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COVID-19 Spike Protein Hydrophobic properties and the Airborne Pollutant PM2.5's Salt Content's Impact on SARS-Cov-2 Evolution

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Abstract

Introduction: Following national lockdown instituting physical distancing, COVID-19 infection and mortality decreased in most countries. Simultaneously due to diminished economic and human activity, the atmospheric levels of particulate matter PM2.5, an important airborne pollutant, decreased significantly. Genes belonging to COVID-19 have been detected on PM2.5 which has been suggested as a vector for viral transmission. PM2.5 has various components including sodium chloride which determines the amount of PM2.5 salt-derived water content. Atmospheric salt content in part determines the sodium chloride content and the consequential hygroscopic properties of PM2.5. COVID-19 possesses a hydrophobic N-terminal spike protein which may have deterred the original Wuhan 1 Clade D variant from adhering to the atmospheric PM2.5. This may not have been the case with the "2nd wave" of COVID-19 where the mutant Clade G displaced Clade D, suggesting differences in the hydrophobic properties in the mutant's spike protein. The mutant Clade G may have utilized a more hydrophyllic form of particulate matter as a vector, such as that emanating from exhaled tobacco smoke. With a background population of 66% of Chinese males smoking and taking in consideration respiratory transmission, this vector change may have had a hand in COVID-19 evolution.

Method: During the "1st wave" of COVID-19 from March till May 2020, the sodium chloride content of the PM2.5 species was obtained from scientific literature for two groups of cities. Cities were chosen as opposed to countries, because substantial regional differences in infection rates were noted between inland and coastal cities. These cities were differentiated by the rate COVID-19 infection and consequent case fatality ratio. Atmospheric salt content was obtained from research that assessed chloride ion wet deposition, a surrogate for atmospheric salinity.

Results: During the first wave, cities with high COVID-19 infection rates had PM2.5 species with significantly lower salt content than the cities with low Covid-19 infection. The PM2.5 salt content in the cities with elevated COVID-19 rates was 0.196μg/m3 SD 0.05μg/m3, while cities with low COVID-19 rates had a PM2.5 salt content of 0.81μg/m3 SD 0.32μg/m3 ($p<0.0001$). There was a significant correlation between atmospheric salinity and the salt content of PM2.5. PM2.5 salt content correlated with minimum atmospheric salinity ($R=0.53$, $p<0.01$) and maximum atmospheric salinity ($R=0.64$, $p<0.002$). During the 1st wave there appeared to be an inverse relationship between COVID-19 infection and atmospheric salt content levels. Cities that were spared high COVID-19 infection rates, have higher maximum/minimum atmospheric salinity content levels (216-1080 mgCl/m^2/day) compared to sodium chloride levels noted in cities with high COVID-19 rates of infection (40-125. mgCl/m^2/day) ($p<0.005$).

Conclusion: During the "1st wave" of COVID-19 between March and May 2020, the airborne pollutant PM2.5 with low salt content, in the presence of low ambient salinity may have been linked to an increased risk of COVID-19 infection in the population. This may explain why coastal cities and islands remained relatively unscathed during the "first wave". This correlation did not prevail during the second wave of COVID-19 pandemic possibly due to the predominance of the mutant Clade G which may have different hydrophobic properties in its spike protein. Clade G's spike protein may possibly adhere to more hydrophilic PM2.5 such as that derived from tobacco smoking which potentially acted as a vector for COVID-19 spread during the "2nd wave" of the pandemic.

Keywords: COVID-19; PM2.5; Hydrophobic properties; Sodium chloride, Atmospheric salt content, COVID-19 evolution, Tobacco smoking

INTRODUCTION

In June 2020, COVID-19 infection appeared to be decreasing in most countries. This decrease is attributed to the social distancing legally enforced in most countries. Concomitant with the reduction in COVID-19 infection incidence, a significant decrease in pollution, including PM2.5 levels, were noted[1-3]. A significant reduction in COVID-19 related mortality was also noted in relation to a reduction in PM2.5[4]. There are some countries and cities which appear to have contained the infection prior and after lockdown, including Malta, Hong Kong, Cyprus, South Korea, Australia, New Zealand and Taiwan[5,6].

There are factors in common to the countries which appear to have contained the COVID-19 pandemic. Geographical isolation, whereby most of these countries are in fact islands, may have prevented large population movements which encourage mass spread of infection–physical distancing on a national scale. Legal implementation of social distancing in small isolated populations may be easier to enforce.

Legislation enforcing public health measures such as physical distancing and procedures to protect the elderly and vulnerable individuals may be more effective[7].

Concomitant with social distancing a reduction in human mobility and economic activity resulted. The reduction in activity in turn led to significant diminution in pollution including PM2.5 levels[1-3]. There lies the possibility that besides, the measures of physical distancing at reducing viral transmission, there may be environmental factors including the level and composition of the pollutant PM2.5 that may impact COVID-19 infection.

COVID-19 has been found adherent to PM2.5[8]. Atmospheric salinity determines the PM2.5's sodium chloride component which in turn determines the water content of PM2.5[9]. The N-terminal of a viral fusion peptide is strongly hydrophobic and the water content of PM2.5 may act as a deterrent to COVID-19 adhesion to this particulate matter[10,11]. The hydrophobic properties of the subsequent mutant may be different[12]. Both clades differ in the spike protein's trimeric peptide configuration with the original Clade D characterized by a "closed" configuration while the mutant Clade G possesses an "open" configuration[13]. For the virus to gain entry into respiratory goblet cells, two of the spike protein's trimeric peptides require to be in an open configuration[14]. Molecular dynamic computer simulation of the spike protein receptor binding domain to the goblet cell angiotensin converting enzyme II indicate an intricate pattern of salt bridges, hydrophobic sites and hydrogen bonding all potentially influenced by the ambient salinity and an altered more hydrophyllic source (exhaled tobacco smoke) of PM2.5[15].

METHODOLOGY

During the "1st wave" of COVID-19 the salt content of particulate matter PM2.5 and atmospheric salt content were assessed in May 2020 from a number of cities, differentially affected by COVID-19 infection. Cities were selected as opposed to countries, because substantial regional differences in infection rates have noted especially between inland and coastal cities[16]. The cities chosen to be assessed were divided into two groups. The first group (Group 1) included Wuhan, Qom, Bergamo, Madrid, New York, Limburg (Vredepeel), Atlanta, and Pretoria, all having significantly high rates of COVID-19 infection, compared to other cities in their respective countries. Group 2 consisted of cities including Seoul, Hanoi, Rehovot, Buenos Aires, Singapore, Beijing, Kanpur, Dhaka, Taipei, Rotterdam, Hong Kong and Malta, all of which have very low rates of COVID-19 infection[16]. An important caveat is that infection and mortality rates very much depend on the level of COVID-19 testing in the individual populations.

The level of atmospheric salt content may be inferred from the chloride ion wet deposition index measured as mgCl/m2/day. Atmospheric salt content levels were obtained from an indexed colour coded map compiled by Fraunhofer ISE, through a site set up by the Galvanizers Association[17]. This map determines the level of salt content in different regions in the world in connection with the risk of automobile corrosion. The data are represented as maximum and minimum levels per day.

RESULTS

There was a significant difference in the salt content of PM2.5 between both groups of cities. Cities with low COVID-19 infection rates had significantly higher salt content in the PM2.5, compared to the cities with high levels of COVID-19 infection. The PM2.5 salt content in the cities with high COVID-19 rates was 0.196µg/m3 SD +/-0.05µg/m3, while cities with low COVID-19 infection rates had a PM2.5 salt content of 0.81µg/m3 SD +/-0.32µg/m3 ($p<0.0001$).

The cities that seem to have contained the pandemic are close or surrounded by sea, and consequently have an elevated atmospheric salt content. Cities that evaded high COVID-19 infection rates have higher atmospheric salt content levels, minimum/maximum 216-1080 mgCl/m2/day compared to minimum/maximum 40-125 mgCl/m2/day ($p<0.005$) noted in cities sustaining high COVID-19 rates of infection.

Normality plots applied to the PM2.5 salt content showed parametric patterns allowing the application of the T-Test. Both the maximum and minimum atmospheric salt content levels were nonparametric whereby the Mann -Whitney U test was applied. Spearman Rank test was applied to assess statistical correlations to nonparametric variables.

The results are demonstrated in two tables designated Figure 1 and Figure 2.

Cities with high COVID-19 infection rates

	Salt Content µg/m3	Minimum Chloride Ion Deposition mgCl/m2/day	Maximum Chloride Ion Deposition mgCl/m2/day
Wuhan	0.3	4	60
Qom	0.19	4	60
Bergamo	0.2	61	300
N. York	0.2	61	300
Madrid	0.2	61	300
Atlanta	0.1	61	301
Pretoria	0.2	4	60
Limburg	0.2	61	301

Cities with low COVID-19 infection rates

	Salt Content in PM2.5 µg/m3	Minimum Chloride Ion Deposition mgCl/m2/day	Maximum Chloride Ion Deposition mgCl/m2/day
Malta	0.62	300	1500
Seoul	0.63	300	1500
Taipei	1.1	300	1500
HongKong	0.46	300	1500
Singapore	1.1	61	301
Kanpur	0.6	4	60
Beijing	1.3	300	1500
Dhaka	1.4	300	1500
B. Aires	0.6	61	301

Rotterdam	0.4	300	1500
Hanoi	0.9	61	301
Rehovot	0.9	300	1500

Figure 1: There was a significant difference in the salt content of PM2.5 between both groups of cities $p<0.0001$. Atmospheric salt content in mgCl/m2/day indicating minimum and maximum levels compared to salt content in PM2.5 species in different cities. The p value for the atmospheric salt content between the two groups of cities is $p<0.005$. References for salt content in PM2.5[9,18-26]

Correlation between PM2.5 content and Atmospheric salt content

	Salt Content in PM2.5 µg/m3	Minimum Chloride Ion Deposition mgCl/m2/day	Maximum Chloride Ion Deposition mgCl/m2/day
Malta	0.62	300	1500
Seoul	0.63	300	1500
Taipei	1	300	1500
HongKong	0.46	300	1500
Singapore	1.1	61	301
Kanpur	0.6	4	60
Beijing	1.3	300	1500
Dhaka	1.4	300	1500
B. Aires	0.6	61	301
Rotterdam	0.4	300	1500
Hanoi	0.9	61	301
Rehovot	0.9	300	1500
Wuhan	0.3	4	60
Qom	0.19	4	60
Bergamo	0.2	61	300
New York	0.2	61	300
Madrid	0.2	61	300
Atlanta	0.1	61	301
Pretoria	0.2	61	301
Limburg	0.2	61	301

Figure 2: The salt content of PM2.5 in both groups of cities correlated with the Atmospheric salt content. Correlation between minimum chloride ion deposition versus salt content in PM2.5 R-value =0.56, ($p<0.01$). Correlation between maximum chloride ion deposition versus salt content in PM2.5 $R = 0.64$, ($p<0.002$). References for salt content in PM2.5[9,18-30]

DISCUSSION

Undoubtedly reduced transmission of COVID-19 followed the institution of social distancing. Besides reducing human to human viral transmission, physical distancing diminished levels of human and economic activity with consequential reduced pollution levels[2]. Coal consumption in China after lockdown decreased by approximately 50%, from 80 thousand tonnes daily to 40 thousand tonnes per day[27]. In India, reductions of the order of 43% and 31% were noted for PM2.5 and PM10 respectively, during lockdown period compared to previous years. During the same period carbon monoxide decreased by 10% and nitrous oxide decreased by 18%. The air quality index (AQI) improved throughout India following lockdown[28]. This study assessed the sodium chloride in the particulate matter (assessed by the AQI) and the salinity in two groups of cities differentiated by the rate of COVID-19 infection from March till May 2020.

PM2.5 and Pulmonary Anti-Microbial Defences

Before statutory lockdown, there may have been synergism between high PM2.5 levels and COVID-19 in infection transmission. This synergism may have also determined the severity of COVID-19 sequelae. Pathological synergism between PM2.5 and respiratory infection has been shown in animal studies. Preclinical studies, exposing mice to cigarette smoke demonstrated that following intratracheal injection of streptococcus pneumoniae, bacterial counts in murine lungs were higher than the control group[29]. Another study showed that air pollution could result in dysfunction of rat tracheal cilia, causing mucus stasis and resultant infection. Moreover a declined nonspecific immune defense, was noted making the rats more prone to secondary infection[30]. Viability and phagocytic activity of alveolar macrophages decreased significantly following the instillation of PM2.5 particles in Wistar rats[31,32].

Similar deleterious effects of PM2.5 have been found in the respiratory system of humans. PM2.5 releases free radicals, metal and the organic components inducing free radical production leading to oxidation of lung tissue[33]. PM2.5-induced inflammation led to an increase in the number of neutrophils, eosinophils, T cells and mastocytes[34,35]. All these cells can result in inflammatory cytokine production and resultant cytokine storm has been responsible for a significant number of COVID-19 related deaths[36].

Addressing this synergism may protect the populations from airborne infections such as the COVID-19 infection and simultaneously foster respiratory health. Prior to the outbreak of COVID-19 in Wuhan, Qom and Bergamo the Air Quality Index demonstrated strikingly elevated pollution levels of PM2.5[41]. These elevated levels of PM2.5 occurred during the winter months possibly due to combustion of fuel for residential heating purposes.

Ambient Salinity and PM2.5 Sodium Chloride Content

Inferring from the fact that coastal cities have been in the main been spared from high COVID-19 infection, another environmental factor may come into play in the form of the level of atmospheric salt content. Chloride ion wet deposition, a variable reflecting the ambient salinity could be obtained from a colour-coded map of the distribution of the earth's salt content[21].

Wuhan has been noted to be one of the cities in China with the least salt content in the air[18]. The cities hard hit by COVID-19 infection are distant from the sea and consequently have low salt content

levels[22-30]. Rotterdam with an elevated salt content PM2.5 (0.4µg/m3) had lower rates of COVID-19 compared to Vredepeel in the inland Limburg region (0.2µg/m3). Beijing with a high salted PM2.5 (1.3µg/m3) had lower levels than Wuhan (0.3µg/m3). It is imperative that the impact of physical distancing due to enforced quarantine be factored in when considering infection rates. South West region of the U.K. with higher saline levels had lower COVID-19 infection rates compared to London which appears to have a lower chloride ion wet deposition. Similarly in Italy whereby the saline deficient Lombardy region was severely affected by COVID-19 infection, while the more saline rich south of Italy have very low infection rates.

Pulmonary Salt Content and COVID-19 Hydrophobic N-terminal fusion peptide

Healthy levels of airway salt content protect the respiratory system disease. Hypertonic saline has been shown to have anti-infective properties[38]. Tracheo-bronchial ciliary action is accentuated when the saline content of the endobronchial tree is optimal. Optimal levels of tracheal sodium chloride are mucolytic making bronchial mucus is less viscous with. Patients with respiratory condition such as asthma and cystic fibrosis benefit from endo-bronchially nebulized saline[39].

A protein component of COVID-19 virus is hydrophobic[10,11]. Viral cell entry is mediated through extensive post-receptor-binding structural readjustments on the cell membrane. The hydrophobic peptides include the N-terminal of a fusion peptide[11,12]. Low concentrations of chlorinated solutions are lethal to the virus[40]. Atmospheric salt content may be another environmental factor that may have protected some populations preventing airborne infection from the COVID-19.

Variable amounts of sodium chloride are found on PM2.5 species[9]. The PM2.5 species vary from city to city, and there are also seasonal changes in the sea salt component of the PM2.5. As a corollary to the variable amount of PM2.5 sodium chloride component, there also a variable degree of hygroscopicity related to the sea salt-derived water content[9]. Besides the lethal effect of sodium chloride on the virus, there may be a connection with aerosol adhesion due to sodium chloride with the pollutant PM2.5. Recent evidence suggests that COVID-19 may adhere to PM2.5 particles to evade the bronchial tree defences and colonize the alveoli[8]. The presence of hygroscopic sodium chloride on PM2.5 may act as a deterrent against the adhesion of the hydrophobic proteins of COVID-19 to these particles, protecting populations living in regions with salt-rich PM2.5 prevalent high salt content regions.

PM2.5 sodium chloride content, Ambient Salinity, Tobacco Smoking and COVID-19 Clade Evolution

As mentioned earlier genes appertaining to SARS-CoV-2 have been detected on the particulate matter, PM2.5[8]. Ambient salinity, especially in coastal regions or close to salty lakes, determines the PM2.5's sodium chloride element which in turn through its hygroscopic properties governs the particle based water content of PM2.5[9]. The N-terminal of a viral fusion peptide in the original coronavirus Clade D is strongly hydrophobic and consequently the salt-derived water content of PM2.5 may act as a deterrent to COVID-19 adhesion to this particulate matter[10,11]. After the SARS-CoV-2 mutant Clade G slowly displaced the original Clade D, this hydrophobic property may have played a smaller role in the transmission of the pandemic as evidenced by the higher infectivity of the mutant[12].

Both of the SARS-Cov-2 clades differ in the spike protein's trimeric peptide configuration. This may be due to the replacement of aspartic acid by glycine at the D614 position of gene coding for the spike protein[41]. The original Clade D is characterized by a "closed" configuration while the mutant Clade D possesses an "open" configuration[13]. Virus entry into respiratory goblet cells requires that two of the spike protein's three peptides are positioned in an open configuration. Spike protein's receptor binding domain had its molecular configuration determined by computer simulation[14]. This indicates a complex series of salt bridges, hydrophobic sites, electrostatic charges and hydrogen bonding between the receptor binding domain to the goblet cell angiotensin converting enzyme II. This stereochemistry of the spike protein's receptor binding domain may all be potentially affected by the ambient salt content[15].

The pattern of COVID-19 infection and mortality rate did not prevail in the 2nd wave where both high and low salinity, coastal and inland regions were affected alike. This may suggest that if particulate matter is indeed a vector for COVID-19 transmission, a more hydrophilic form, such as PM2.5 originating from tobacco smoking. Particulate matter originating from exhaled tobacco smoke expands 1.5 times in volume due the addition of water gained through the passage in the humid respiratory tract[42]. The importance of this change in particulate matter acting as COVID-19 vector became more likely with a background smoking population of 66% of Chinese males who are smokers. The evolutionary predominance first noted in China of the potentially more hydrophyllic Clade G may have come about in part because of the modified vector in the form of particulate matter arise from exhaled tobacco smoke.

A recent publication compared states with higher smoking populations, with states with a lower proportion of smokers possibly determined by legislation banning smoking in public places. This study indicated that the incidence of COVID-19 infection was significantly elevated in the States with partial bans on tobacco smoking compared to highly regulated States ($p<0.038$)[43]. The incidence of COVID-19 infection in the States with partial bans was 2046/100,000 (sd+/-827), compared to 1660/100,000 (sd+/-686) in States with more severe smoking prohibitions. A significant difference of 23% in the COVID-19 infection rate[43].

CONCLUSION

The COVID-19 pandemic may have occurred due to the combination of a number of environmental factors besides the virulence of the virus. During the 1st wave, besides social distancing, a decrease in airborne PM2.5 possibly in the presence of sodium chloride rich species of PM2.5 and high ambient salinity may have protected some populations from the COVID-19 pandemic. This protective effect did not prevail during the second wave of the COVID-19 pandemic possibly due to the predominance of the SARS-CoV-2 mutant Clade G. The mutant Clade G may have different hydrophobic properties in its spike protein, consequently altering its adherence PM2.5.

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