Revista da Sociedade Brasileira de Medicina Tropical 46(3):281-287, May-Jun, 2013
http://dx.doi.org/10.1590/0037-8682-0026-2013

Major Article

Prevalence and factors associated with HCV infection among elderly individuals in a southern Brazilian city

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ABSTRACT

Introduction: Few Latin American studies have assessed the prevalence of hepatitis C virus (HCV) infection in elderly individuals, in whom the highest rates are expected. We aimed to investigate the prevalence of and factors associated with HCV infection in elderly residents in the municipality of Tubarão, Santa Catarina. **Methods:** This cross-sectional study included 820 individuals (aged ≥ 60 years) who were selected by simple random sampling. The presence of anti-HCV antibodies was tested by chemiluminescence, and HCV RNA detection was performed for the anti-HCV-reactive subjects. Those individuals who were anti-HCV reactive but had undetectable HCV RNA levels were tested using a third-generation recombinant immunoblot assay. The variables were compared using the chi-squared test or Fisher's exact test, and those variables with $p < 0.05$ were included in the logistic regression model. **Results:** The mean patient age was 68.6 years (SD 7.0 years); 39% were men, and 92% were Caucasian. Eighteen subjects were anti-HCV positive. Among these individuals, 4 were characterized as false-positives, leaving 14 (1.7%) individuals with confirmed infections for analysis. HCV infection was associated with an age older than 65 years, households with 3 or more residents and the previous transfusion of blood products. In the logistic regression analysis, the following variables were independently associated with HCV infection: households with 3 or more residents (OR 7.9, 95% CI 1.7–35.9, $p = 0.008$) and previous blood transfusion (OR 6.2, 95% CI 2.1–18.6, $p = 0.001$). **Conclusions:** The HCV prevalence in the elderly population in the municipality of Tubarão was higher than that found in previous studies of blood donors in the same region. Although exposure to contaminated blood products remained important, other transmission routes, such as household transmission, could play a role in HCV infection.

Keywords: Hepatitis C. Epidemiology. Transmission. Prevalence. Risk factors.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a major cause of liver disease worldwide. Although the prevalence varies geographically, it is estimated that 2.3% of the world's population are HCV carriers, a percentage that represents over 160 million infected people worldwide[1]. The primary risk factors for HCV infection include the transfusion of blood products from unscreened donors, intravenous drug use, organ transplantation and hemodialysis[2,3]. Significant proportions of chronically infected individuals are asymptomatic and remain undiagnosed for many years, representing a natural reservoir for the further transmission of HCV[4]. Approximately 20% of chronic HCV carriers will progress to cirrhosis, and the 5-year risk of progressing to decompensation is approximately 18% in those subjects with established cirrhosis[5,6]. In addition, there is an increased risk of developing hepatocellular carcinoma in individuals with cirrhosis caused by HCV, with an estimated annual incidence of 1-5% per year[7]. Overall, it is estimated that over 350,000 deaths occur annually as a result of HCV infection[8].

Studies in various parts of the world have found a higher prevalence of hepatitis C in elderly populations[9-13]. This epidemiological profile is most likely related to increased exposure to specific risk factors, such as the transfusion of blood products before HCV screening was introduced in blood banks and having undergone medical or therapeutic procedures performed under poor conditions and without the current standard precautionary measures. In addition, older age is associated with disease progression and is related to more advanced stages of fibrosis, higher rates of fibrosis progression and an increased incidence of hepatocellular carcinoma[14]. Because of these relationships, it is likely that the prevalence of and factors associated with HCV infection among elderly individuals will provide important tools for developing health policy strategies.

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Received 5 February 2013

Accepted 4 June 2013

A population-based study conducted by the Ministry of Health found a prevalence of 1.38% for anti-HCV antibodies, with significantly higher rates among those older than 60 years[15]. However, that study included only individuals aged up to 69 years and was performed in state capitals; therefore, it did not reflect the situation in less urbanized regions and inner cities. The purpose of this study was to estimate the prevalence of and investigate possible factors associated with hepatitis C infection among elderly individuals in the municipality of Tubarão, southern Brazil.

METHODS

Study population

An observational cross-sectional population-based study was conducted between June 2010 and March 2011 in the municipality of Tubarão, located in the State of Santa Catarina, southern Brazil. Individuals aged 60 years (completed in 2010) and older who were enrolled in the Family Health Strategy (FHS) Program were included in the study. Individuals who refused or declined to participate and those who did not attend the blood collection appointment were excluded. The FHS program is a strategic initiative of the Brazilian Public Health System; it involves multidisciplinary teams and aims to promote health, disease prevention and recovery and rehabilitation after illness. The FHS Program of Tubarão has a good coverage rate, totaling 9,009 elderly subjects in June 2010 (75.4% of elderly residents in the municipality according to the last Brazilian census).

Assuming an estimated prevalence rate of 1.5% for HCV in the population[16] and using a 95% confidence interval (CI) and an accuracy of 0.8%, the estimated sample size was 808 individuals[17]. Anticipating possible losses, 207 additional subjects (25%) were included, totaling 1,015 subjects who were selected by simple random sampling. A pre-survey of all elderly subjects enrolled in the health units was conducted, and the data were entered into a spreadsheet to perform random sampling using the Random Generator for Microsoft® Excel® (Add-in Express Ltd, Homel, Belarus).

Data collection

Participants were interviewed at home after being informed that all data collected would be kept strictly confidential. The structured questionnaire consisted of closed questions on sociodemographic characteristics, medical and surgical history and risk behaviors, including drug use and sexual behavior. Blood transfusions prior to 1993 are considered to be greater risk factors for HCV transmission because HCV screening tests were introduced in the majority of Brazilian blood banks in 1992. Alcoholism was defined as ≥ 2 affirmative responses to the CAGE questionnaire[18]. Individuals who reported having smoked ≥ 100 cigarettes in their lifetimes were considered smokers (current or previous)[19].

In the present study, invasive procedures were defined as all surgical procedures during which the skin or mucous membranes were incised or an instrument was introduced through a natural body orifice. This definition includes all surgical procedures, minimally invasive dermatological interventions, techniques such as percutaneous transluminal angioplasty and cardiac catheterization, minimally invasive procedures involving biopsies or the placement of probes or catheters requiring entry into a body cavity through a needle or trocar and endoscopic procedures.

After the interview, the participants were scheduled for blood collection at an FHS unit. Home collections were performed for the individuals with limited mobility. The samples were centrifuged *in situ* by the collection team and transported to the Clinical Laboratory of Unisul for testing. In the case of household blood collection, the samples were immediately transported to the laboratory and then centrifuged and processed.

Laboratory analyses

The presence of anti-HCV antibodies was assessed using enhanced chemiluminescence (Vitros. Eci, Johnson & Johnson, USA). A qualitative analysis of the HCV RNA level was performed for those individuals who were anti-HCV reactive. The polymerase chain reaction (PCR) method was used and was performed with the AMPLICOR® HCV Test 2.0 kit (Roche Molecular Systems, Branchburg, NJ, USA), which has a lower detection limit of 50 IU/mL. Individuals who were anti-HCV reactive but had an undetectable HCV RNA level were tested using a third-generation recombinant immunoblot assay (HCV RIBA) to differentiate between previous contact with HCV and false-positivity for anti-HCV antibodies. Subjects who were anti-HCV reactive and positive according to the HCV RNA PCR or the HCV RIBA were considered to have a confirmed HCV infection (current or previous).

Statistical analysis

Numerical variables were expressed as the mean and standard deviation (SD), and categorical variables were expressed as absolute numbers and proportions. Pearson's chi-squared and Fisher's exact tests were used to compare proportions, and $p < 0.05$ was considered statistically significant.

To identify the parameters independently associated with HCV infection, the variables with $p < 0.050$ in the univariate analysis were evaluated by logistic regression using the Enter method. The discrimination capability of the final model was evaluated using the area under the receiver operating characteristic curve (AUROC), and the goodness-of-fit of the logistic model was verified by the Hosmer-Lemeshow test ($p > 0.05$ indicated a model with appropriate adjustment). All statistical tests were performed using SPSS, version 17.0 (SPSS, Chicago, IL, USA).

Ethical considerations

The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration and the Brazilian regulations concerning research involving human subjects. This study was approved by our institutional review board.

RESULTS

Sample characteristics

Among the 1,015 subjects randomly selected for inclusion in the study, 122 did not attend the interview for the following reasons: 8 died, five moved to other cities, 11 were not found and 98 refused to participate. Of the 893 individuals who were interviewed, 73 did not attend the appointment for blood collection and were excluded from the final analysis.

The characteristics of the included subjects and the comparison of these subjects with the excluded individuals (73 interviewed) are shown in **Table 1**. Compared with the excluded subjects, the subjects included in the analysis had a higher proportion of stable relationships (married or cohabiting) and were less likely to share personal items with non-household members. No differences were observed with respect to the other variables.

Among the included subjects, the mean age was 68.6 years (SD 7.0 years), 38.5% were men, and 92.4% were Caucasian. The mean duration of schooling was 4.2 years (SD 3.4 years), and 51.5% of the subjects had 4 or more years of schooling. With regard to marital status, 66% were living in stable relationships (married or cohabiting couples), and 43% were living with 3 or more people in the household.

Concerning the variables related to exposure and risk factors, alcohol abuse was observed in 10.1% of the subjects, current or previous smoking was observed in 40.6%, and illicit drug use was observed in only 0.4%. None of the subjects reported intravenous drug use. Only 2.5% of the surveyed participants reported having 2 or more sexual partners during the past year, and 97.8% reported that they did not use condoms regularly. A history of blood transfusion was observed for 15.8% of the subjects, 49.6% of whom had undergone transfusions before 1993 (7.8% of all included subjects). Invasive procedures were reported by 60.8% of participants, 8% had undergone acupuncture treatments, and 0.2% had been tattooed. The survey indicated that 30.5% of the subjects routinely shared personal items, 16.9% of whom shared these items with family members; 15.1% shared them with unrelated people. In addition, 9.1% of the respondents had already undergone human immunodeficiency virus (HIV) screening, and 2 individuals were HIV positive.

Prevalence of markers for HCV infection

As shown in **Figure 1**, anti-hepatitis C virus reactivity detected by chemiluminescence was observed for 18 subjects

TABLE 1 - Characteristics of the included subjects and a comparison with the excluded individuals.

	Included (n = 820)		Excluded (n = 73)		
Variable	n	%	n	%	p*
Age ≥ 65 years	530	64.6	47	64.4	0.966[q]
Male	316	38.5	23	31.5	0.244[q]
Caucasian*	756	92.4	65	89.0	0.343[q]
Schooling < 4 years	422	51.5	30	41.7	0.255[q]
Stable relationship	541	66.0	36	49.3	0.007[q]
Household with 3 or more residents	353	43.0	32	43.8	0.881[q]
Alcoholism	83	10.1	7	9.6	0.911[q]
Current/previous smoking	331	40.6	34	47.2	0.271[q]
History of drug use	3	0.4	0	0.0	1.000[f]
No regular use of condoms	795	97.8	64	95.5	0.210[f]
Two or more sexual partners[#]	20	2.5	3	4.3	0.416[f]
Transfusion of blood products	129	15.8	7	9.7	0.173[q]
Transfusion before 1993	64	7.8	3	4.2	0.262[f]
History of invasive procedures	497	60.8	42	58.3	0.671[q]
Acupuncture	68	8.3	8	11.1	0.413[q]
Tattoo	2	0.2	1	1.4	0.225[f]
Sharing personal hygiene objects	249	30.5	24	33.8	0.550[q]
Sharing with household members	138	16.9	8	11.3	0.226[q]
Sharing with non-household members	123	15.1	18	25.4	0.023[q]
Previous HIV testing	74	9.1	5	7.0	0.560[q]

[q]Chi-squared test;*Self-reported; [f]Fisher's exact test; [#]Within the past 12 months; HIV: human immunodeficiency virus.

(2.2%, 95% CI 1.2% – 3.2%). Among these subjects, 14 had an HCV RNA detectable by PCR (1.7%, 95% CI 1.0% – 2.9%); and HCV RNA was undetectable in the remaining subjects (0.5%). For the 4 subjects with undetectable HCV RNA levels, the HCV RIBA was performed. RIBA was negative for 2 subjects and indeterminate for the remaining 2. The individuals with indeterminate RIBA results had anti-HCV by chemiluminescence near the lower limit of detection (cut-off points 1.79 and 2.16); thus, these individuals were considered negative in the analysis of risk factors. The prevalence of confirmed HCV infection was 0.6% among the subjects aged 60-65 years and 2.5% among those over 65 years.

Analysis of factors associated with HCV infection

As detailed in **Table 2**, compared with elderly subjects without HCV infection, the infected subjects included a higher percentage of individuals over age 65 years (85.7% vs. 58.3%, p = 0.039), a higher proportion of households with 3 or more residents (85.7% vs. 42.3%, p = 0.001) and a higher proportion of subjects who had undergone transfusions of blood products (57.1% vs. 15%, p < 0.001). When the subjects were evaluated only regarding blood transfusion prior to 1993, there was a trend toward a higher frequency of exposure to blood products among those with HCV infection than their uninfected counterparts (23.1% vs. 7.7%, p = 0.076). No differences were observed with respect to the other variables.

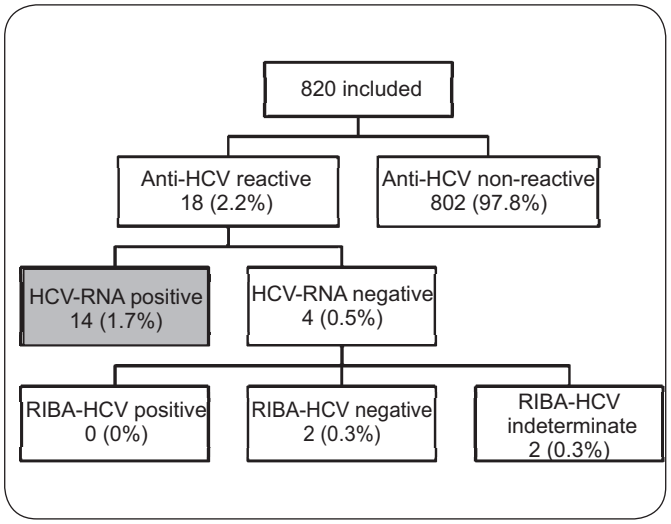

FIGURE 1 - Flowchart detailing the included subjects according to HCV infection markers. RIBA: recombinant immunoblot assay; HCV: hepatitis C virus; RNA: ribonucleic acid.

TABLE 2 - Univariate analysis of factors associated with HCV infection.

	HCV - (n = 806)		HCV + (n = 14)		
Variable	n	%	n	%	p
Age > 65 years	470	58.3	12	85.7	0.039[q]
Male	309	38.3	7	50.0	0.374[q]
Caucasian*	744	92.5	12	85.7	0.287[f]
Schooling < 4 years	394	48.9	4	28.6	0.132[q]
Stable relationship	530	65.8	11	78.6	0.403[f]
Household with 3 or more residents	341	42.3	12	85.7	0.001[q]
Alcoholism	81	10.1	2	14.3	0.645[f]
Current/previous smoking	324	40.4	7	50.0	0.468[q]
History of drug use	3	0.4	0	0.0	1.000[f]
No regular use of condoms	782	97.9	13	92.9	0.271[f]
Two or more sexual partners[#]	19	2.4	1	7.1	0.296[f]
Transfusion of blood products	121	15.0	8	57.1	0.001[f]
Transfusion before 1993	61	7.7	3	21.1	0.076[f]
History of invasive procedures	488	60.7	9	64.3	0.785[q]
Acupuncture	68	8.4	0	0.0	0.621[f]
Tattoo	2	0.2	0	0.0	1.000[f]
Sharing personal hygiene objects	245	30.5	4	28.6	1.000[f]
sharing with household members	136	17.0	2	14.3	1.000[f]
sharing with non-household members	120	15.0	3	21.4	0.455[f]
Previous HIV testing	72	9.0	2	14.3	0.369[f]

[q]Chi-squared test;*Self-reported; [f]Fisher's exact test; [#]Within the past 12 months; HIV: human immunodeficiency virus; HCV: hepatitis c virus.

TABLE 3 - Logistic regression analysis of the factors associated with hepatitis c virus infection (variables with p < 0.050 in the univariate analysis were included).

Factor	Odds Ratio	95% CI	p
Age > 65 years	4.1	0.9 – 18.8	0.069
Household with 3 or more residents	7.9	1.7 – 35.9	0.008
Transfusion of blood products	6.2	2.1 – 18.6	0.001

CI: confidence interval.

A multiple logistic regression analysis was performed using the presence of HCV infection as the dependent variable. The following variables with p < 0.050 were included in the regression model: age > 65 years, households with 3 or more residents and the transfusion of blood products. The variable transfusion before 1993 was not included because the blood transfusion variable was already included in the model. In the multivariate analysis **(Table 3)**, the variables that were independently associated with HCV infection were households with 3 or more residents (OR 7.9, 95% CI 1.7 – 35.9, p = 0.008) and a history of blood transfusion (OR 6.2, 95% CI 2.1 – 18.6, p = 0.001). The Hosmer-Lemeshow goodness-of-fit test yielded a p value of 0.179; thus, the final model was considered adequate. The AUROC of the final model was 0.827 (95% CI 0.690 – 0.965, p < 0.001), which was indicative of good discrimination capability.

DISCUSSION

To our knowledge, this is the first population-based study in a Brazilian city with the aim of determining the prevalence of HCV infection among elderly individuals. Most previous Brazilian studies on the prevalence of hepatitis C have significant limitations and have been generally conducted with small groups that were usually not representative of the general population (e.g., blood donors). In addition, the inappropriate sample sizes and the inadequate methodology that were often used may have restricted the validity and generalizability of the results of these studies.

The seroprevalence of hepatitis C (anti-HCV reactive in the chemiluminescence assay) in this study was 2.2% (95% CI 1.2% – 3.2%), and the prevalence of confirmed current HCV infection was 1.7% (95% CI 1.0% – 2.9%). These prevalences are significantly higher than those observed in the studies of blood donors, which found a seroprevalence of 1.2% in Brazil, 0.3% in the State of Santa Catarina and 0.3% in the City of Tubarão[20,21]. A population-based study conducted in São Paulo, Brazil, found that the prevalence of anti-HCV antibodies was 1.4% in the general population and 3.2% among individuals older than 60 years, suggesting that the prevalence is higher than that observed in studies of blood donors and is even higher among elderly individuals[22]. However, only 90 individuals older than 60 years were tested in that study, which may have adversely impacted the reliability and validity of the outcomes. The divergence between the prevalence detected among blood donors and that observed in the present study suggests that there is a higher prevalence of hepatitis C among elderly individuals. However, this difference may reflect other specific characteristics of blood donors in whom lower HCV infection rates are often observed.

In 2011, the data from the largest study ever conducted in Brazil on the prevalence of viral hepatitis were released[15]. The study included 19,634 individuals aged between 10 and 69 years who were residents of Brazilian state capitals. The overall prevalence of anti-HCV positivity was 1.4%, ranging from 0.7% in the Northeast Region to 2.1% in the North Region. In the South Region, the prevalence was 1.2%, which is lower than that observed in the elderly population in the municipality of Tubarão. When considering only subjects aged between 60 and 69 years, the study conducted by the Ministry of Health found a global seroprevalence of 3.3%. However, no data regarding the different age groups were reported specifically for the South Region[15]. Despite its undeniable importance, that study had several major limitations, such as the inclusion of individuals aged only up to 69 years and the relatively small number of elderly individuals who were included (862 individuals). In addition, because the study included only populations from large cities, the results most likely do not accurately reflect the epidemiological characteristics of small- and medium-sized cities.

In the univariate analysis, the variables age older than 65 years, households with 3 or more residents and the transfusion of blood products were associated with HCV infection. The last 2 variables remained independently associated with HCV infection in the logistic regression analysis.

Although the direct relationship between age and the prevalence of hepatitis has already been described, few studies have specifically investigated older age as a factor associated with HCV infection[23]. However, as previously mentioned, the 2 main Brazilian population-based studies have identified a higher prevalence of HCV among older individuals[15-24]. It is possible that elderly subjects have lived most of their lives exposed to potential risk factors, such as the transfusion of contaminated blood products and medical and therapeutic procedures performed without standard precautionary measures. Although age was not associated with HCV infection in the multivariate analysis, in this study, the prevalence in individuals older than 65 years was 4 times greater than that in the other age groups (2.5% vs. 0.6%). These findings are most likely related to a shift in the incidence curve toward older age after appropriate measures to control HCV transmission were implemented in Brazil, particularly the implementation of anti-HCV screening in blood banks in the early 1990s[23].

In the present study, an association between HCV infection and living in a household with 3 or more residents was observed. There have been few studies on the relationship between HCV infection and the number of people in the household. Presumably, this finding may be linked to the household transmission of HCV, either related or unrelated to sexual activities[25]. Another plausible explanation is that the number of people in the household is a confounding variable that may be connected to other socioeconomic or exposure variables that were not investigated in this study.

Previous blood transfusions were independently associated with HCV infection in this study. The variable transfusion before 1993 was not included in the regression analysis because the blood transfusion variable was already included in the model. The broader variable (previous blood transfusions) was used in an attempt to overcome a possible recall bias related to the more restricted variable (transfusion before 1993). An important study that investigated the period between 1960 and 1991 demonstrated the relevance of blood transfusion as a risk factor for HCV infection[26]. In that study, between 5% and 15% of blood-product recipients were infected with HCV[26].

After the adoption of anti-HCV screening tests, the risk of HCV infection through blood transfusion was significantly reduced and it is currently about one in 1,657,722 units of blood transfused in the United States of America[27]. A Brazilian study showed that the probability of contamination by HCV was still 1 in 13,721 units of blood transfused[28], a significantly higher rate than that observed in developed countries[28]. In Brazil, the routine use of molecular biology-based tests to screen blood units has been implemented over the past years. This procedure will most likely reduce the rate of post-transfusion HCV infection. It is likely that the findings of the present study reflect greater exposure to contaminated blood products before the beginning of systematic HCV screening. This hypothesis is corroborated by the trend toward a higher frequency of exposure to blood products before 1993 observed among those with HCV infection than among their uninfected counterparts.

There was no association between a history of invasive procedures and HCV infection. Despite the lack of reliable data, it is estimated that approximately 2 million individuals worldwide are infected by HCV each year because of invasive procedures[29]. It is possible that these findings are related to the high proportion of individuals that were previously subjected to procedures that were considered invasive. Thus, it is likely that a more detailed variable that includes the type of procedure (e.g., in-patient or out-patient), the magnitude or extent (small, medium and large) and the individual who performed the procedure (i.e., health professional or layperson) would provide better information on this risk factor.

Other important exposure variables, such as illicit drug use, acupuncture and tattooing, were also not associated with HCV infection. With regard to drug use and a history of tattooing, the low frequencies observed in this study explain the outcomes. However, intravenous drugs and inhaled cocaine have been found to be associated with HCV infection and remain important risk factors[15,30]. Acupuncture remains controversial as a risk factor for HCV infection. Although some studies have observed an association between HCV infection and acupuncture treatment, this association was weak and predominantly found in Asian studies[31].

Some limitations of this study should be discussed. First, the differences between the included and excluded individuals regarding marital status and the proportion of subjects who share personal items with non-household members may suggest selection bias. However, such bias is unlikely because the proportion of excluded subjects was relatively small, and these excluded individuals were similar to the included individuals with respect to all other major characteristics. Finally, the study design used does not allow the establishment of direct causal relationships or the temporal sequence between the variables investigated and HCV infection. This issue is a common limitation of cross-sectional studies and can be resolved with cohort studies[32]. Nevertheless, cross-sectional population-based studies represent useful tools for public health planning, understanding disease etiology and hypothesis generation[33].

In conclusion, the prevalences of positive serology for hepatitis C and confirmed HCV infection in the elderly population of the municipality of Tubarão were 2.2% and 1.7%, respectively, which are substantially higher than the results observed in studies of blood donors performed in the same municipality. HCV infection was associated with older age (univariate analysis), a higher number of people in the household and the transfusion of blood products (in univariate and multivariate analyses). The association between a greater number of household members and HCV infection may be related to household HCV transmission or other socioeconomic and exposure variables that were not investigated in this study. The association between older age and blood-product transfusion confirms the importance of transfusion risk, particularly among elderly individuals, and reinforces the hypothesis that the prevalence of HCV is higher in older age groups.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

This study was supported by *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina* (FAPESC) and Tubarão City Hall.

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