SARS-CoV-2 Antibody Prevalence in the Portuguese Municipality of Vila Nova de Gaia after the First Wave of the Pandemic

Prevalência de Anticorpos Contra SARS-CoV-2 na População Residente no Município Português de Vila Nova de Gaia após a Primeira Onda da Pandemia

Álvaro DE CARVALHO✉[1], Ana VIRGOLINO[2,3], Paula QUEIRÓS[4], Ana Rita HENRIQUES[5,6], Helena CANHÃO[5,6], Ana Maria RODRIGUES[5,6], Veneranda BARBOSA[1], Joana RODRIGUES[4], José GERMANO DE SOUSA[7], Miguel GUIMARÃES[8]

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ABSTRACT

Introduction: Assessment of SARS-CoV-2 seroprevalence may detect the real spread of the virus because antibody data can provide a long-lasting measure of infection. Existing serological studies in Portugal have tested new serology methods, albeit with small sample sizes and a lack the focus on geographical regions with a high rate of infection cases. The aim of this study was to estimate the serological prevalence of SARS-CoV-2 in Vila Nova de Gaia, the most populous municipality in the north of Portugal and one of those most affected during the first pandemic wave.

Material and Methods: A cross-sectional observational study was conducted between June 23rd and July 17th, 2020. Included in the cohort were 18- to 74-year-old men and women living in the municipality of Vila Nova de Gaia, who were sampled through a non-probabilistic quota-based approach. Cases with a previous RT-PCR diagnosis of COVID-19 were excluded. Sociodemographic and clinical information was collected using a self-administered, written questionnaire. Blood samples were collected for serological laboratory analysis to detect and quantify SARS-CoV-2 anti-IgG antibodies.

Results: We tested 2754 participants. Our results show a SARS-CoV-2 seroprevalence of 3.03% (95% confidence interval: 2.37% – 3.87%). Being a smoker (odds ratio: 0.382, 95% confidence interval: 0.147 – 0.99) and having symptoms of COVID-19 (odds ratio: 2.480, 95% confidence interval: 1.360 – 4.522) were consistently associated with lower and higher odds of SARS-CoV-2 antibody presence, respectively, regardless of the analytic design. Moreover, without adjusting for any variables, having had contact with an infected person within the household was associated with increased odds of a positive test (odds ratio: 9.684, 95% confidence interval: 4.06 – 23.101); after adjusting, having self-reported chronic diseases (odds ratio: 0.448, 95% confidence interval: 0.213 – 0.941) was associated with decreased odds.

Conclusion: This was the first study to estimate the serological prevalence of SARS-CoV-2 in one of the most populous municipalities in Portugal, representing the first step in the development of an epidemiological surveillance system in Portugal, which can help to improve the diagnosis of COVID-19.

Keywords: Antibodies, Viral; COVID-19; Epidemiological Monitoring; Portugal; SARS-CoV-2; Seroepidemiologic Studies

RESUMO

Introdução: A estimativa da seroprevalência de SARS-CoV-2 pode detetar a real disseminação do vírus uma vez que os dados sobre anticorpos podem permitir determinar a evolução da infeção ao longo do tempo. Em Portugal, os estudos serológicos existentes têm sido utilizados sobretudo para testar novos métodos, sendo, no entanto, realizados com amostras de pequena dimensão. Além disso, estes estudos não se têm focado nas regiões geográficas com o maior número de casos de infeção. Este estudo teve como principal objetivo estimar a prevalência serológica de SARS-CoV-2 em Vila Nova de Gaia, Portugal, o município mais populoso do norte do país e um dos mais afetados pela primeira onda da pandemia.

Material e Métodos: Estudo observacional transversal conduzido entre 23 de junho e 17 de julho de 2020. Foram incluídos adultos, com idades compreendidas entre os 18 e os 74 anos, de ambos os sexos, residentes numa das 15 freguesias do município de Vila Nova de Gaia. Foi seguida uma amostragem com recurso a uma abordagem não probabilística por quotas. Casos de indivíduos com um diagnóstico prévio de COVID-19 com teste RT-PCR foram excluídos. Os dados sociodemográficos e clínicos foram recolhidos através de questionário autopreenchido, em papel. Foram ainda recolhidas amostras de sangue para análise laboratorial serológica para deteção e quantificação de anticorpos anti-IgG contra SARS-CoV-2.

Resultados: Foram testados 2754 participantes. Os nossos resultados mostram uma seroprevalência de SARS-CoV-2 de 3,03% (intervalo de confiança 95%: 2,37 – 3,87%). Ser fumador (OR: 0,382, intervalo de confiança 95%: 0,147 – 0,99) e apresentar sintomas de COVID-19 (OR: 2,480, intervalo de confiança 95%: 1,36 – 4,522) foram observados como estando associados a menor e maior probabilidade de presença de anticorpos SARS-CoV-2, independentemente do desenho analítico. Sem ajustamento para qualquer variável, o contacto com uma pessoa infetada dentro do domicílio (OR: 9,684, intervalo de confiança 95%: 4,06 - 23,101) esteve

1. Fundação Álvaro Carvalho. Lisboa. Portugal.
2. Instituto de Saúde Ambiental. Faculdade de Medicina. Universidade de Lisboa. Lisboa. Portugal.
3. Laboratório Associado TERRA. Faculdade de Medicina. Universidade de Lisboa. Lisboa. Portugal.
4. Fundação Vox Populi. Lisboa. Portugal.
5. EpiDoC Unit. Chronic Diseases Research Center (CEDOC). NOVA Medical School. Universidade NOVA de Lisboa. Lisboa. Portugal.
6. Comprehensive Health Research Center (CHRC). NOVA Medical School. Universidade NOVA de Lisboa. Lisboa. Portugal.
7. Germano de Sousa Group. Centro de Medicina Laboratorial. Pólo Tecnológico de Lisboa. Lisboa. Portugal.
8. Ordem dos Médicos Portugueses. Lisboa. Portugal.

✉ **Autor correspondente:** Álvaro de Carvalho. alvaro.e.carvalho@gmail.com

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associado com aumento da chance de ter um teste positivo. Após ajustamento, ter doenças crónicas autorreportadas (OR: 0,448, intervalo de confiança 95%: 0,213 - 0,941) esteve associada à diminuição da chance de ter um teste COVID-19 positivo.
Conclusão: Este foi o primeiro estudo a estimar a prevalência serológica do SARS-CoV-2 num dos municípios mais populosos de Portugal, constituindo o primeiro passo para o desenvolvimento de um sistema de vigilância epidemiológica em Portugal, que pode ajudar a melhorar o diagnóstico da COVID-19.
Palavras-chave: Anticorpos Antivirais; COVID-19; Estudos Seroepidemiológicos; Portugal; SARS-CoV-2; Vigilância Epidemiológica

INTRODUCTION

At the end of 2019, the World Health Organization (WHO) was officially notified of the occurrence of a new pneumonia cluster of unknown etiology in Wuhan, China.[1] The agent responsible for this new infectious disease, later labeled as coronavirus disease 2019 (COVID-19), was a new coronavirus—severe acute respiratory syndrome coronavirus 2, or SARS-CoV-2.[2] Following the rapid spread of the virus in several countries worldwide, the WHO declared COVID-19 to be a pandemic in March 2020.[3,4] Since the reported outbreak, the virus has caused more than 5 million deaths worldwide and almost 466 million confirmed cases of COVID-19, according to data from the WHO.[5]

At the diagnostic level, the COVID-19 pandemic presents certain key challenges. Several diagnostic strategies can be used to confirm or discard the infection and test previous infections and immune responses.[6] Serological tests to detect antibodies against SARS-CoV-2 are useful tools for epidemiological surveillance and can improve the diagnosis of COVID-19.[7] Many SARS-CoV-2 infections are not reported due to a considerable proportion of asymptomatic cases. To address this, seroprevalence testing may be able to detect the real spread of the virus because antibody data provide a long-term measure of SARS-CoV-2 infection. Most infected people have an IgG antibody response detectable 14 to 21 days after infection. Furthermore, although IgG levels may start to decrease approximately 90 days after infection, they remain detectable for much longer.[8] Population serological testing that measures SARS-CoV-2–specific IgG antibody titers can therefore be used to estimate the total number of infections by assessing the number of individuals who have mounted an immune response regardless of whether their infections were subclinical or happened in the recent past.[9] By providing estimates of who is and is not immune to SARS-CoV-2, serological data can be used to estimate several epidemiological variables, such as the attack rate and case fatality rate, which are necessary to assess the rate of community transmission and its burden.[10]

In the first seroepidemiological studies carried out in Europe, SARS-CoV-2 seroprevalence varied between 0% and 8.5%, depending, for example, on the interval between the date of the study and the beginning of the epidemic, the target population under study, and the type of tests used.[11] Two serological studies have been reported so far in Portugal.[12,13] Figueiredo-Campos *et al*[12] reported the development of a new SARS-CoV-2 serology method that enabled the identification of peak antibody production three weeks after infection. The antibody response was still detectable after at least six months and was correlated with the severity of the disease. However, the study only targeted COVID-19 patients and healthy volunteers from a specific location - the University of Lisbon - leaving other affected regions of the country unobserved. On the other hand, Kislaya *et al*[13] included a national sample of the Portuguese population, but the small number of participants from each region was a major limitation of the study.

This study aimed to estimate the serological prevalence of SARS-CoV-2 in Vila Nova de Gaia, Portugal, in June and July 2020, in individuals who had been neither diagnosed with COVID-19 nor hospitalized. Vila Nova de Gaia is the most populous municipality in the north of Portugal (according to 2021 data)[14] and provides a detailed socioeconomic picture of the country as it is characterized by primary, secondary, and tertiary economical activities and both urban and rural areas. Furthermore, it was one of the most affected municipalities by the first pandemic wave.[15] At that time, and given the lack of knowledge about the pandemic evolution, efforts were made to develop an information system for immunity to COVID-19 in Portugal. For this purpose, immunity tests were performed on the population of Vila Nova de Gaia.

MATERIAL AND METHODS

A cross-sectional observational study was undertaken between June 23rd and July 17th, 2020, with individuals aged 18 years or older living in any of the 15 parishes of Vila Nova de Gaia, Portugal. The considered period began after the end of the first national lockdown implemented by the Portuguese Government, when the imposed restrictive measures were eased.[16]

Study participants and sampling

The population under study included men and women between 18 and 74 years of age living in any of the 15 parishes of the municipality of Vila Nova de Gaia, Portugal. Individuals with a previous diagnosis of COVID-19 (determined by the RT-PCR diagnostic test) were excluded.

The sampling followed a non-probabilistic quota-based approach by sex, age groups (18 – 44, 45 – 64, and 65 – 74), and parishes. Key social institutions in Vila Nova de Gaia invited the individuals that they provided support to within their regular activities (sequential sampling procedure) to take part in the study and directed them to the venues prepared for data collection. Regular contact with these institutions was ensured by the research team in order to recruit potential participants within the necessary quotas. For households with more than one eligible family member, participation was limited to one person from each family.

Considering a sample of 2500 people and an immunity rate of 2%, there is a 95% probability that the real prevalence estimate is between 1.44% and 2.56%. To reduce the

Table 1 – Seroprevalence of SARS-CoV-2 by smoking habits and sociodemographic and professional characteristics (weighted data). Univariate logistic regression model.

	Seroprevalence (IgG) n	Seroprevalence (IgG) (95% CI)	*β*	*p*-value	OR[a]	95% CI[b]
Total	83	3.03 (2.37 – 3.87)	-	-	-	-
Sex						
Female	46	3.19 (2.35 – 4.32)	Ref			
Male	37	2.87 (1.92 – 4.27)	−0.12	0.658	0.89	0.531 – 1.492
Age group (years)						
18 – 34	21	3.89 (2.25 – 6.63)	Ref			
35 – 54	33	2.33 (1.6 – 3.37)	−0.528	0.128	0.59	0.299 – 1.164
≥ 55	29	3.74 (2.5 – 5.55)	−0.039	0.914	0.962	0.479 – 1.933
Household size						
1 element	9	5.54 (2.29 – 12.42)	Ref			
2 elements	17	2.51 (1.5 – 4.16)	−0.805	0.127	0.447	0.159 – 1.258
≥ 3 elements	53	2.96 (2.17 – 4.02)	−0.637	0.188	0.529	0.205 – 1.365
Education level						
Basic or upper secondary education	54	3.06 (2.26 – 4.13)	Ref			
Higher (university) education	29	2.98 (1.95 – 4.53)	−0.025	0.926	0.975	0.572 – 1.662
Professional situation						
Professionally active	58	2.78 (2.08 – 3.7)	Ref			
Other	25	3.84 (2.41 – 6.07)	0.336	0.244	1.399	0.795 – 2.462
Workplace[c]						
At home	28	2.63 (1.68 – 4.07)	Ref			
On-site	23	2.67 (1.75 – 4.04)	0.017	0.958	1.017	0.545 – 1.899
Other	4	6.25 (1.53 – 22.26)	0.906	0.233	2.473	0.557 – 10.977
Smoking habits						
Never smoker	58	3.52 (2.62 – 4.73)	Ref			
Former smoker	17	3.08 (1.77 – 5.32)	−0.138	0.675	0.871	0.457 – 1.661
Smoker	8	1.56 (0.78 – 3.1)	−0.837	**0.032**	0.433	0.202 – 0.93

[a]OR: odds ratio; [b]CI: confidence interval; [c]: Only for professionally active.
Note: Statistically significant values are denoted in bold; Sample size is not constant due to missing data: total (n = 83); sex (n = 83); age group (n = 83); household size (n = 79); education level (n = 83); professional situation (n = 83); workplace (n = 55); smoking habits (n = 83).

range of the weighting matrix coefficients and minimize the sampling error associated with the total sample size, an attempted sample with proportional size to the resident population and a quota matrix (sex/age) was designed for each parish.

Study procedures and data collection

Data collection took place between June 23rd and July 17th, 2020, including participants from the 15 parishes in the municipality of Vila Nova de Gaia.

Participants were asked to answer a self-administered, paper-and-pencil questionnaire before the collection of blood samples. A trained person from the research team remained present to answer questions that arose while participants completed the questionnaire. The questionnaire comprised questions related with: i) sociodemographic information (age, sex, parish of residence, education, and professional situation); ii) history of chronic non-communicable diseases; iii) smoking habits, due to their association with the transmissibility of the virus and the disease progression; iv) symptoms compatible with COVID-19; and v) epidemiological history (namely, contact with a confirmed case of COVID-19, inside or outside the household).

A 5 mL blood sample was collected from each participant after completing the survey. Serological laboratory analysis was performed for all samples. Venous blood was collected in BD Vacutainer® SST® tubes, which contain a clot activator and separator gel to obtain serum. The tests used were the LIAISON® SARS-CoV-2 IgG tests from DiaSorin, performed on Liaison XL. All tests met the requirements of the manual of good laboratory practices and were carried out in duplicate whenever required to confirm the result. A chemiluminescence immunoassay was used to test participants for antibodies to SARS-CoV-2. The test detects and quantifies anti-SARS-CoV-2-IgG antibodies. A case was considered seropositive for SARS-CoV-2 when concentrations ofanti-SARS-CoV-2-IgG antibodies were greater than or equal to 15 U/mL, which was established by the manufacturer

(DiaSorin). All reagents used were *in-vitro* diagnostics that were subject to previous performance tests. Internal validation of the reagents was performed for all tests (correlation tests). The test has a clinical sensitivity of 98.7% and a clinical specificity of 99.5%.

Up to 72 hours after blood sample collection, participants received their test results by e-mail.

Statistical analysis

All results were weighted by sex (male, female), age group (18 – 44, 45 – 64, 65 – 74 years), and parish of residence using data from the Portuguese 2011 Census.[17] Weighting allowed the sociodemographic variables of the sample distribution to more closely match the distribution of those of the Portuguese population. For the weighted sample, the final margin of error was established as two times the standard deviation of the binomial distribution of the means for the *p*-value obtained in the study. The degree of confidence that the real value is in the range was 95%.

Relative and absolute frequencies of the categorical variables were reported using descriptive statistics. All parameters and estimations are presented with 95% confidence intervals (95% CIs).

The seroprevalence for IgG antibodies was estimated for the total population and stratified by sex (male and female), age group (18 – 34, 35 – 54, and ≥ 55), household size (1, 2, and ≥ 3 elements), education level [basic or upper secondary education and higher (university) education], professional situation (professionally active and other), workplace (at home, on-site, and other), smoking habits (non-smoker, ex-smoker, and smoker), self-reported chronic diseases, COVID-19 related symptoms, and contact with an infected person. For all point estimates, 95% CIs were calculated and weighted according to the sampling design.

Participants with positive and negative results were compared through univariate logistic regression for complex sample design considering each of the variables under study.

Logistic regression models for complex sample design were used to assess the characteristics associated with seropositivity. For the model, those variables with a significant bivariate test in simple models ($p < 0.25$) were selected as candidates for the multiple regression analysis; the significance level in the multiple regression analysis was set to $\alpha = 0.05$. The variables representing sex and contact with an infected person were forced into the model.

All statistical analyses were performed using STATA/IC 16.1 software (StataCorp, 2019) with Stata's facilities for survey data analysis, *svy*. A significance level of $\alpha = 0.05$ was considered.

Ethical approval

The study was carried out following the Declaration of Helsinki principles.[18] Furthermore, the study received approval from the National Medical Ethics and Deontological Council of the Portuguese Medical Association (Conselho Nacional de Ética e Deontologia Médicas da Ordem dos Médicos Portugueses) before it began. All participants who agreed to take part in the study gave their written informed consent before their participation.

RESULTS

Overall, 2754 participants were tested, nearly 70% of whom were 40 years of age or over. The detailed sociodemographic characterization of the sample with both unweighted and weighted data is presented in Appendix 1, Tables A.1 and A.2 (https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/17676/Appendix_01.pdf).

The seroprevalence of SARS-CoV-2 by smoking habits and sociodemographic and professional characteristics is detailed in Table 1. No statistically significant differences were observed between the two groups (i.e., negative test and positive test) for most of the considered variables, except for smoking habits. Smokers presented lower odds of receiving a positive test [odds ratio (OR): 0.433, 95% CI: 0.202 – 0.93] than non-smokers.

Table 2 presents the seroprevalence of SARS-CoV-2 by clinical variables, namely self-reported chronic disease, self-reported COVID-related symptoms, and previous contact with an infected person. According to the univariate logistic regression model, having had COVID-19–related symptoms and having had contact with an infected person within the household increased the odds of receiving a positive test (OR: 2.54, 95% CI: 1.479 – 4.361; OR: 9.684, 95% CI: 4.06 – 23.101, respectively).

When adjusting for the variables under study, both being a smoker and having chronic diseases (self-reported) lowered the odds of receiving a positive test (OR: 0.382, 95% CI: 0.147 – 0.999; OR: 0.448, 95% CI: 0.213 – 0.941, respectively), whereas those who reported having had COVID-19–related symptoms had higher odds for presenting a positive test result (OR: 2.48, 95% CI: 1.36 – 4.522), as shown in Table 3.

Smoking habits and presence of self-reported comorbidities stratified by having had contact with an infected person and living with an infected person are presented in the Appendix 1, Table A.3 (https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/17676/Appendix_01.pdf). The seroprevalence in participants with contact with an infected person or who lived with an infected person for the factors - smoking habits and presence of self-reported comorbidities, are also presented in Appendix 1, Table A.4 (https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/17676/Appendix_01.pdf).

DISCUSSION

This study was the first to investigate the serological prevalence of SARS-CoV-2 in one of the Portuguese regions most affected by the first wave of the COVID-19 pandemic. Our results show a prevalence of 3.03% (95% CI: 2.37% – 3.87%), which is slightly higher than that reported by results from a previous study conducted nationwide in Portugal, which estimated 2.1% IgG positivity against

Table 2 – Seroprevalence of SARS-CoV-2 by clinical variables (weighted data). Univariate logistic regression model.

	Seroprevalence (IgG) n	Seroprevalence (IgG) (95% CI)	β	*p*-value	OR[a]	95% CI[b]
Self-reported chronic disease						
Yes (total)	23	2.23 (1.45 – 3.42)	−0.446	0.120	0.64	0.365 – 1.123
Diabetes	6	3.49 (1.50 – 7.93)	0.158	0.730	1.171	0.477 – 2.875
Arterial hypertension	7	1.63 (0.75 – 3.47)	−0.719	0.086	0.487	0.215 – 1.106
Other cardiovascular disease	2	1.70 (0.5 – 5.45)	−0.612	0.323	0.542	0.161 – 1.827
Respiratory disease	10	3.36 (1.68 – 6.61)	0.119	0.758	1.126	0.528 – 2.402
Chronic kidney disease	1	5.54 (5.46 – 38.49)	0.634	0.552	1.885	0.234 – 15.191
Oncological disease	2	2.97 (0.4 – 19)	−0.021	0.983	0.979	0.133 – 7.215
Autoimmune disease	1	0.97 (0.23 – 3.96)	−1.186	0.105	0.305	0.073 – 1.283
Obesity	-	-	-	-	-	-
Other	4	1.88 (0.64 – 5.39)	−0.521	0.357	0.600	0.196 – 1.802
Having (self-reported) COVID-19–related symptoms	27	5.98 (3.92 – 9.00)	0.932	**0.001**	2.54	1.479 – 4.361
Having had contact with an infected person						
Yes (total)	44	3.53 (2.52 – 4.93)	0.291	0.261	1.337	0.805 – 2.220
Within household	11	19.99 (9.63 – 36.93)	2.270	**< 0.001**	9.684	4.06 – 23.101
A relative (outside the household)	10	3.53 (1.60 – 7.62)	0.083	0.858	1.087	0.439 – 2.686
Friend	8	2.38 (0.98 – 5.65)	−0.449	0.370	0.638	0.239 – 1.703
Colleague	19	5 (3.10 – 7.96)	0.704	0.058	2.021	0.977 – 4.182
Other	13	3.39 (1.83 – 6.17)	0.031	0.937	1.032	0.477 – 2.23

[a]OR: odds ratio; [b]CI: confidence interval

Note: Statistically significant values are denoted in bold; self-reported chronic disease (n = 68); diabetes (n = 83); arterial hypertension (n = 83); other cardiovascular diseases (n = 83); respiratory disease (n = 83); chronic kidney disease (n = 83); oncological disease (n = 83); autoimmune disease (n = 83); obesity (n = 83); other (n = 83); COVID-19–related symptoms (n = 83); contact with an infected person (n = 83); within household (n = 42); relative (n = 40); friend (n = 40); colleague (n = 40); other (n = 40).

SARS-CoV-2 for the whole northern region of the country.[13] This difference can be explained by the fact that the northern region includes 86 Portuguese municipalities in addition to Vila Nova de Gaia (according to the Nomenclature of Territorial Units for Statistics), which, when considered, may have affected the serological results as Vila Nova de Gaia was one of the most affected municipalities during the period of data collection.[15] Our results are in line with those of a previous study conducted by Portuguese healthcare professionals, which reported a SARS-CoV-2 seroprevalence rate of 3.1%.[19]

A result of this study worthy of note is that smokers had a lower odds of SARS-CoV-2 antibody presence. A recent systematic literature review shed some light on this paradoxical effect (also known to be associated with cardiovascular-related outcomes)[20,21] by concluding that the potential protection from COVID-19 through interaction with smoking can be attributed to specific biological mechanisms.[22] First, a smoker's immune system presents a decreased response due to chronic inflammation, which can decrease the production of TNF, IL-1, and IL-6 and play a protective role against cytokine storm syndrome, a hyperinflammatory response responsible for the pathophysiology of severe cases of COVID-19.[22–25] Another explanation may be that the high concentrations of nitric oxide in smoke hinder the replication and entry of SARS-CoV-2 into the cells by maintaining airway dilation and filtration before nitric oxide enters the lungs.[22,26,27] Our results are supported by previous studies conducted with patients hospitalized with COVID-19, which reported only a small number of smokers.[28] Furthermore, smokers have a decreased risk of SARS-CoV-2 antibody presence compared with non-smokers.[29] However, as most of these studies were published at the beginning of the pandemic when scientific information was being published at a fast pace, the conclusions drawn must be considered with caution. Furthermore, several methodological flaws of the early studies have been acknowledged, namely that i) smoking was not often one of the variables of primary interest, resulting in a vast amount of missing data on smoking status; ii) the extrapolation in the determination of the prevalence of COVID-19 in hospitalized individuals was based on the prevalence of smoking in the general population; iii) no adjustment was made for several factors that are known to have an association with smoking status; and iv) there was bias related with selection and misclassification.[30] Moreover, some authors raised the question of pressure from the tobacco industry, which benefited from the media coverage because claims about a protective effect of smoking were associated with an increase in tobacco consumption.[30,31] Against this background, similar evidence that smokers are more vulnerable to infections, including respiratory infections, has been noted in previous pandemics (Middle East respiratory syndrome coronavirus, influenza, and pneumococcal disease).[30,32,33]

Table 3 – OR for the likelihood of being seropositive (IgG positive; ≥ 15 U/mL) according to demographic and clinical characteristics. Multivariable logistic regression model.

	n (%)	β	*p*-value	OR[a]	95% CI[b]
Sex					
Female	1150 (52.05%)	Ref			
Male	1059 (47.95%)	−0.022	0.945	0.978	0.522 – 1.831
Age group (years)					
18 – 34	456 (20.64%)	Ref			
35 – 54	1112 (50.33%)	−0.425	0.256	0.654	0.314 – 1.361
≥ 55	641 (29.03%)	0.082	0.830	1.086	0.512 – 2.302
Educational level					
Basic or upper secondary education	1407 (63.70%)	Ref			
Higher (university) education	802 (36.30%)	−0.139	0.671	0.871	0.459 – 1.650
Professional situation					
Professionally active	1662 (75.25%)	Ref			
Other	547 (24.75%)	0.223	0.492	1.25	0.662 – 2.359
Household size					
1 element	133 (6.02%)	Ref			
2 elements	579 (26.23%)	−1.071	0.065	0.343	0.110 – 1.070
≥ 3 elements	1497 (67.75%)	−0.916	0.072	0.400	0.148 – 1.085
Having had contact with an infected person					
No	1210 (54.76%)	Ref			
Yes	999 (45.24%)	0.175	0.544	1.192	0.676 – 2.101
Smoking habits					
Never smoker	1351 (61.16%)	Ref			
Former smoker	459 (20.77%)	−0.227	0.535	0.797	0.388 – 1.635
Smoker	399 (18.07%)	−0.962	**0.048**	0.382	0.147 – 0.99
Having (self-reported) COVID-19–related symptoms					
No	1845 (83.5%)	Ref			
Yes	364 (16.50%)	0.908	**0.003**	2.480	1.360 – 4.522
Self-reported chronic diseases					
No	1223 (55.38%)	Ref			
Yes	986 (44.62%)	−0.803	**0.034**	0.448	0.213 – 0.941

[a]OR: odds ratio; [b]CI: confidence interval

In our study, we also observed that participants with comorbidities showed decreased odds of receiving a positive test. Although evidence points to a higher susceptibility to infection and worse disease progression in patients with pre-existing chronic conditions, namely diabetes, hypertension, chronic respiratory illness, and chronic kidney and liver conditions (associated with increased receptor expression of the angiotensin-converting enzyme 2, which facilitates the entry of the virus, cytokine storm syndrome, and possible interaction of medications),[34,35] it has also been observed that individuals with chronic diseases tend to adhere to COVID-19 preventive behaviors, although to different degrees depending on the health condition,[36,37] which can justify our findings. Nevertheless, a worrisome conclusion from the existing studies shows that compliance with recommendations can go from wearing a mask, washing hands, and avoiding crowded places to postponing healthcare appointments, hospital visits, or work.[37] This signals a major impact of COVID-19 on general health as well as mental health and population-wide well-being.

Finally, our findings suggest that COVID-19–related symptoms are associated with SARS-CoV-2 antibody presence. While this is not a novel result, it reinforces the need to adopt individual protective behaviors to prevent the spread of the virus. Several studies have shown that even asymptomatic patients can be a source of transmission.[38–40]

Our study has some limitations that should be considered when interpreting the results. First, it has a cross-sectional design, hindering the possibility of studying the disease progression and establishing causality. As a result, disease determination and the identification of associated risk factors were conducted simultaneously. Conclusions, such as the decreased risk of SARS-CoV-2 infection in smokers versus non-smokers and in individuals with chronic

health conditions, should always be considered along with the study design. However, the data were collected while restrictive measures were easing after the first national lockdown in one of the most affected Portuguese municipalities (data from June 23rd, 2020, show that Vila Nova de Gaia had the third highest number, in absolute terms, of reported COVID-19 cases of all Portuguese municipalities)[15] and from a rather large number of people, which provided an important, although static, picture of the situation at that moment. Second, selection bias may have occurred due to the voluntary nature of participation. Even so, we ensured that only one person from each household took part in the study to minimize clustering bias. On the other hand, as individuals with a previous diagnosis of COVID-19 were excluded, selection bias may have contributed to the absence of statistically significant differences in the observed results. The decreased odds could also be related with the characteristics of the population under study, for example individuals who were unaware they were infected. Comorbidities are also a risk factor for more severe disease, so it was expected that individuals with these characteristics would not be included in the sample.

CONCLUSION

This was the first study to estimate the serological prevalence of the SARS-CoV-2 in one of the most populous municipalities in Portugal and one of the e most affected during the first pandemic wave. Overall, it was observed that smokers and people with chronic diseases were more likely to be protected against SARS-CoV-2 infection, whereas having COVID-19–related symptoms was associated with an increased risk of being infected. More regular monitoring of the pandemic evolution is needed in order to develop an information system on immunity to COVID-19 in Portugal.

AUTHORS CONTRIBUTION

AC: Conceptualization; methodology; investigation; writing review and editing; supervision; approval of the final version.

AV: Writing original draft; writing review and editing; approval of the final version.

PQ: Conceptualization; investigation; resources; data curation; writing review and editing.

ARH: Formal analysis; data curation; writing original draft preparation; writing-review and editing.

HC: Formal analysis; writing review and editing; supervision; approval of the final version.

AMR: Writing review and editing, approval of the final version.

VB: Research; resources; writing review and editing.

JR: Data curation; research; resources; writing review and editing.

GS, MG: Conceptualization; research; resources; writing review and editing; supervision.

PROTECTION OF HUMANS AND ANIMALS

The study was conducted according to the guidelines of the Declaration of Helsinki updated in 2013 and approved by the National Ethical and Deontological Committee of the Portuguese Medical Association.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication. The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy reasons.

PATIENT CONSENT

Informed consent was obtained from all subjects involved in the study.

COMPETING INTERESTS

The authors declare no conflicts of interest.

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