Journal of Oral Research

ORIGINAL ARTICLE

Analysis of risk factors associated to cytomegalovirus infection in dentistry students

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Receipt: 05/20/2015 **Revised:** 06/08/2015
Acceptance: 06/15/2015 **Online:** 06/15/2015

Abstract: Objective: The purpose of the study was to analyze the association between cytomegalovirus (CMV) infection in dental students with occupational risk factors and a genetic trait (NKG2C gene deletion). Study design: Case-control study. 176 students were included and divided in two groups according to CMV serological results: those with CMV infection (case group) and those without prior infection (control group). Demographic, occupational, and the presence of NKG2C gene deletion were compared between both groups. Results: The presence of CMV IgG antibodies was detected in 104 (59.1%) students (case group) while 72 (40.9%) students were CMV negative (control group). The frequency of patient contact, the use of protective barriers, and the number of reported accidents was compared between the study groups; no significant differences were noted. The appropriate use of infection-control measures was observed in the majority of students in both study groups. In the case group the frequency of NKG2C deletion was 9.7% compared to 5.6% in the control group (p=0.33). Conclusion: No association between the presence of CMV infection with occupational and genetic risk factors was found in this population. Dentists should be aware of the CMV prevalence and risks factors associated to this infection, particularly among child-bearing age dentist women.

Keywords: *Cytomegalovirus; NKG2C; NK cells; occupational risk factors.*
DOI: *10.17126/joralres.2015.039*

Cite as: *De la Tejera-Hernández C, Noyola D, Sánchez-Vargas L, Nava-Zárate N, De La Cruz-Mendoza E, Gómez-Hernández A & Aranda-Romo S. Analysis of risk factors associated to cytomegalovirus infection in dentistry students. J Oral Res 2015; 4(3): 197-204.*

INTRODUCTION.

Cytomegalovirus (CMV) is a DNA virus that belongs to the Herpesviridae family and betaherpesvirinae subfamily[1]. Innate immunity plays a fundamental role in the response to viral pathogens. A subpopulation of NK cells that express the NKG2C receptor has been reported to expand in individuals after CMV infection[2]. Although the impact of the expansion of this cell population has not been established, it has been suggested that these cells may play a role in infection control. Of note, a significant proportion of the population lacks the gene that encodes for this receptor either as a heterozygous or homozygous trait. Given the fact that the deletion of this gene (even in heterozygosis) is associated with a lower number of NKG2C positive cells, it is possible that this trait could be a risk factor for the acquisition or control of viral infections[3].

CMV is frequently excreted in saliva, particularly in children and individuals who suffer from significant immune suppression[4]. However, 11 to 33.5% of the healthy population can also excrete this virus[5]. Therefore, dentists may be at higher risk of CMV infection due to frequent contact with saliva of infected persons. Primary infections that occur during pregnancy are of particular concern since viremia occurs frequently during acute infection and may lead to transplacental transmission of

the virus causing congenital CMV infection. Although most infected infants are asymptomatic, 10% of affected children will present with diverse symptoms such as intrauterine growth restriction, microcephaly, hepatosplenomegaly, petechiae, jaundice, chorioretinitis, thrombocytopenia, and anemia[6]. In addition, there is a significant risk for the development of neurologic sequelae, including intellectual disabilities, hearing loss, and/or visual impairment; these may occur even in asymptomatic newborns[6].

Several studies have highlighted the occupational risks associated to dental practice. The risks of infections caused by blood-borne pathogens (hepatitis B virus, HIV, and hepatitis C virus) have been studied extensively[7-9]. Another group of ubiquitous pathogens which are also important for health-care workers are herpes viruses[10]. Nevertheless, there are very few studies that evaluate the risk of transmission of CMV among dentists, especially in Mexico.

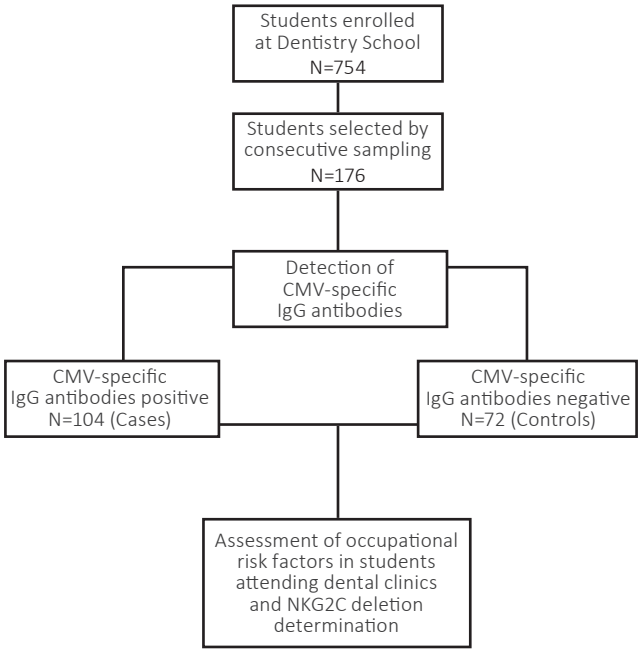
The objective of this study was to analyze the association between occupational risk factors and a genetic trait (NKG2C gene deletion) with CMV infection in a population of dentistry students. The hypothesis was that CMV infection would be more frequent in students with occupational risk factors or those with deletion of the NKG2C gene.

MATERIALS AND METHODS.

Subjects and samples.

This study was carried out at the Dentistry School (Universidad Autónoma de San Luis Potosí). The study protocol was reviewed and approved by the Ethics Committee. Students of all academic years were invited to participate, the objectives of the study as well as information regarding CMV infection were explained to them, and signed informed consent was obtained. The study was designed as a case control study. Subjects were enrolled between March 5th and March 20th, 2013. 176 students were included and divided in two groups according to CMV serological results: those with CMV infection (case group) and those without prior infection (control group). Demographic and occupational information was obtained through a brief questionnaire. A 6ml blood sample was obtained by venipuncture for determination of serological status and the presence of NKG2C gene deletion. (Figure 1).

Figure 1. Study design and sample selection.



Anti-CMV antibody determination.

Blood samples were centrifuged and serum separated and stored at -20°C until the antibody detection assay was carried out. CMV-specific IgG antibodies were determined using a solid-phase, sequential chemiluminescent enzyme immunoassay (IMMULITE 1000 CMV IgG assay; Siemens Healthcare Diagnostic Products GmbH, Marburg, Germany). Positive and negative controls were included in every assay. In this assay results from controls are used to establish a cutoff value. Emission detected from subjects samples are divided by the cutoff value to obtain an index which is interpreted as follows: a ratio >1.1 is considered as a reactive result; a ratio <0.9 is considered as negative; samples with ratios between 0.9 and 1.1 are considered indeterminate and were retested. Calculation and reporting of qualitative results (reactive/nonreactive/indeterminate) is handled automatically by

the IMMULITE/1000 system.

Determination of NKG2C gene copy number.

Genomic DNA was extracted from blood samples as previously described[11]. Assessment of the copy number of the NKG2C gene was carried out using PCR SSP analysis using previously reported primers as described by Moraru *et al.*[12]. This assay uses two sets of primers: a set that amplifies a 411-bp fragment when the NKG2C gene is absent and another set of primers that amplifies a 201-bp fragment of the NKG2C gene. The PCR conditions used were those described by Rangel-Ramírez *et al.*[13].

Statistical analysis. Demographical and clinical variables, as well as occupational risk factors were compared between study groups. Categorical variables were compared using Fisher's exact test or the chi-squared test while continuous variables were compared using Students t test. Odds ratios and 95% confidence intervals were calculated. Statistical analyses were carried our using SPSS 14.00 for Windows (IBM, USA) and OpenEpi (OpenEpi, USA).

RESULTS.

Demographic characteristics.

At the time there were 754 undergraduate students enrolled at the Dentistry School. A total of 176 dental students were included in the study by consecutive sampling; 127 (72%) females and 49 (28%) males. The mean age of study participants was 21.5 years (SD, 3.18). The frequency of students per academic grade (the professional study program is divided into 5 grades, one per school year) was as follows: 62 (35%) of the students were in 1st grade of college, 20 (12.5%) in 2nd grade, 29 (14%) in 3rd grade, 15 (10%) in 4th grade, and 50 (28.4%) in 5th grade. Students in 1st and 2nd grades do not attend dental clinics (preclinical group students) while those in 3rd, 4th, and 5th grade perform clinical practices (clinical group students).

Frequency of CMV-specific antibodies.

Blood samples were evaluated to detect the presence of CMV-specific antibodies. Positivity for CMV antibodies was detected in 104 (59.1%) while 72 (40.9%) were negative. There was no difference in age between CMV positive and CMV negative students (mean 21.59 years (SD, 3.37) and 21.39 years (SD, 2.89), respectively; p=0.69). Seventy-five percent of CMV positive students were female while this proportion was 68% among those with negative results (p=0.31). In addition, no significant difference in the frequency of CMV antibodies was observed between the preclinical student group (61%) and those in the clinic group (57.4%; p=0.6). Based on these results the study participants were categorized in two groups: cases (positive for CMV-specific antibodies) and controls (negative for CMV-specific antibodies).

Occupational risks factors and CMV infection in dental students.

The use of barrier techniques to prevent transmission of infection was assessed only in students that were already participating in clinical practices; the use of gloves, masks, safety goggles, protective face shield, and long sleeve coat was evaluated. Also, information regarding the accidents that had occurred during the use of dental instruments was collected. The most commonly reported accident was the puncture with the dental explorer, followed by the accidental puncture with the dental syringe at the moment of application of anesthetics. Comparisons of the occupational findings among case and control students that are already participating in clinical practices are shown in Table 1. When evaluating the frequency of accidents, it was observed that the number of reported accidents was higher in students who had been involved in dental practices for a longer period of time: 79.3% of students in the 3rd grade mentioned to have suffered some accident during dental practice and the frequency increased to 96% in the students in the 5th grade. However, there was no difference in the presence of CMV infection in relation to the number of accidents. The number of accidents per college grade is shown in Table 2. In regards to infection-control measures used during clinical practice, there were no significant differences between the two groups. All students wore gloves and surgical masks routinely. However, not all students used other preventive measures systematically. For ins-

Table 1. Characteristics of students attending clinics at the dentistry school according to CMV infection status.

Characteristics	Cases (CMV +) N= 54	Controls (CMV -) N= 40	OR	95% CI
History of occupational accidents	48 (88.9)*	38 (95)*	0.42	0.08-2.2
Number of accidents				
1-5	23 (47.9)	13 (34.2)	1.0	
6-10	10 (20.8)	12 (31.6)	0.47	0.16-1.39
>10	15 (31.3)	13 (34.2)	0.65	0.24-1.78
Use of gloves on a routine basis	54 (100)	40 (100)	NA**	NA
Use of surgical mask on a routine basis	54 (100)	40 (100)	NA	NA
Use of safety glasses on a routine basis	48 (88.9)	35 (87.5)	1.14	0.32-4.05
Use of face shield on a routine basis	2 (3.7)	3 (7.5)	0.47	0.08-2.98
Use of medical coat on a routine basis	51 (94.4)	40 (100)	0.18	0.009-3.62

* Numbers in parentheses are percentages. ** NA, not applicable

Table 2. Number of accidents that occurred in dental practice according to college grade.

Number of accidents	College Grade 3rd grade (N=29)	4th grade (N=15)	5th grade (N=50)	Total (N=94)
None	6 (20.7)*	0 (0)	2 (4)	8 (8.5)
1-5 accidents	17 (58.6)	7 (46.6)	14 (28)	38 (40.4)
6-10 accidents	5 (17.2)	4 (26.6)	11 (22)	20 (21.3)
>10 accidents	1 (3.4)	4 (26.6)	23 (46)	28 (29.8)

* Numbers in parentheses are percentages.

Table 3. Frequency of routine use of protective barriers for patient care.

Type or barrier	College Grade 3rd grade (N=29)	4th grade (N=15)	5th grade (N=50)	Total (N=94)
Gloves	29 (100)*	15 (100)	50 (100)	94 (100)
Surgical mask	29 (100)	15 (100)	50 (100)	94 (100)
Goggles	27 (93.1)	14 (93.3)	44 (88)	85 (90.4)
Face shield	0 (0)	2 (13.3)	2 (4)	4 (4.2)
Medical coat	27 (93.1)	15 (100)	48 (96)	90 (95.7)

* Numbers in parentheses are percentages.

Table 4. Frequency of NKG2C genotypes in students with and without CMV infection

Genotype	CMV + N= 104	CMV -N= 72	OR
NKG2C			
+/+	94 (90.3)*	68 (94.4)	1.0
+/-	9 (8.6)	4 (5.6)	1.63 (0.48-5.5)
-/-	1 (1)	0 (0)	2.17 (0.09-54.2)

* Numbers in parentheses are percentages.

tance, a medical coat was used by 95.7% of students in the clinic group. The use of protective goggles was reported in 90.3% of 3rd grade students and in 88% of 5th grade students; face shields were not used by any 3rd grade student and only 4% of 4th and 5th grade students reported their use. The protective barriers used for clinical practice per grade of college are shown in Table 3.

NKG2C gene copy number and CMV infection.

The NKG2C gene copy number determination was carried out in duplicate and the following frequencies were obtained: 162 (92%) were homozygous for the presence of the NKG2C gene (+/+; these students have two copies of the gene that encodes for the NKG2C receptor); 13 (7.4%) were heterozygous (+/-; students with only one copy of the NKG2C gene); one (0.6%) of the students was homozygous (-/-; for the deletion of the gene). The comparative frequencies between the case and control groups are shown in Table 4.

DISCUSSION.

In this study occupational exposures as well as a genetic trait (deletion of the NKG2C gene) in association to CMV infection in dental students were evaluated. First, the presence of IgG CMV specific antibodies was evaluated to identify students with past (persistent) infection; since the incidence of acute primary infections in healthy adults is very low, and the presence of IgM antibodies may also be found during CMV reactivation/reinfection, this determination was not included in this study[14,15]. The frequency of CMV infection in this group of students was 59%; this figure is relatively low when compared to previous reports in our country in which CMV antibodies have been reported in 89.2% of the population[16]. This could be attributed to the fact that, unlike other reports, most of the subjects in our study are of middle-upper socioeconomic status. Studies carried out in the United States have found significant differences in the prevalence of CMV infection according to socio economic status[17].

Although a higher CMV seroprevalence has been reported in women compared to men, this difference is usually very small[17]. No significant difference in CMV infection was found according to sex. Nevertheless, it is noteworthy that in our study 68% of women were negative for CMV antibodies. A substantial percentage of women of reproductive age in other populations have also been reported to be seronegative[17]. This is very relevant in the dental field since many professional are women and primary infection during pregnancy carries a high risk of transmission to the fetus. In addition, the severity of infection in the infant appears to be higher in women who suffer a primary infection during pregnancy. Future vaccine or educational campaigns to prevent primary infection in pregnant women may need to be tailored to suit the needs of specific populations, such as health care workers. Despite the frequency of congenital CMV infection and the serious consequences that may result from it, a large proportion of the population is not aware of the impact of this infection[18]. In fact, most gynecologists do not counsel women regarding congenital CMV infection[19]; nevertheless, most women are receptive to informative messages about prevention of this infection.

Our study provided information to the students about their serological status and about the importance of CMV infection and its consequences, especially regarding the possibility of CMV transmission during pregnancy and the risk that congenital CMV infection represents. Once the proportion of the population infected was determined, the possible association between occupational exposure factors and a genetic trait with CMV infection was evaluated. CMV is frequently excreted in saliva and, therefore, it was hypothesized that occupational exposure during dental procedures might be associated to increased risk of acquiring this infection. However, despite contact with patients the clinic group did not show a higher proportion of CMV infection compared to the pre-clinic group. Our results are consistent with those reported in a study conducted in the UK in which no occupational risk of infection by CMV, herpes simplex virus, or human herpes virus-6

was found; however, a possible risk of Epstein Barr virus infection was suggested[10]. Therefore, the importance of infection control measures needs to be stressed among all health-care workers.

With regards to measures to reduce the risk of infections an excellent adherence to the use of basic protective measures (such as the use of gloves and surgical masks) by students participating in the study was found. The use of medical coats and protective eyewear was also high among students in our study. It is possible that the routine use of protective barriers by students of the dentistry school has prevented CMV infection resulting in the lack of increase in the prevalence of infection in the clinical group compared to the preclinical group.

The history of accidents during dental practice was also evaluated. Not surprisingly, students who have attended the dental clinics for longer periods of time reported a history of one or more accidents more frequently. The most common reported accidents were punctures with the dental explorer and dental needle when applying anesthesia. An association between a history of accidents and increased frequency of CMV infection was not found. A study in the UK reported that 30% of dentists have had an accident, especially with the dental needle[10]. The frequency of accidents in our study population was notably higher compared to that report; therefore, it is necessary to take additional measures in order to avoid accidents when washing dental instruments or when using needles during anesthesia. Of interest, the frequency of the NKG2C gene deletion in participating students was low in contrast to the prevalence reported in European and Asian populations[20]. These results confirm findings from a recent report in which the prevalence of the NKG2C gene deletion in Mexican individuals was approximately 50% lower than in other populations[13]. A slight difference in the proportion of subjects with two copies between students with and without CMV infection was observed in the present study; however, this was not statistically significant. However, it may be of interest to conduct studies with a larger sample size to assess whether the NKG2C gene copy number may have a modulatory effect on the risk or outcome CMV infections.

One of the limitations of our study is that the determination of antibodies was performed only once, which may not be sufficient to reflect the occupational risk for CMV infection in the dentist; therefore, a cohort study to monitor all seronegative students until their senior year in order to verify the frequency of seroconversion and to determine the risk of transmission of CMV to the dentist is planned.

In conclusion, CMV infection in Mexican dentistry students was lower than previously reported. No association between occupational and genetic factors, and the presence of CMV infection was found in this population. Dentists should be aware of the prevalence and risks of acquiring this infection, particularly among child-bearing age women, and also the potential risk of transmission of these pathogens to patients during clinical practice, especially when barriers are not used routinely. It is relevant that a high percentage of dentistry students report puncture accidents during their clinical activities, which should be considered as a potential risk for acquiring CMV infection and other pathogens.

Análisis de factores de riesgo asociados a la infección por citomegalovirus en estudiantes de odontología.

Resumen: Objetivo: El objetivo de este estudio fue analizar la asociación entre la infección por citomegalovirus (CMV) en estudiantes de odontología con factores de riesgo ocupacionales y un polimorfismo genético (deleción del gen NKG2C). Diseño del Estudio: Estudio de casos y controles. 176 estudiantes fueron incluidos y divididos en dos grupos de acuerdo al resultado serológico para CMV: aquellos con la infección por CMV (grupo de casos) y aquellos que no presentaron infección por CMV (grupo control). Las características demográficas, ocupacionales y la presencia de la deleción del gen NKG2C

fueron comparadas entre ambos grupos. Resultados: La presencia de anticuerpos IgG de CMV fue detectada en 104 (59.1%) estudiantes (grupo de casos) mientras que 72 (40.9%) estudiantes fueron negativos a CMV (grupo control). Se comparó la frecuencia de contacto con el paciente, el uso de barreras protectoras y el número de accidentes ocupacionales reportados entre los grupos de estudio. No fueron detectadas diferencias significativas. El uso apropiado de las medidas de control de infección fue observado en la mayoría de los estudiantes de ambos grupos. En el grupo de casos la frecuencia de la deleción de NKG2C observada fue de 9.7% comparada con un 5.6% en el grupo control (p=0.33). Conclusión: No se encontró asociación entre la infección por CMV con los factores de riesgo ocupacionales y genéticos de esta población. Los dentistas deben conocer la frecuencia de la infección por CMV, así como los factores de riesgo asociados, particularmente las mujeres odontólogas en edad fértil.

Palabras clave: *Citomegalovirus; NKG2C; Células NK; Factores de riesgo ocupacionales.*

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