Bacillus Calmette-Guérin Vaccination Policy and Consumption of Ammonium Chloride-Enriched Confectioneries May Be Factors in Reducing COVID-19 Death Rates in Europe

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Abstract

Background: The 2019 severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic continued into 2020, and the coronavirus disease-2019 (COVID-19) associated death toll increased. Preparations for a second outbreak were considered as a population-based preventative measure.

Objectives: To analyze COVID-19 rates in European countries or regions to determine whether there was a significant association between bacillus Calmette-Guérin (BCG) vaccination policy and lower rates of COVID-19 related deaths.

Methods: Certain Northern European countries or regions had low death rates regardless of BCG policy. The authors assumed the consumption of foods containing salmiak (NH_4Cl) was a common and peculiar cause of the reduced COVID-19 related death rates. Lysosomotropic agents have been shown to inhibit or prevent SARS-CoV infection. To check the possible effectiveness of salmiak consumption against COVID-19 related death, the authors used a linear regression model with the death rate as the dependent variable and BCG-policy and salmiak consumption score as independent variables. Using least squares regression and a robust standard error algorithm, the authors found a significant effect exerted by the independent variables ($P < 0.0005$ for BCG and $P = 0.001$ for salmiak). Salmiak score alone was significant ($P = 0.016$) when using least squares regression with robust error algorithm.

Conclusions: Despite some methodological limits, the results seem to confirm an association between BCG-positive vaccination policy and salmiak consumption, and lower death rates from COVID-19. Implementing BCG vaccination policy and fortification of foods with salmiak (NH_4Cl) may have a significant impact on the control of SARS-CoV epidemic.

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KEY WORDS: coronavirus disease-2019 (COVID-19), severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), bacillus Calmette-Guérin (BCG), ammonium chloride (NH_4Cl), lysosomotropic agent.

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From the beginning of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) outbreak, which started in December 2019, there have been hundreds of thousands of coronavirus disease-2019 (COVID-19) related deaths reported worldwide [1]. This pandemic is believed to have started in Wuhan, Hubei province, China. The danger of a similar second outbreak is imminent; thus, preparations for this outcome are essential. Revealing factors that affect COVID-19 related mortality rate is important. Any possible intervention that might reduce the COVID-19 death rate (calculated as number of COVID-19 associated deaths per 1 million population) would be considered as population-based preventive measures.

In this article, we focused on Europe only, and investigated two factors that we think favorably influenced COVID-19 survival rates: nationwide bacillus Calmette-Guérin (BCG) vaccination policy and widespread consumption of foods (confections) containing ammonium chloride (NH_4Cl).

It has been shown that long-term BCG vaccination also induces beneficial nonspecific immunological effects, other than just prevention of tuberculosis, in immunized individuals [2]. Based on this premise, and by analyzing COVID-19 mortality rates versus BCG vaccination policies, Miller and colleagues [3] found significant correlations between uninterrupted BCG policy in certain countries and reduced COVID-19 mortality in the same countries. The introduction or re-implementation of BCG vaccination policies for curbing COVID-19 morbidity and mortality has been suggested, although this recommendation has been challenged by others [4-6]. Iceland has been presented as a prime example of a location where COVID-19-related death rates have remained low, although the country has never implemented a national BCG policy [7]. Iceland's timely testing and containment policies were important aspects that favorably influenced infection incidence stabilization and death rate in the current epidemic [8].

We assumed that there may be a common and unusual cause for the reduced COVID-19 related death rates in Iceland and other Northern European countries or regions where no BCG policy is in effect: the widespread consumption of salmiak (ammonium chloride; NH_4Cl). In these Nordic regions, salmiak-enriched foods, such as liquorices and similar confectioneries containing up to 7.99% of food-grade NH_4Cl, are enjoyed on a daily basis [9]. In fact, NH_4Cl is an approved food flavoring substance in Europe and this ingredient may be added to confectionery products quantum satis (in the amount which is enough) [10]. For example, a representative strong version of salmiakki candies (made in Finland), contained the following ingredients (in decreasing order): sugar, ammonium chloride (salmiak), liquorice extract, salt, anti-caking agent, and flavor. It has been shown that NH_4Cl effectively reduces endosome mediated entry thus, the infectivity of SARS-CoV in Vero cells. NH_4Cl may inhibit SARS-coronavirus infection as strongly as chloroquine does [11]. Unlike chloroquine, which is a drug with curative potential in COVID-19, NH_4Cl, when used as flavoring, is considered to be a food not a drug. Chloroquine is currently recommended in Israel and in other countries [12] for the treatment of COVID-19 patients with various disease grades, from asymptomatic to more severe forms (see https://clinicaltrials.gov/ct2/show/NCT04333628). To the best of our knowledge, this article is the first attempt to imply an association between salmiak consumption and COVID-19 death rate.

PATIENTS AND METHODS

Our analysis comprised data from the following countries or regions (in decreasing order of the total population): Russia, Turkey, United Kingdom, France, Italy, Ukraine, Spain, Poland, Romania, Nordhein-Westfalen, Netherlands, Bayern, Belgium, Baden-Württenberg, Czech Republic, Greece, Portugal, Hungary, Austria, Serbia, Switzerland, Niedersachsen, Bulgaria, Hessen, Denmark, Finland, Slovakia, Norway, Croatia, Rheinland-Pfalz, Sachsen, Moldova, Berlin, Bosnia-Herzegovina, Schleswig-Holstein, Albania, Lithuania, Brandenburg, Sachsen-Anhalt, Thüringen, Slovenia, Latvia, Hamburg, Mecklenburg-Vorpommern, Estonia, Saarland, Bremen, Montenegro, Luxembourg, Malta, and Iceland.

Cyprus and Ireland were excluded from the analysis due to the lack of reliable BCG immunization data. Sweden was also omitted from analysis because this country implemented a SARS-CoV-2 policy that was opposite of the rest of Europe [13]. Germany, as a whole, was also excluded because the former West (FRG) and East (DDR) Germany had different BCG policies. In the former FRG, BCG immunization was discontinued in 1975; whereas, in the former DDR, immunization was only discontinued after 1990. Each of the German Federal States, however, were included in the BCG policy-based analysis. Two German states from northern Germany (Schleswig-Holstein [ex-FRG] and Mecklenburg-Vorpommern [ex-DDR]) were included in the salmiak analysis as well because of their widespread salmiak consumption. In the past, countries that were part of the Soviet bloc had uniform national BCG policies with mandatory vaccination for newborns. For the present analysis thus, all ex-Soviet bloc countries were regarded as BCG-vaccination-positive.

We considered BCG to be a binary variable (1 = active BCG vaccination policy, 0 = no active BCG vaccination policy).

Countries or regions where consumption of salmiak containing foods was common were referred to as "salmiak territory" [14]. These regions comprised primarily the Nordic countries (i.e., Iceland, Norway, Sweden, Finland, Denmark) and to a lesser extent Northern Germany (i.e., Schleswig-Holstein, Mecklenburg-Vorpommern), the Netherlands, and two of the Baltic states (i.e., Estonia, Latvia). Based on internet searches, including social media, we ranked the degrees of salmiak consumption as 1 for the Nordic countries, 0.5 for the Northern German states, 0.1 for the Baltic states, and 0 for all other countries. Netherlands was not included in the salmiak analysis due to its multicultural food consumption habits that made the estimation of salmiak consumption practically impossible.

Country-specific COVID-19 death rates and data on BCG vaccination policies and practices were extracted mostly from two sources–https://www.worldometers.info/coronavirus/ (USA) and http://www.bcgatlas.org/ (edited and launched by Canada), respectively–at 01:00 (CET) 26 April 2020. For particular European aspects, other sources were also used to report BCG policies [15-19]. Data for COVID-19-related death rates in German states were obtained from https://www.rki.de/.

Usual descriptive statistics techniques were used for collected data before inferential analysis. First, Student's *t* test was used to evaluate the difference in deaths per million inhabitants between BCG vaccination positive vs. negative countries. Logistical regression with a receiver operating characteristic (ROC) curve was used to check these results. Secondly, a linear regression model with robust algorithm was used for modeling deaths in which BCG policy and salmiak score were the independent variables.

RESULTS

In the 51 different countries and regions analyzed, we found a mean value of 84.24 deaths per million (lowest value 3.0, highest 597.0, standard deviation = 128.17; relative standard deviation was 152.15%). Median deaths per million was 34.0. In the BCG-policy negative countries (n=23) mean deaths per million was 158.78 and median was 76.0 (range 29–597), while in the BCG-positive countries (n=28) mean deaths per million was 21.43 and median was 16.5 (range 3–86).

These values were not normally distributed (Shapiro-Wilk test: $P < 0.0001$); however, we conducted a Student's *t* test comparing the mean in BCG-positive vs. BCG-negative countries using the logarithm of the data, since the logarithm-transformed data were normally distributed (Shapiro-Wilk test: $P = 0.3514$) and since also the variances were found homogeneous (Levene test: $P = 0.262$). The two-tailed Student's *t* test on the logarithm-transformed data was $P < 0.0001$, thus showing a significantly lower death rate in the BCG-positive countries.

We tried to verify these results using logistic regression using BCG policy (1 vs. 0) as dependent variable and death rate per million as independent variable. We found that any single unitary increase in death rate was associated with a 8.3% reduction in the odds of living in a BCG-positive country (95% confidence interval [95%CI] for odds reduction: from 2.8% to 13.5%; Wald's test: $P = 0.0035$) and a ROC curve analysis found area under the curve (AUC) = 0.946 (95%CI 0.845–0.990).

To check the possible effect of salmiak score on death rate per million, we used a least squares regression model using the death rate as the dependent variable with BCG (1 vs. 0) and salmiak score as independent variables. Notably, BCG positivity and salmiak scores were not correlated since we had Spearman's $P = -0.030$ (95%CI -0.298–0.242, $P = 0.830$).

With the least squares regression, using a robust standard error algorithm, we found a significant effect exerted by the independent variables ($P < 0.0005$ for BCG and $P = 0.001$ for salmiak score). The salmiak score alone (not adjusted by BCG) was significant ($P = 0.016$) when using least squares regression with robust error algorithm.

DISCUSSION

The results of our study seem to confirm an association between BCG-positive vaccination policy and lower death rates from COVID-19, even if other variables might have influenced death rates. However, the measured effect in these statistical evaluations appears highly significant.

The effect exerted by the salmiak consumption score could be considered somehow less determined since the values we used were established in an empirical manner, without having a direct measure of its consumption. However, its inclusion in the model seems justified by the biological effects of the ammonium chloride.

Although glycyrrhizic acid, the main component of liquorice extract, is a potent inhibitor of SARS-CoV replication in Vero cells [20], NH_4Cl is even more significant in this respect. Ammonium chloride is a lysosomotropic agent that alters intracellular vesicular traffic. Like other acidotropic weak bases, such as chloroquine, NH_4Cl is concentrated in acidic intracellular vesicles, endosomes, and lysosomes, which raises/neutralizes their intra-compartmental pH. Because the spike-mediated entry of SARS-CoV-2 into host cells is pH-dependent and requires acidification of the endosomes, NH_4Cl has been thought to inhibit SARS-CoV-2 infection [21]. Most importantly, it has also been demonstrated that host cells that had been pre-treated with NH_4Cl became resistant to SARS-coronavirus infection [11]. This finding also suggests a prophylactic value for salmiak consumption in the context of coronavirus pandemics. Consequently, fortification of foods, like certain popular confectioneries, with salmiak may have a significant impact on the control of SARS-coronavirus epidemics in the future.

One of the caveats of the COVID-19 mortality based studies or calculations is the lack of a generally accepted post-mortem protocol by which deaths should or should not be counted as caused by the SARS-CoV-2. For example, in a television interview it was explained that in any Italian region, if someone died of myocardial infarction and was positive to SARS-CoV-2, the deceased person was counted as a COVID-19 death; however, if in any German region someone died by myocardial infarction and was positive for COVID-19, this death was counted as cardiovascular [22]. In Belgium, unlike in other countries, deaths that were suspected as COVID-19 associated cases, and happened in care homes, were uniformly counted as COVID-19 ones [23].

CONCLUSIONS

The present data refer to different infection outbreak dates, thus we compared countries that were at different stages of epidemic evolution. Such analysis of disease outcomes should be performed at a definitive date at the decline of the current epidemic. Moreover, there are variables that are probably fundamental in terms of deaths, which we did not consider in our analysis. Such variables include the mix of acute beds, ICU beds, and CC beds, with some possible differences in their classification depending on the country and the local protocols for admission and discharge [24]; or the unevenness of the median age of infected patients, which, for example, is 62 in Italy [25] and 49 in Germany [26]. Despite of these evident methodological paucities, we still decided to make our preliminary results public as the current SARS-CoV-2 epidemic continues.

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Capsule

Predicting epidemics

Modeling an emerging infectious disease is an inexact science. At an early stage of an epidemic, researchers only have sparse data, little knowledge of the mechanisms driving emergence, and an urgent need to devise control measures that will be effective. Using epidemiological incidence reports, **Brett** and **Rohani** developed a detection algorithm for disease (re)emergence that is agnostic to the mechanisms involved. This supervised statistical learning algorithm was trained on data collected for mumps outbreaks in England and resurgent pertussis in the United States. The algorithm successfully anticipated reemergence of mumps 4 years in advance, which would have given plenty of time for mitigation efforts to be implemented. The algorithm also performed well for vector-borne diseases, including dengue in Puerto Rico, and predicted the rapid emergence of plague in Madagascar. The success of this approach stems from the common statistical properties of incidence data across disease emergence contexts and has obvious application for monitoring waves of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) reemergence.

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Eitan Israeli

It is not the mountain we conquer, but ourselves.

Sir Edmund Percival Hillary KG ONZ KBE (1919–2008),
New Zealand mountaineer, explorer, and philanthropist; one of the first climbers confirmed to have reached the summit of Mount Everest