.01

**Table 5** Multiple logistic regression and multiple Poisson Regression Analysis of two risk factors for herpes simplex virus, varicella zoster virus and cytomegalovirus.

	HSV1-2; VZV		HSV1-2; VZV	
	CMV		CMV	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	1.07 (1.06-1.08)	$<.0001$	1 (0.99-1.01)	0.98
Gender	0.82 (10.56-1.22)	0.33	0.87 (0.78-0.97)	$<.0001$

A final analysis about these selected patients was made considering a multiple Poisson Regression Analysis, in order to establish the statistical significance of how many different kinds of seropositivity (HSV1-2, VZV, CMV) were acquired by the patients vs. the gender and the age. The results in **Table 5b**, show that gender is now the more significant risk factor.

## Discussion

This study is the first, to our knowledge, to report the comparative seroepidemiology among HHV in Southern Italy. Few data are available on HSV1-2, VZV, CMV seroprevalence in Italy. The main importance of this study is that the detection of specific antibodies against HHV was conducted on samples randomly collected from a general population among both female and male of different age and not in subgroups even if some age groups are numerically different depending on the subjects coming to the Hospital.

Previous studies in Europe and USA reported that women have a high seropositivity against HSV1-2 than men probably because they are more biologically susceptible to HSV infections [12-14]. We found females had a higher risk of HSV1-2 infection compared to males in South Italy agreeing with previous studies in other countries. This may reflect the differential role of gender on clinical presentation, with men more likely to have asymptomatic HSV1-2 infection and could result in higher rates of male to female transmission [15-17].

Similar to other countries in Europe, varicella immunity in Italy is acquired gradually starting in early age, showing an increase around 3-5 years because in this age they are increasing possibilities for exposure and at about six years, they are already immune at school entry. Studies demonstrated that in 11 European countries with a temperate climate, VZV seroprevalence shows rapid increase during the first decade of life, while in tropical and subtropical areas, varicella affect mainly adolescents and adults [18-20]. Over than 90% of children were seropositive for VZV at 15 years with a lower proportions of seropositive individual at 5-years-old children in Italy (38%) and in Poland (48%) and a highest proportion in Netherlands (95-97%). The wide variation in transmissibility observed in different European countries is due to varying population densities, social mixing, climate differences. In our study, the proportion of immunized patients increase by age even if the immunity gained through vaccination is not considered to affect the results of our study since so far the vaccine was not widely used.

CMV prevalence increases gradually with age so that at old age the majority of people in South Italy are infected and with a higher females compared to males. The increasing age associated with increased seroprevalence for proportion of CMV is in line with reports of a significant rate of seroconversion in adults [21]. We noticed a higher incidence of infection in adult women than men. Furthermore, the increasing seroprevalence by age

is in contrast with VZV for which over 50% of the population is infected at young age but is comparable with HSV1-2 with a slow increase age-specific seroprevalence. The main implication is that the majority of CMV infections is not only caused by transmission among children after primary infection, but also by older subjects going through one or more reactivation episodes. A corollary is that persistence of CMV in the population is not possible with transmission from primary infected persons only, and is dependent on infectious reactivation.

Our sampling is subject to a selection bias because samples were collected from people seeking medical helps, limiting generalization of results. Convenience of this kind of study is less costly, time-consuming and render easier the process to obtain samples. To increase study validity, we analyzed samples coming from a big hospital that reaches patients from all south Italy to receive both primary and hospital care. A high proportion of susceptible individuals in certain age of groups detected in our study, underlines a need to revise vaccine recommendations and to consider the need of a screening above all between non-immune women before pregnancy but also between all susceptible individuals because at older ages there is an increase in the risk of complications.

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