



# There Are No Correlations Between Biosafety Standards Acknowledgement and the Prevalence of Hepatitis B and C in Manicures/Pedicures Professionals Working in Beauty Salons of Maringá (Paraná-Brazil)

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## Abstract

**Background:** Since manicure and pedicure professionals are exposed to blood and to other human body fluids, these workers are susceptible to hepatitis viruses (HBV, HCV) when they fail to follow standard safety procedures (SSP) in their work routine. Knowledge on SSP is one of the main factors to reduce contamination risks.

**Objectives:** The current study investigated correlations between knowledge on SSP and the occurrence of HBV/HCV in manicure/pedicure professionals working at beauty salons in Maringá (Paraná-Brazil).

**Methods:** A cross-sectional study was conducted between March 2015 and March 2016. Beauty salons (n = 30) were visited and 150 professionals completed a questionnaire and a rapid test for HBV/HCV detection.

**Results:** Data indicated that there were no correlations ( $P < 0.01$ ) between knowledge about transmission and prevention of viral hepatitis and professional behaviors of manicure/pedicure in beauty salons of Maringá. There was low adherence ( $P < 0.01$ ) to SSP and to procedures that would guarantee an adequate sterilization of the materials used. One out of 150 individuals showed reactivity for HBV (genotype D by HBV DNA).

**Conclusions:** Manicure and pedicure professionals in Maringá were aware of the correct procedures to warrant personal protection, yet such knowledge is not adequately applied in their work routine.

**Keywords:** Hepatitis B, Hepatitis C, Manicure and Pedicure Service, Biosafety

## 1. Background

Hepatitis affects a large number of individuals and is acknowledged as a public health problem (1). Approximately, 257 and 71 million people worldwide have been infected by hepatitis B (HBV) and C (HCV) virus, respectively (2), while several virus types have killed about one million people.

Although hepatitis may be caused by different etiological agents, there is one general and common hepatotropism among them. Furthermore, agents may reveal clinical-laboratorial similarities despite their different epidemiologies (3). Great progress in the prevention and control of viral hepatitis has occurred during the last decades, comprised of the identification of viral agents, develop-

ment of specific laboratory tests, individual infection test screening, and the emergence of protective vaccines (4, 5).

The number of infected patients in Brazil is unknown (3). Lack of precise statistics is generally related to the failure of health authorities in several states and municipalities to identify the etiologic agent through laboratory techniques in molecular biology (4). However, an increasing integration of states and municipalities in surveillance and control programs coupled with partnership of research groups will provide more reliable national databases (5, 6).

Hepatitis B Virus and HCV may be transmitted by unprotected sexual intercourse and through parenteral routes, sharing syringes, needles and non-sterilized surgical materials (6). However, unprotected sexual intercourse

in the transmission of hepatitis C has not received enough attention due to the fact that the infection route has been considered less important (2, 7).

A common habit among Brazilian women is the removal of cuticles from the fingernails and toenails prior to the application of nail polish. In Brazil, such procedures are normally performed in beauty salons by specialized professionals, known as manicure and pedicure professionals. Consequently, Brazilian manicure and pedicure professionals are more susceptible to hepatitis virus (HBV, HCV) when they fail to follow standard safety procedures (SSP) in their working routine. Infection mainly occurs when professionals handle non-sterilized or inadequately sterilized materials (pliers, wooden sticks) and when they do not use personal protective equipments (PPE) (8). Pliers and wooden sticks are actually the main means of transmission of the disease (9).

Taken into account that there are no scientific work, at least until now, that has objectified the investigation between knowledge on the use of PPE and the occurrence of hepatitis (HBV, HCV) in manicure and pedicure professionals working in Maringá, PR, Brazil, and considering the importance of this data in public health policies of developing countries, the current study was conducted to determine whether manicure and pedicure professionals working in Maringá use their knowledge about PPE in their daily practice to protect themselves from HBV or HCV infections. The occurrence of HBV and HCV in manicure and pedicure professionals that participated in current work was also investigated.

## 2. Methods

The current study was approved by the Committee for Ethics in Research with Humans Beings of the Universidade Estadual de Maringá (ECRDH-UEM-1.573.796). The data (31st March 2015 to 31st March 2016) analyzed in the current work were obtained from a questionnaire administered to manicures and pedicures professionals over 18 years old (n = 150) living in Maringá city (Paraná state, South of Brazil) that agreed to participate in the study. The answers obtained by the application of the questionnaire were compared with the behaviors of the professionals in their work places. Such study could verify how knowledge about PPEs is regularly applied by manicure/pedicure professionals in their work environments. Immunochromatography virus test was also applied for rapid screening of HBV and HCV.

### 2.1. Minimal Ideal N Number

Open Source Epidemiologic Statistics for Public Health (OpenEpi- 3.01) (10) was used to determine the minimal sample size to be used in this study. Serum prevalence

for HBV (9.59% for anti-HBc and 0.48% for HBsAg) and HCV (1.19% for anti-HCV) (1) markers in the capital cities of the states in southern Brazil (Paraná, Santa Catarina and Rio Grande do Sul) were taken in account to calculate the sample size. Although the minimum number was 126 ( $P < 0.05$ ), a greater number (150) of individuals participated in the current study. Socio-demographic data and knowledge on HBV and HCV infections (transmissibility and prevention methods) were obtained from responses in a specific questionnaire (11, 12). The epidemiological occurrences of HBV and HCV in the manicure and pedicure population was studied by blood sample collection.

### 2.2. Analyses from Blood Sample Collection

The lateral immunochromatography virus test was used that permitted a rapid screening test (HBsAg) to detect HBV (VIKIA<sup>®</sup> HBsAg, bioMérieux SA, Marcy-L'etoile, France) and HCV (WAMA Diagnostica, São Carlos SP Brazil). When positive, the blood sample was confirmed serologically at the Testing and Counseling Center (CTA) and at the Clinical Analyses and Research Laboratory (LEPAC) of Universidade Estadual de Maringá. Serum and plasma aliquots were removed from the samples and stored at  $-80^{\circ}\text{C}$  until serological tests were performed. Since only one of 150 professionals participating in the study had positive results for HBV (HBsAg) rapid screening test, the blood sample was collected from venous access in the arm. HBsAg and total anti-HBc serological markers were collected for HBV confirmation or exclusion by chemiluminescent microparticle immunoassay (CMIA) (Abbott, Diagnostics Division, Wiesbaden, Germany), real-time polymerase chain reaction (qPCR) with Abbott RealTime VHB kit (Abbott Laboratories of Brazil Ltda, Division of Diagnostics; sensitivity  $> 12 \text{ IU/mL}$  and m2000sp and m2000rt automation) and genotyping sequencing methodology.

### 2.3. Detection, Sequencing and Phylogenetic Analysis of HBV DNA Sequences

DNA extraction of HBV was performed by the QIAmp<sup>®</sup> DNA Mini Kit (QIAGEN, Courtaboeuf, France). DNA was amplified by nested-PCR reaction, involving S-polymerase (S-Pol) gene region (primers PS3132F - 5'-CCT YGC HTC YAC CAA TCG-3'; nt 3132-3151/2920R - 5'-ACG TCC YKC KHA GDA TCC AG-3'; nt 1417 - 1398; PS3201F - 5'-CAY CCH CAG GCM ATG CAG TGG -3'; nt 3201-3221 / P1285R - 5'-CWA GGA CCG CAG TAT GG -3'; nt 1285-1266) of 1306 base pairs. Polymerase chain reaction fragments were observed under ultraviolet light with 1% agar gel electrophoresis and stained with ethidium bromide.

#### 2.4. Sequencing of S/Pol (RT Domain) Regions

The technique of the current study to obtain sequencing of S/Pol (RT domain) was that of Sanger (1977). According to ABI PrismR BigDye™ Terminator kit (Applied Biosystems), dideoxynucleotides (ddNTPs) containing fluorescent labels was employed. Three pairs of primers, generating sequences around 500 bp superimposable at both ends, were used: 1- PS3132F (5'-CCT YGC HTC YAC CAA TCG-3'; Nt 3132-3151) and HBV477R (5'-GGA CAV ACG GGC AAC ATA CCT T-3'; nt 477-456); 2- L372 (5'-TCG YTG GAT GTR TCT GCG GCT TTT-3'; nt 370-396) and RADE2M (5'-TGR CAN ACY TTC CAR TCA ATN GG-3'; nt 781-804) and PI285R (5'-CWA GGA CCG CAG TAT GG-3'; nt 1285-1266). Electrophoresis and fluorescence reading of DNA molecules were performed on ABI 3500 automated sequencer (Applied Biosystems, Foster City, CA).

#### 2.5. Gene Sequence Analysis

Genetic sequences were analyzed for quality by Phred-Phrap program, and consensus sequence of each sample was assembled by the CAP3 program. Identification of HBV genotype and sub-genotype was performed by consensus sequence similarity analysis with the NCBI sequence database, using the nucleotide blast tool (Basic Local Alignment Search Tool).

#### 2.6. Statistical Analysis

Chi<sup>2</sup> and Fisher tests (SAS 4, Statistical Analysis System, version 9.3) (13) were used to compare data at P < 0.05 significance.

### 3. Results

Questionnaire and previous interviews verified that the manicure and pedicure professionals (n = 150.0, 96.0% manicure and 4.0% pedicure exclusively), who accepted to participate in current study, were all females, aged 30 to 39 years (n = 75; 50.3%), married (n = 80; 53.6%), living in rented residence (n = 98, 66.2%), with a family receiving from one to three Brazilian minimum wages (n = 64, 44.4%) and individual income of one to three Brazilian minimum wage (n = 132, 88.6%) (Table 1). Furthermore, it was verified that 50.4% of the professionals had less than 10 years of work experience (Table 2). Furthermore, 34.9% (52) declared to be single and 19.2% (23) admitted to having sexual intercourse with partners, who were not using condoms (Table 2).

Ninety-four (62.7%) out of 150 interviewed professionals reported that they obtained knowledge on hepatitis B and C acquisition and transmission from different sources (TV, magazines, newspapers); only 40 (42.6%) professionals attended courses to obtain such information (Table 3). Sixty-six (36.0%) out of 94 professionals reported that the

**Table 1.** General Characteristics of the Study Participants

Characteristics	No. (%)
<b>Age</b>	
< 30	59 (39.60)
30 - 49	75 (50.34)
≥ 50	15 (10.07)
<b>Civil status</b>	
Married	80 (53.69)
Separated	14 (9.40)
Single	52 (34.90)
Widow	3 (2.01)
<b>Number of residents</b>	
1 to 3	98 (66.22)
4 to 7	42 (28.38)
8 to 10	1 (0.68)
Alone	7 (4.73)
<b>House</b>	
Rented	76 (51.35)
Own	6 (4.05)
Lent	66 (44.59)
<b>Level of education</b>	
Below-high-school	42 (28)
High school diploma	89 (59.33)
Academic	19 (12.67)
<b>Total income</b>	
1 minimum wage	6 (4.17)
1 - 3 minimum wage	61 (42.36)
3 - 6 minimum wage	64 (44.44)
6 - 9 minimum wage	9 (6.25)
9 - 12 minimum wage	4 (2.78)
<b>Income</b>	
1 minimum wage	7 (4.70)
1 - 3 minimum wage	132 (88.59)
3 - 6 minimum wage	10 (6.71)
<b>Worrrk</b>	
Manicure	144 (96.00)
Other	6 (4.00)
<b>Years of work</b>	
< 5	31 (21.09)
5 to 9	43 (29.25)
10 to 14	20 (13.61)
15 to 19	20 (13.61)
> 20	33 (22.45)

main form of transmission was contact with blood (Table 3). However, 49 (43.0%) did not know the answer to the question (Table 3). Although 55 (36.2%) out of a total of 94 professionals, answered that vaccine against HBV was the main form of prevention, 87 (58.0%) failed to take the vaccine and 94 (62.7%) professionals felt exposed to the disease (Table 3).

Standard wear for manicure and pedicure profession-

**Table 2.** Distribution of Salon Professionals (Manicure/Pedicure) According to Sexual Intercourse Without Condoms, Schooling and Marital Status<sup>a</sup>

	Intercourse Without the Use of Condom		P Value
	No	Yes	
<b>Schooling</b>			< 0.001 <sup>b</sup>
Under 8 years	1 (3.5)	23 (19.0)	
Over 8 years	28 (96.5)	98 (81.0)	
<b>Marital status</b>			< 0.001 <sup>b</sup>
Married	0 (0.0)	80 (66.7)	
Single	29 (100.0)	23 (19.2)	
Other	0 (0.0)	17 (14.1)	

<sup>a</sup>Values are expressed as No. (%).<sup>b</sup>Chi-square association test. Significance level at 95% confidence.**Table 3.** Distribution of Salon Professionals (Manicure/Pedicure) According to the Relationship Between Schooling and Knowledge of the Disease and Other Associated Variables<sup>a</sup>

	Knowledge of HBV and HCV		P Value
	No	Yes	
<b>Exposure to disease</b>			< 0.001 <sup>b</sup>
I don't know	2 (3.6)	7 (7.5)	
No	47 (83.9)	0 (0.0)	
Yes	7 (12.5)	87 (92.5)	
<b>Schooling</b>			< 0.001 <sup>b</sup>
Under 8 years	22 (39.3)	2 (2.1)	
Until 8 years	34 (60.7)	92 (97.9)	
<b>Vaccination</b>			< 0.001 <sup>b</sup>
No	43 (87.8)	44 (46.8)	
Yes	6 (12.2)	50 (53.2)	
<b>Use of nail file</b>			< 0.001 <sup>b</sup>
One for each customer	1 (1.8)	59 (62.8)	
One for all customers	55 (98.2)	35 (37.2)	
<b>Use of cuticle pusher</b>			< 0.001 <sup>b</sup>
One for each customer	1 (1.8)	56 (59.6)	
One for all customers	55 (98.2)	38 (40.4)	
<b>Is vaccine a preventive method?</b>			< 0.001 <sup>b</sup>
No	55 (98.2)	75 (79.8)	
Yes	1 (1.8)	19 (20.2)	

<sup>a</sup>Values are expressed as No. (%).<sup>b</sup>Fisher's Exact test. Significance level at 95% confidence.

als in the current study comprised of a long-sleeve lab coat, a pair of gloves used for each client, correct procedures for hand hygiene, use of closed shoes during work, nail file for each client, cuticle pusher for each client, and cuticle pliers and cuticle stick for each client. However, 130 (82.7%) professionals did not use a lab coat, 131 (77.3%) did

not wear gloves, 84 (56.0%) did not practice hand hygiene, 101 (67.3%) did not wear closed shoes, 90 (60%) used nail files, 93 (62.0%) used cuticle pusher, 78 (52.0%) used cuticle pliers, and 72 (48.0%) used the same cuticle stick for several clients (Table 3).

Other risk behaviors observed, which did not comply

with biosafety norms, were also checked. They included the use of the same pair of gloves to manipulate cuticles and other objects (7% to 18.9%), not covering the lab coat sleeves with gloves (28% to 75.6%), failing to disinfect gloves (7% to 18.9%), failing to take gloves off immediately at the end of the service (17% to 46%), and failing to wash hands prior to donning gloves (20% to 54%) (Table 4).

Taking into consideration standard use of autoclave and best temperature and sterilization time of materials for each client, the current study demonstrated that 123 (82.0%) of the 150 professionals under analysis used an autoclave as a sterilization method, yet 130 (86.7%) did not know, which was the best time and temperature for the sterilization of each equipment. None (0%) used alcohol (70%) to disinfect the table in spite of schooling level and knowledge of the disease.

It was verified that there was a close correlation (Table 5) between schooling level and the correct use of closed shoes, towels for each client, gloves, lab coat, and autoclaves. In fact, 20 professionals (99.0%) with higher education complied with biosafety criteria.

Hepatitis B Virus in only one professional (0.7% of individuals analyzed) belonged to genotype D.

#### 4. Discussion

Several professionals working in beauty salons had general knowledge on the risks of disease transmission proper to their activities. In spite of this, they adopted risk behavior that conflicts with correct theoretical knowledge, or rather, a significant majority of these individuals failed to use lab coats, gloves, closed shoes, and practice of hand hygiene. Furthermore, it has also been detected that 90 professionals used nail files, cuticle pusher (93), cuticle pliers (78), and cuticle stick (72) in more than one client. Contrary to declared knowledge on the risk of disease transmission in their professional routines, it has also been observed that their behavior was not according to biosafety norms. In fact, 39% of the professionals used the same pair of gloves to manipulate cuticles and other objects, 75.6% did not cover the lab coat sleeves with gloves; 19% did not discard gloves after use, 46% did not remove immediately their gloves at the end of service, and 54% did not wash their hands prior to donning gloves. Results discussed above are in accordance with previous reports by other authors (14, 15), who revealed that manicure and pedicure professionals featured low adherence for adopting biosafety norms and for using PPE. Furthermore, the same studies have also revealed that the small number that used PPE, failed to do so correctly.

Oliveira and Focaccia (12) reported that 66% of 100 manicure/pedicure professionals working in São Paulo,

Brazil, stated that hand washing is not in itself an important activity to prevent infections. These data are similar to those obtained by the current study since 44% of manicure/pedicure professionals in Maringá failed to wash their hands before attendance and after each care. Another important aspect detected in the current analysis was the professionals' scanty knowledge on HBV/HCV (62.7%) and their transmission (57.0%). These results are similar to that of Oliveira and Focaccia (12), who reported that 72% of manicure/pedicure professionals did not know the routes of hepatitis B transmission, 93% did not know how to prevent it, 85% did not know how hepatitis C was transmitted, and 95% did not know anything about hepatitis C prevention methods. Furthermore, Oliveira and Focaccia (12) admitted that only 3% of manicure/pedicure professionals had knowledge of the manner of its transmission and what to do to prevent the disease and other data. This study was in accordance with the current data and the studies of Garbaccio and Oliveira (15) and Oliveira et al. (16).

Although 62.7% of respondents stated that they received information on HBV/HCV through health courses and campaigns, a low (37%) adherence to vaccination was noted. These data suggest the need for greater health attention during training courses for manicure/pedicure professionals. Furthermore, it may be hypothesized that beauty salons are not adequately prepared to train professionals. In fact, Oliveira and Focaccia (12) also registered low adherence to vaccination in these professionals in São Paulo, Brazil.

A similar study conducted in Belo Horizonte MG Brazil, by Garbaccio and Oliveira (17) also revealed low (34%) level of adherence of manicure/pedicure professionals to use of closed shoes, uniforms or aprons over clothing (68.1%), whilst 37% admitted they removed all the apparatus sets during work. Other authors (8, 12, 18) also recorded predominance of inadequate practices by manicures.

Although 123 respondents admitted sterilizing of materials by an autoclave, only 20 had knowledge on the correct temperature and the appropriate sterilization time for the equipments. Oliveira and Focaccia (12) reported that 60% of professionals working in the city's districts used a stove to sterilize the tools. However, only 7.14% of professionals using the stove knew the appropriate time and the correct temperature. Furthermore, it has been reported (13) that no professional, who used the autoclave, had any knowledge on the correct time and temperature for adequate sterilization of materials. de Melo and Isolani (8), Moraes et al. (18) and Harumi Yoshida et al. (19) also emphasized the professionals' low knowledge in the sterilization of materials. Since small skin lesions during shaving may contaminate a razor (19) and small cuts on the hands of clients (cuticles) may also contaminate the instruments, the risk of contamination of clients and professionals in-

**Table 4.** Distribution of Salon Professionals (Manicure/Pedicure) According to the Relationship Between the Use of Gloves and Other Hygiene Procedures<sup>a</sup>

	Gloves		P Value
	Correct Use	Incorrect Use	
<b>Handling other objects</b>			< 0.01 <sup>b</sup>
No	19 (100.0)	11 (61.1)	
Yes	0 (0.0)	7 (38.9)	
<b>Covering the lab coat sleeve with gloves</b>			< 0.001 <sup>b</sup>
No	10 (52.6)	18 (100.0)	
Yes	9 (47.4)	0 (0.0)	
<b>Disinfecting of gloves</b>			< 0.01 <sup>b</sup>
No	19 (100.0)	11 (61.1)	
Yes	0 (0.0)	7 (38.9)	
<b>Removing gloves immediately after care</b>			< 0.001 <sup>b</sup>
No	0 (0.0)	17 (100.0)	
Yes	19 (100.0)	0 (0.00)	
<b>Washing hands prior to putting on gloves</b>			< 0.001 <sup>b</sup>
No	2 (10.5)	18 (100.0)	
Yes	17 (89.5)	0 (0.0)	

<sup>a</sup>Values are expressed as No. (%).

<sup>b</sup>Fisher's Exact test. Significance level at 95% confidence.

creases when the instruments are reutilized (20).

It has also been verified that none of the professionals performed correct disinfection of the work table, indicating lack of information on disinfection and sterilization processes, and on the possible contamination of clients or professionals. Harumi Yoshida et al. (19) also underscored lack of knowledge in professionals on the difference between disinfection and sterilization.

Schooling level plays a key role in assessing equilibrium between practical use and theoretical information, since all participants reported that they knew how to use PPE, even though only twenty, with a university and/or specialization degree, used a lab coat and gloves correctly. A study carried out in beauty salons of the city of Isfahan, Iran, showed that the level of education may influence knowledge on viruses and their transmission (21).

Although rapid HBV and HCV tests revealed only one positive result for HBV, the information obtained in the questionnaire showed that several professionals were susceptible to future infections. Data demonstrated permanent neglect of professionals to use their individual protection apparatus set. The current results are different from those obtained from manicure/pedicure professionals in the city of São Paulo, Brazil, on this particular item. In this case, one in ten manicure/pedicure professionals interviewed had serologic markers for hepatitis B or C; 8% had hepatitis B, and 2% were infected by hepatitis C (12).

Another study performed in the Brazilian Amazon region showed the prevalence of HBV surface antigen (HBsAg) and two (4%) out of 50 professionals presented positive results for HBV surface antigen (HBsAg); 64% presented serological scarring for hepatitis B, seven (14%) had anti-HBs antibody alone (vaccine immunity), and nine (18%) were susceptible to hepatitis B virus (22). The conflict between the current results and those obtained by other authors (REF) may be explained by the fact that distribution of infection by HBV was lower in southern Brazil, specifically in Maringá (23).

The current study recorded genotype D in one professional. The occurrence of this particular genotype was expected since a study on HBV genotypes performed with blood donors in the state of Paraná, Brazil, characterized 228 samples with D as the most common genotype (82.9%, 189/228) (24).

In general, the current study showed that HBV and HCV infection risk associated with blood is still not well understood by many manicure and/or pedicure professionals working in beauty salons in Maringá PR Brazil. The sterilization instruments used in commercial establishments that offer public manicure/pedicure services have presented important deficiencies related to cleaning and sterilization. Since the current analysis showed several results similar to those obtained by other authors in other areas of Brazil (8, 12, 15-20) and in other parts of the world (14,

**Table 5.** Distribution of Salon Professionals (Manicure/Pedicure) According to the Relationship Between Schooling and Knowledge of the Disease and Other Hygiene Procedures<sup>a</sup>

	Schooling		P Value
	Under 8 Years	Over Than 8 Years	
<b>Closed shoes</b>			< 0.01 <sup>b</sup>
No	21 (87.5)	80 (63.5)	
Yes	3 (12.5)	46 (36.5)	
<b>Towel use</b>			0.001 <sup>b</sup>
One for each client	0 (0.0)	84 (66.7)	
One for all client	24 (100.0)	42 (33.3)	
<b>Availability of disposable gloves</b>			< 0.001 <sup>b</sup>
No use of gloves	130 (100.0)	0 (0.0)	
One for each client	0 (0.0)	20 (100.0)	
<b>Use of laboratory coat</b>			< 0.023 <sup>b</sup>
No	24 (18.5)	106 (81.5)	
Yes	0 (0.0)	20 (100.0)	
<b>Use of autoclave</b>			< 0.001 <sup>b</sup>
No	21 (77.8)	6 (22.2)	
Yes	3 (2.4)	120 (97.6)	
<b>Knowledge of HBV and HCV</b>			< 0.001 <sup>b</sup>
No	22 (39.3)	34 (60.7)	
Yes	2 (2.1)	92 (97.9)	

<sup>a</sup>Values are expressed as No. (%).

<sup>b</sup>Fisher's Exact test. Significance level at 95% confidence.

20, 24-29), data suggested that the sources of information used or offered to professionals working in beauty salons seem to be only informative, since they practically have a slight impact on risk behavior adopted by professionals in beauty salons. In spite of this fact, only one case of hepatitis B virus contamination was recorded, revealing low virus dissemination in the city of Maringá.

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### Footnotes

**Authors' Contribution:** Amauri Donadon Leal Junior Donadon Leal Junior, Eraldo Schunk Silva, Eliane A Biazon and Sonia Kaori Miyamoto made substantial contributions to conception and design, and/or acquisition of

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**Ethical Considerations:** This work was approved by the Standing Committee on Ethics in Research involving Human Beings of the State University of Maringá to evaluate human research in accordance with Resolution No. 466 of December 12, 2012 on Research Involving Human Beings/National Health Council/Ministry of Health/Brasilia/2012 (n: 1,573,796) (<http://www.saude.sp.gov.br/centro-de-referencia-e-treinamento-dstaids-sp/pesquisa/comite-de-etica-em-pesquisa/projetos-de-pesquisa-plataforma-brasil>).

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