Impact of Saharan dust exposure on airway inflammation in patients with ischemic heart disease

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Epidemiological studies found that increases in the concentrations of airborne particulate matter (PM) smaller than 10 microns diameter (PM_{10}) in the ambient air due to desert dust outbreaks contribute to global burden of diseases, primarily as a result of increased risk of cardiovascular morbidity and mortality. No studies have investigated the possible association between desert dust inhalation and airway inflammation in patients with ischemic heart disease (IHD). Induced sputum was collected in 38 patients and analyzed to determine markers of airway inflammation (Transforming Growth Factor-β1 (TGF-β1) and hydroxyproline) concentrations. For the purpose of the investigation, PM_{10} and reactive gases concentrations measured in the European Air Quality Network implemented in the Canary Islands were also used. We identified Saharan desert dust using meteorology and dust models. Patients affected by smoking, chronic obstructive pulmonary disease (COPD), asthma, pulmonary abnormalities, acute bronchial or pulmonary disease were excluded. The median of age of patients was 64.71 years (56.35−71.54) and 14 (38.84%) of them were women. TGF-β1 and hydroxyproline in sputum were highly associated to PM_{10} inhalation from the Saharan desert. According to a regression model, an increase of 1 $\mu g/m^3$ of PM_{10} concentrations due to desert dust, results in an increase of 3.84 pg/gwt of TGF-β1 (R2 adjusted = 89.69%) and of 0.80 $\mu g/gwt$ of hydroxyproline (R^2 adjusted = 85.28%) in the sputum of patients. The results of this study indicate that the exposure to high PM_{10} concentrations due to Saharan dust events are associated with intense inflammatory reaction in the airway mucosae of IHD-patients. (Translational Research 2020; 000:1−10)

Keywords: Saharan dust; Particulate matter; Induced sputum; Airway inflammation; Ischemic heart disease

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Submitted for Publication April 13, 2020; received submitted May 26, 2020; accepted for publication May 27, 2020.

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1931-5244/$ - see front matter

https://doi.org/10.1016/j.trsl.2020.05.011

INTRODUCTION

The exposure to ambient air pollution has become in a global threat to public health.[1] It is associated with ~4 million deaths and the development of chronic obstructive pulmonary disease (COPD), ischemic heart disease (IHD), stroke, acute lower respiratory infection and lung cancer, according the World Health Organization (WHO).[2] Particulate matter (PM, a mixing of solids and liquid particles -micrometer size- mostly linked to combustion, agriculture and soil dust) is a major prompter of such health effects, specifically its respirable fraction, i.e. PM smaller than 10 microns diameter (so-called PM_{10}). Much of the attention has been paid to the health effects linked to combustion PM.[1]

Recently, attention is also being drawn to the air quality impairment due to airborne desert dust and the associated adverse health effects.[3,4] In Europe, health effects due to the exposure to desert dust outbreaks is been investigated. Stafoggia et al.[5] addressed the short-term effects of PM_{10} on mortality and hospital admissions in 13 Southern European cities, distinguishing between PM_{10} originating from deserts and other sources (i.e. PM emitted by vehicle exhausts, industry, road dust etc...). They found that increases of 10 $\mu g/m^3$ in PM_{10}-desert dust were associated with increases in premature mortality between 0.55% and 0.65%.[5] Satellite observation shows that large-scale desert dust outbreaks occur frequently from North Africa to the Atlantic and to Southern Europe, in the Middle East and in Asia. These intense dust events result in high concentrations of airborne particles, visibility reduction and an orange atmosphere (Fig 1).

Long term exposure to ambient PM has been associated with chronic airflow obstruction.[6] Airway inflammation refers to the infiltration and accumulation of granulocytes and macrophages in the airway tissue in response to inhaled irritants or pathogens. The inflammatory process contributes to the thickening of airway walls and airway narrowing. It may lead to irreversible changes and long-term loss of lung function.[7] Airway inflammation can be assessed using a noninvasive procedure that involves sputum collection after the inhalation of a hypertonic salt solution.[7] During the last 2 decades there has been an increasing trend in the analysis of markers in sputum samples in a wide range of airway diseases studies, both for diagnosis and scientific research.[7] Two interesting markers involved in the airway inflammation are the Transforming Growth Factor-β1 (TGF-β1),[8] a dimeric polypeptide of 25 KDa, and the hydroxyproline.[9] It would be interesting to evaluating the airway inflammation in subjects with IHD living outside North Africa, frequently exposed to high concentrations of inhalable desert dust.[10,11] Therefore, the aim of this study is to investigate the influence of the exposure to airborne desert dust on the airway inflammation in subjects with IHD.

METHODS

Study population. This study was prospectively conducted in patients with IHD in Tenerife (28.5°N, 16.35°E, Canary Islands, located off North Africa) (Fig 1), between June 29, 2017 and November 22, 2017. In this region, meteorology is dominated by the

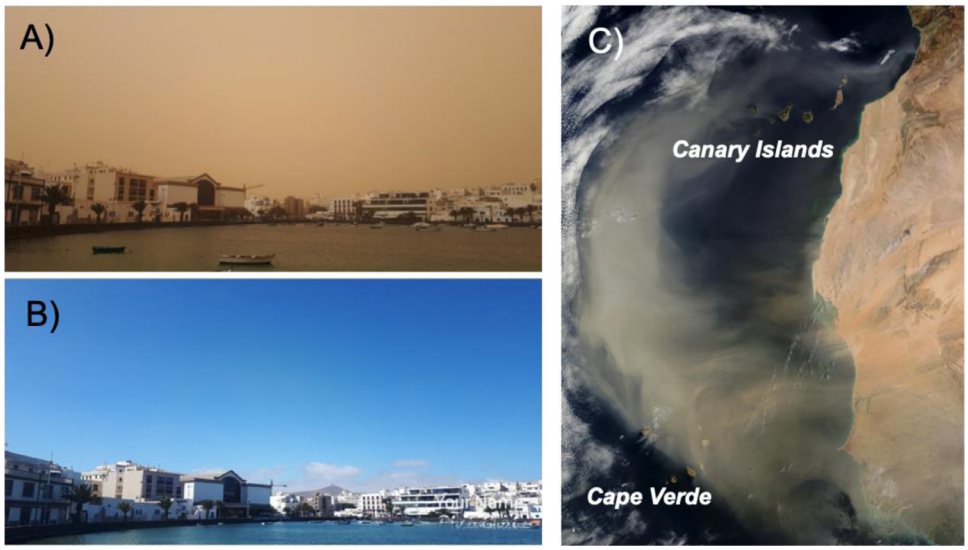

Fig 1. View of El Charco bay in Arrecife -Lanzarote, Canary Islands, during a Saharan dust event (**A**) and under dust-free regular conditions (**B**). Satellite view of a Saharan dust outbreak (**C**), courtesy of NASA, sensor MODIS. Pictures were taken the February 22, 2020 (**A**), $PM_{10} = 360$ $\mu g/m^3$, and February 26, 2020 (**B**), $PM_{10} = 19$ $\mu g/m^3$. Satellite image of NASA obtained with MODIS sensor (March 2, 2003).

North Atlantic anticyclone, which prompts trade winds blowing. Episodically, easterly winds bring high loads of airborne desert dust particles from the North Africa.[10,11]

Participants in this pilot study were recruited through a local cardiologist in the outpatient cardiology clinic at the University Hospital of the Canaries. They were required to be males or women aged 50 o more with physician-diagnosed IHD, stable angina pectoris or prior myocardial infarction (more than 12 months ago). Based on the study objectives, smokers or patients exposed to second-hand smoke at home or school, COPD, asthma, others pulmonary abnormalities and patients with acute bronchial or pulmonary disease were excluded from participation. Thus, 232 candidates were excluded from a total of 270 participants. Hence, the data from 38 patients were analyzed. These individuals were invited to participate in collection of sputum samples. Our research was carried out according to The Code of Ethics of the World Medical Association (Declaration of Helsinki), that informed consent was obtained, and that the author's institutional review board has approved the study.

Dust events. The arrival of Saharan dust to the Canary Islands is enhanced in 2 seasons: (1) December to March linked to the Harmattan easterly winds [12] and (2) July and August, under a complex meteorological scenario linked to the West African monsoon.[13]

We used the observations of the air quality network of the Canary Islands, where PM_{10}, $PM_{2.5}$ (PM with an aerodynamic diameter smaller than 2.5 microns), meteorology and gaseous pollutants (CO, NO_x, O_3, SO_2) are monitored following the mandatory reference methods of the European Union (Directive 2008/50/EC). This network provides hourly resolution data, which are transmitted to the European Environmental Agency (Air Quality in Europe, 2019). We determined and analyzed daily averaged values. We selected the data from the station with the highest data availability, Tena Artigas (availability = 98.4% from 2013 to 2017). Because of the large-scale nature of Saharan dust events (10^3 s km), the PM_{10} records from this site exhibits a high correlation with the PM_{10} data from the other air quality monitoring stations of Tenerife (0.76−0.84, from 2013 to 2017). Concentrations of coarse (size 2.5−10 μm) particles ($PM_{2.5-10}$) were determined as the difference between PM_{10} and $PM_{2.5}$.

We identified the arrival of Saharan dust to the Canary Islands using modeling products provided by the Giovanni Earth data system of NASA: column dust and surface dust concentrations obtained with MERRA-2 model. Days with the presence of Saharan dust were identified. Those Saharan events prompting PM_{10} concentrations >50 $\mu g/m^3$ were flagged and labeled as "Saharan dust event." During the study period, 23 events with $PM_{10} > 50$ $\mu g/m^3$ occurred, all them prompted by the arrival of Saharan dust, according to MERRA-2 modeling (Fig 2). In these dust events, PM_{10} is basically constituted by mineral desert dust, mixed with traces amounts of other particle types.[10,11]

Sputum processing. Sputum samples were induced using 3% (weight/volume) nebulized hypertonic saline and frozen immediately and stored at −70°C until use.

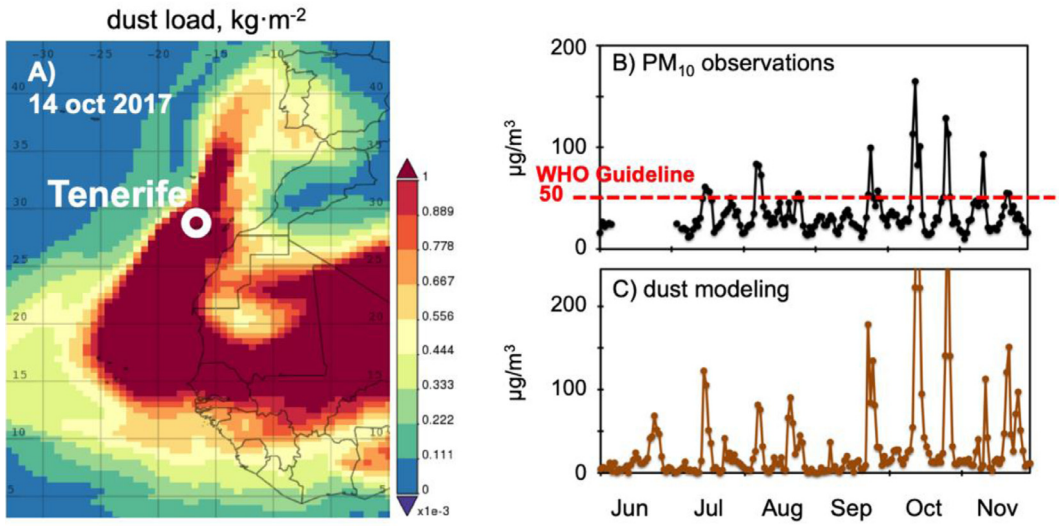

Fig 2. Map showing the location of Tenerife (white circle) and the column dust load (the vertically integrated dust concentrations) during the Saharan dust episode occurred the 14-oct-2017 (**A**). Time series of (**A**) PM_{10} observations in the air quality network in Tenerife and of (**B**) surface dust concentrations according to MERRA-2 model of NASA during the study period (Jun−Nov 2017).

Samples were thawed, cleaned with saline solution (NaCl 0.9%) twice and dried over filter paper. Individual sputum was homogenized by sonication at 100 watts during 5 second in 0.5 ml of cold saline solution. The suspensions were filtered through nylon gauze to remove debris tissue and they were centrifuged at 1000 g 5 minutes at 4°C. Aliquots of supernatant were stored at −70°C until analysis.

Induced sputum concentrations of TGF-β1 were analyzed by enzyme-linked immunosorbent assay with a use of a commercially available kit according to the manufacturer's instructions (IBL International Gmbh, Hamburg, Germany). The coefficients of variation intra-assay and inter-assay, respectively, were 6.1% and 5.3%. The limit of detection was established in 3.4 pg/ml. The concentrations of TGF-β1 in sputum were expressed as pg/gwt, where gwt is grams of wet tissue.

In order to determine the level of collagen degradation, the amount of hydroxyproline was measured in sputum by spectrophotometric determination using the method of Reddy and Enwemeka.[14] The coefficients of variation intra-assay and inter-assay, respectively, were 2.7% and 3.4%. The limit of detection was established in 1.47 μg/ml. The concentrations of hydroxyproline in sputum are expressed as μg/gwt.

Statistical analysis. Categorical variables were presented as *n* (%) and quantitative variables were presented as median (Q1−Q3). To know the influence of PM_{10} on the levels of the sputum variables, we first created a maximum model with TGF-β1 as the dependent variable and PM_{10}, $PM_{2.5}$, $PM_{2.5-10}$, NO, NO_2, SO_2 and O_3 as independent variables. Collinearity was considered to exist when tolerance or 1 / variance inflation factor (VIF) < 0.1 or when VIF > 10.[15] If collinearity was detected, one or several variables were excluded based on the purposes of the work or the theorical knowledge. We detected high levels of collinearity between PM_{10}, $PM_{2.5}$ and $PM_{2.5-10}$. So, we excluded $PM_{2.5}$ and $PM_{2.5-10}$ of the maximum model.

Once this regression analysis was performed, any cofounding factor was removed if 2 of the following conditions were met: (a) that variable did not cause an important modification [| (b − $b_{adjusted}$)/$b_{adjusted}$ | >0.10] over the association PM_{10} and the sputum variable and (b) similar or smaller standard error was achieved for that b.[16] If several models fulfilled these conditions, the most parsimonious model was selected. Statistical significance of independent variables was assessed using the Wald test. Finally, linearity, homogeneity of variances, independence and collinearity were checked again for this reduced model. We calculated R^2, R^2 $_{adjusted}$, AIC (*Akaike's Information Criteria*) and BIC (*Bayesian information criteria*) of the models. All tests were 2-sided.

Table 1. Sample characteristics, air quality and sputum variables

Population	No Saharan dust events (*n* = 22)	Saharan dust events (*n* = 16)	*P* value
Age	63.08 (55.31−68.97)	69.91 (60.96−72.59)	0.08
Women	9 (40.91%)	5 (31.25%)	0.74
Diabetes	4 (18.18%)	4 (25%)	0.69
Hypertension	13 (59.09%)	9 (56.25%)	1
Dyslipidemia	10 (45.45%)	8 (50%)	1
Body mass index	24.3 (21.89−27.56)	26.31 (19.91−30.58)	0.44
Weight			
Normal	12 (54.55%)	8 (50%)	
Overweight	6 (27.27%)	4 (25%)	
Obesity	4 (18.18%)	4 (25%)	0.69
Metabolic syndrome	2 (9.09%)	1 (6.25%)	1
FEV1	85 (82−86.5)	85 (82−86.75)	0.49
Air composition			
PM_{10} $\mu g/m^3$	15.5 (14.75−23)	57.5 (55.25−107.25)	<0.01
$PM_{2.5}$ $\mu g/m^3$	4 (3−7)	20 (13.25−28.75)	<0.01
$PM_{2.5-10}$ $\mu g/m^3$	12 (10−14.25)	43.5 (36.75−77.75)	<0.01
SO_2 $\mu g/m^3$	1.83 (1−4.83)	2.38 (1−5.23)	0.87
NO $\mu g/m^3$	1.63 (1.38−1.73)	1.58 (1.45−2)	0.4
NO_2 $\mu g/m^3$	7.7 (6.65−10.38)	9.54 (4.96−12.5)	0.51
O_3 $\mu g/m^3$	71.27 (62.79−80.68)	70.87 (63.17−77.92)	0.77
Sputum variables			
TGF-1 pg/gwt	130.65 (103.95−183)	352.54 (254.2−451.6)	<0.01
Hydroxyproline μg/gwt	16.14 (10.8−25.65)	54.5 (39.54−69.51)	<0.01

FEV1, forced expiratory volume in 1 second; NO, nitrogen monoxide; NO_2, nitrogen dioxide; O_3, ozone; PM_{10}; $PM_{2.5}$ and $PM_{2.5-10}$, particulate matter with an aerodynamic diameter < 10. < 2.5 and between 2.5 and 10 microns. respectively; SO_2, sulphur dioxide; TGF-β1= Transforming Growth Factor-β1.

Finally, we repeated the same process considering PM_{10} as a categorical variable named "dust event days" with average levels of PM_{10} over 50 $\mu g/m^3$, and we also repeated all the statistical process with hydroxyproline as the dependent variable.

All analyses were performed with STATA v.15 (Stata Corp, Texas) and the best model was selected with the user-written command confound.[17]

RESULTS

Characteristics of the sample. A total 38 sputum samples were taken from 38 different individuals. Median of age was 64.38 years (56.57−71.07) and 14 (36.84%) were women. The median of PM_{10} was 23 $\mu g/m^3$ (15−57) and the sputum of 16 (42.11%) patients was taken during days of Saharan dust event over 50 $\mu g/m^3$. The median of TGF-β1 was 198.69 pg/gwt (124.05−337.73) and the median of hydroxyproline was 32.99 μg/gwt (14.75−47.39). All characteristics of the patients were similar between dust and nondust days. We found differences in all PMs and the sputum variables between these 2 types of days. Characteristics of the population, environmental and sputum variables were shown in Table 1.

Influence of PM_{10} and dust on the levels of TGF- β1 in sputum. Concentrations of PM_{10} were highly correlated with those of $PM_{2.5}$ and $PM_{2.5-10}$. Tolerance (1/VIF) for $PM_{2.5}$ = 0.02 and VIF = 49.31. Tolerance (1/VIF) for $PM_{2.5-10}$ = 0.01 and VIF = 194.42. So, $PM_{2.5}$ and $PM_{2.5-10}$ were excluded of the maximum model. The maximum model showed that PM_{10} was highly associated with the levels of TGF- β1 in sputum.

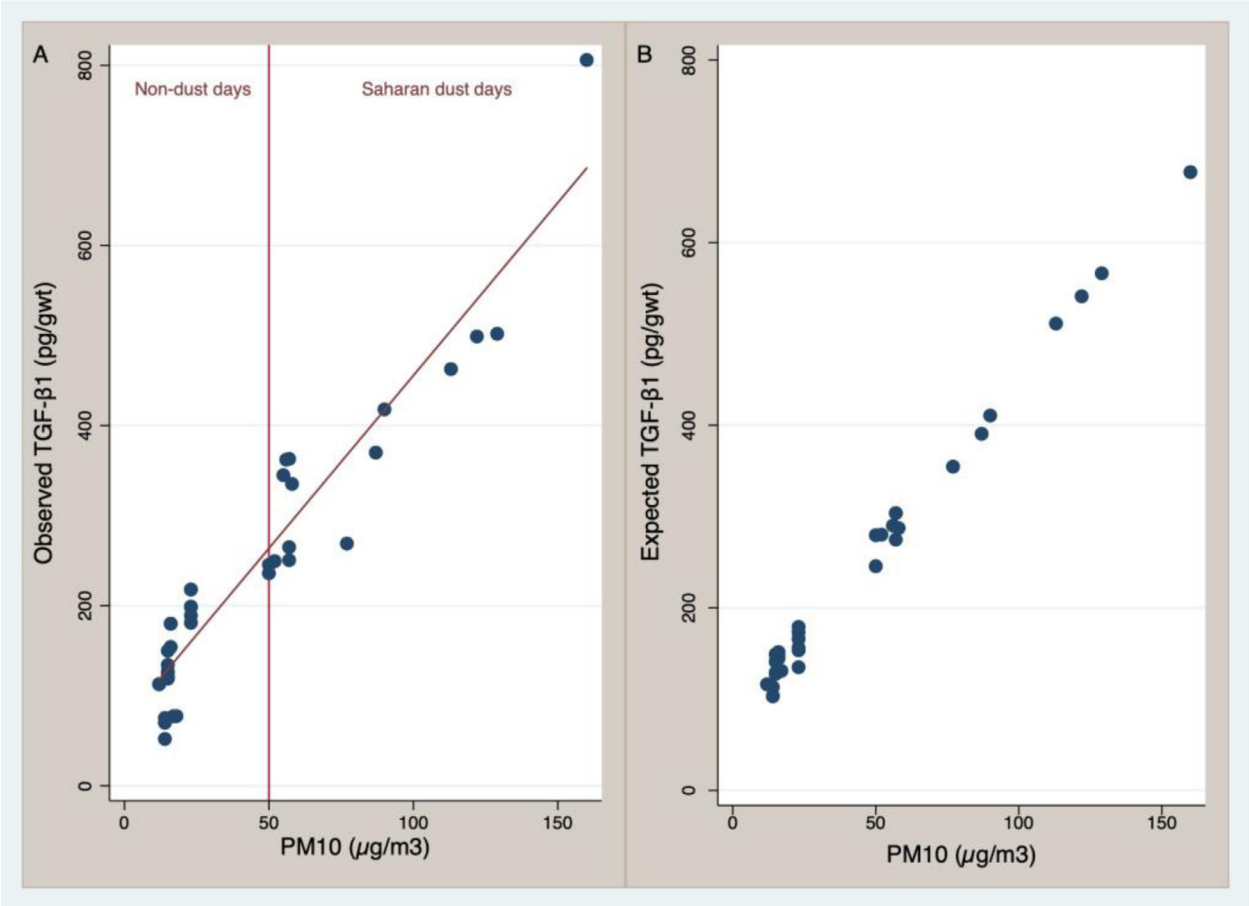

Fig 3. A: Observed values of TGF-β1 in the sputum of patients vs PM_{10} concentrations in the ambient air. Measurements performed during Saharan dust events are differentiated from those collected during nondust days. Red line is the univariate linear regression model. **B**: Predicted or expected value of TGF-β1 in the sputum of patients vs PM_{10} concentrations by controlling for all variables of the maximum model (SO_2, NO_2, NO, and O_3). Data are a total of 38 sputum samples. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Coefficient = 3.79 (CI 95% 3.34−4.24; $P < 0.001$). No other environmental variable was associated with TGF-β1. The reduced model used PM_{10} as the only independent variable, and a clear linear relationship between PM_{10} and the levels of TGF- β1 was found. Coefficient = 3.84 (CI 95% 3.41−4.27; $P < 0.001$). Fig 3 shows the observed levels of TGF- β1 vs PM_{10} (A) and the predicted levels of TGF- β1 vs PM_{10} (B), after controlling for all variables of the maximum model. Final results of the maximum model and the reduced model are shown in Table 2. $R^2_{adjusted}$ of the reduced model = 89.69%, which means that PM_{10} explained the 90% of the TGF-β1 variability in the sputum of the studied individuals.

Levels of TGF-β1 collected in the sputum of patients was 130.65 pg/gwt (103.95−183.1) during no Saharan dust event and 352.54 pg/gwt (254.21−451.6) Saharan dust event (Fig 4A).

A similar statistical modeling was performed to study the influence of dust intrusions ($PM_{10} > 50$ $\mu g/m^3$) on the levels of TGF-β1. The occurrence of Saharan dust events was highly correlated with $PM_{2.5}$, and $PM_{2.5-10}$. $PM_{2.5}$ had a tolerance (1/VIF) = 0.09; VIF = 10.69. $PM_{2.5-10}$ had a tolerance (1/VIF) = 11.99; VIF = 0.08. So, these 2 PM were eliminated of the maximum model. The maximum model showed that Saharan dust events were highly associated with the levels of TGF-β1. Coefficient = 248.45 (CI 95% 172.25−324.65; $P < 0.001$). No other environmental variable was associated with the levels of TGF-β1 and $R^2_{adjusted}$ = 54.78%. The reduced model used Saharan dust events as the only independent variable showing a coefficient = 234.14 (CI 95% 166.20−302.09; $P < 0.001$). $R^2_{adjusted}$ = 56.39%.

Table 2. Maximum model and reduced model for the relationship between PM_{10} and TGF-β1

Maximum model			
Variable	Coefficient	95% Confidence interval	*P*
PM_{10} $\mu g/m^3$	3.79	3.34−4.24	< 0.001
SO_2 $\mu g/m^3$	−0.03	−7.23−7.16	0.99
NO_2 $\mu g/m^3$	2.29	−4.72−9.32	0.51
NO $\mu g/m^3$	−8.05	−35.27−19.17	0.55
O_3 $\mu g/m^3$	−0.88	−2.46−0.69	0.26

Model performance	R^2	$R^2_{adjusted}$	AIC	BIC
	91.13%	89.65%	389.24	398.75

Reduced model			
Variable	Coefficient	95% Confidence interval	*P*
PM_{10} $\mu g/m^3$	3.84	3.41−4.27	<0.001

Model performance	R^2	$R^2_{adjusted}$	AIC	BIC
	89.97%	89.69%	406.46	409.73

NO, nitrogen monoxide; NO_2, nitrogen dioxide; O_3, ozone; PM_{10}, particulate matter with an aerodynamic diameter smaller than 10 microns; SO_2, sulphur dioxide.

Influence of PM_{10} and dust on the levels of hydroxyproline in sputum. Concentrations of $PM_{2.5}$ and $PM_{2.5-10}$ were excluded of the maximum model due to the aforementioned reasons.

The maximum model showed that PM_{10} was associated with the levels of hydroxyproline in the sample. Coefficient = 0.80 (CI 95% 0.67−0.92; $P < 0.001$). No other environmental variable was associated with the levels of hydroxyproline.

The reduced model used PM_{10} as the only independent variable and it showed a high relationship between PM_{10} and the levels of hydroxyproline. Coefficient = 0.80 (CI 95% 0.69−0.91; $P < 0.001$) Fig 5 shows the observed levels of hydroxyproline in sputum vs PM_{10}(A) and the predicted levels of hydroxyproline vs PM_{10}(B) by controlled for all variables of the maximum model. Final results of the maximum model and the reduced model were shown in Table 3. $R^2_{adjusted}$ was = 85.68% suggesting that almost 90% of the variability in the levels of hydroxyproline in sputum is conditioned by the concentration of PM_{10}.

Levels of hydroxyproline in sputum was 16.14 μg/gwt (10.8−25.65) during no Saharan dust events and 54.5 μg/gwt (39.54−69.51) during Saharan dust events (Fig 4 B).

The same process of statistical modeling was performed to study the influence of Saharan dust events ($PM_{10} > 50$ $\mu g/m^3$) on the levels of hydroxyproline. Because these dust events are highly correlated with $PM_{2.5}$ and $PM_{2.5-10}$ they were removed of the maximum model. This maximum model showed that dust events are highly associated with the levels of hydroxyproline in sputum. Coefficient = 49.19 (CI 95% 30.79−67.60; $P < 0.001$). No other environmental variable was associated with the levels of hydroxyproline. The $R^2_{adjusted}$ was= 43.78%. The reduced model was formed by Saharan dust events as the only independent variable showing a coefficient = 45.62 (CI 95% 29.40−61.84; $P < 0.001$). $R^2_{adjusted}$ = 46.01%.

Influence of PM_{10}, TGF-β1 and hydroxyproline in sputum in relation to the obesity as important risk factor for IHD. We calculated the rate of overweight and obesity (50% of patients with overweight or obesity more less). There are no differences between both groups in this variable (Table 1). Nevertheless, as a sensitivity analysis, we performed the regression model controlling for weight and for age (trend towards difference between groups). We found no differences in the

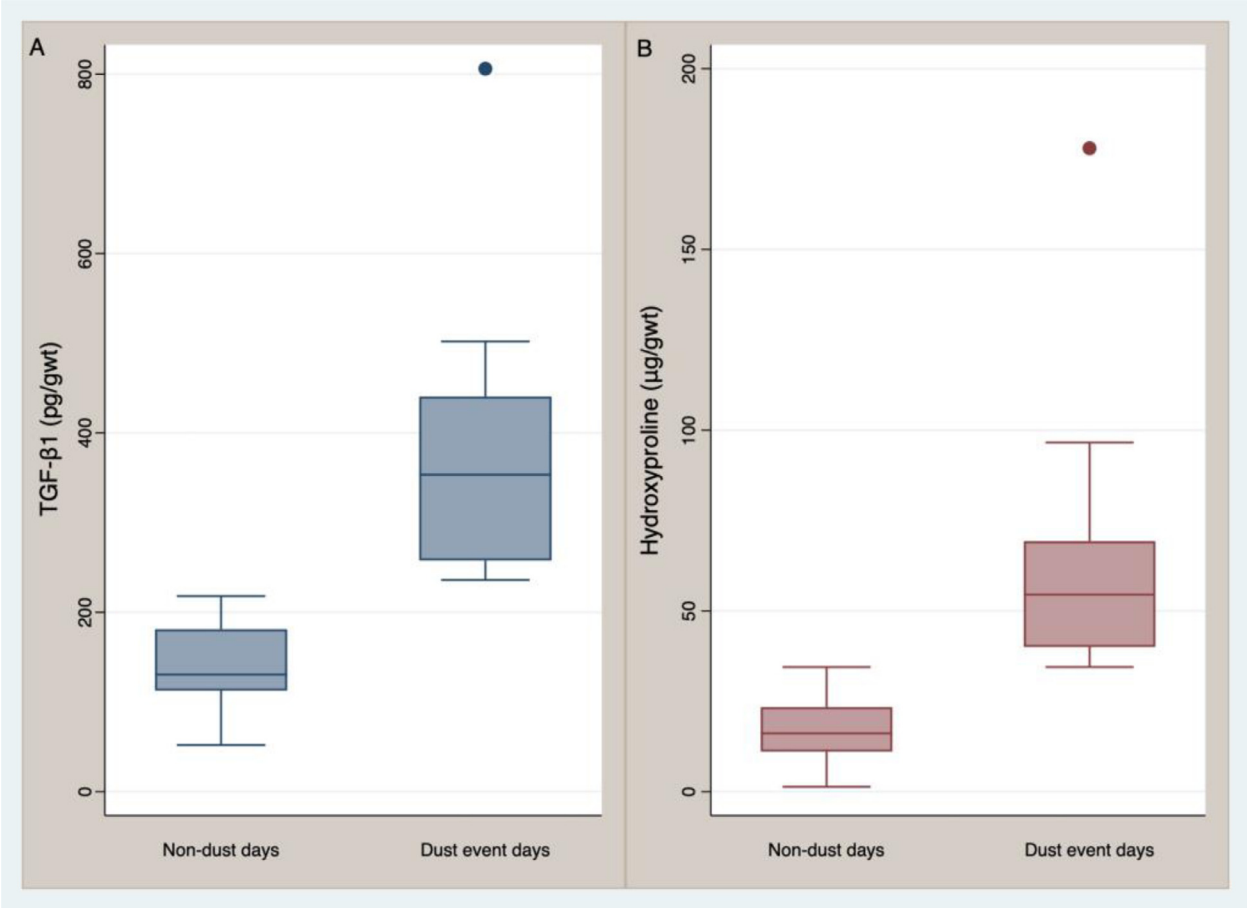

Fig 4. **A**: Concentrations of TGF- β1 in the sputum of patients during Saharan dust events and during no dust events. **B**: Concentrations of hydroxyproline in the sputum of patients during Saharan dust events and during no dust events. Data are shown as median (Q1−Q3), n = 38 sputum samples.

influence of PM_{10} on TGF-β1: coefficient = 3.75 (CI 95% 3.25 - 4.25), similar to the coefficient without controlling for these 2 variables: Coefficient = 3.79 (CI 95% 3.34−4.24; $P < 0.001$). Something similar occurs with the hydroxyproline. Controlling for these 2 variables, the influence of the PM_{10} on hydroxyproline has a coefficient: 0.77 (CI 95% 0.63−0.90). Without controlling for these 2 variables: Coefficient = 0.80 (CI 95% 0.69−0.91). Therefore, in our study these 2 variables are not acting as confounding factors.

DISCUSSION

In the present study, we have shown for the first time that the deleterious effect of the exposure to airborne desert dust in PM_{10} is associated with airway inflammation. These findings reveal a characteristic signature of bronchial inflammation inflicted by Saharan dust events and could provide new insights into potential mechanisms by which inhalation of airborne desert dust increases cardiovascular disease risk.

Most of desert dust sources are located in the so-called ⟨dust belt⟩, that expands through North Africa, the Middle East to inner Asia.[18] Dust from North Africa is mostly exported to the Atlantic, resulting in frequent dust concentrations of (1) up to 1000s $\mu g/m^3$ in Western North Africa, and (2) tens to hundreds $\mu g/m^3$ in the Canary Islands.[13,19] Off North Africa (including the Canary Islands), background levels of PM_{10} are usually low, 15−17 $\mu g/m^3$ (i.e. < 50 $\mu g/m^3$ guidelines of the WHO for human health protection), as result of the contributions of sea salt (~30% of PM_{10}), fuel oil combustion (25%), vehicle exhausts (12%) and local dust (12%).[10] During Saharan dust events, PM_{10} concentrations increase up to reach values within the ranges 50−100s $\mu g/m^3$. In these cases PM_{10} is -by far- constituted by mineral dust.[10] This is an ideal scenario (i.e. airborne particle population dominated by desert dust) for studying the health effects linked to desert $dust_{10}$. A previous study of our group found that exposure to desert dust events with $PM_{10} > 50$ $\mu g/m^3$ was a precipitating factor for admission due to heart failure.[20]

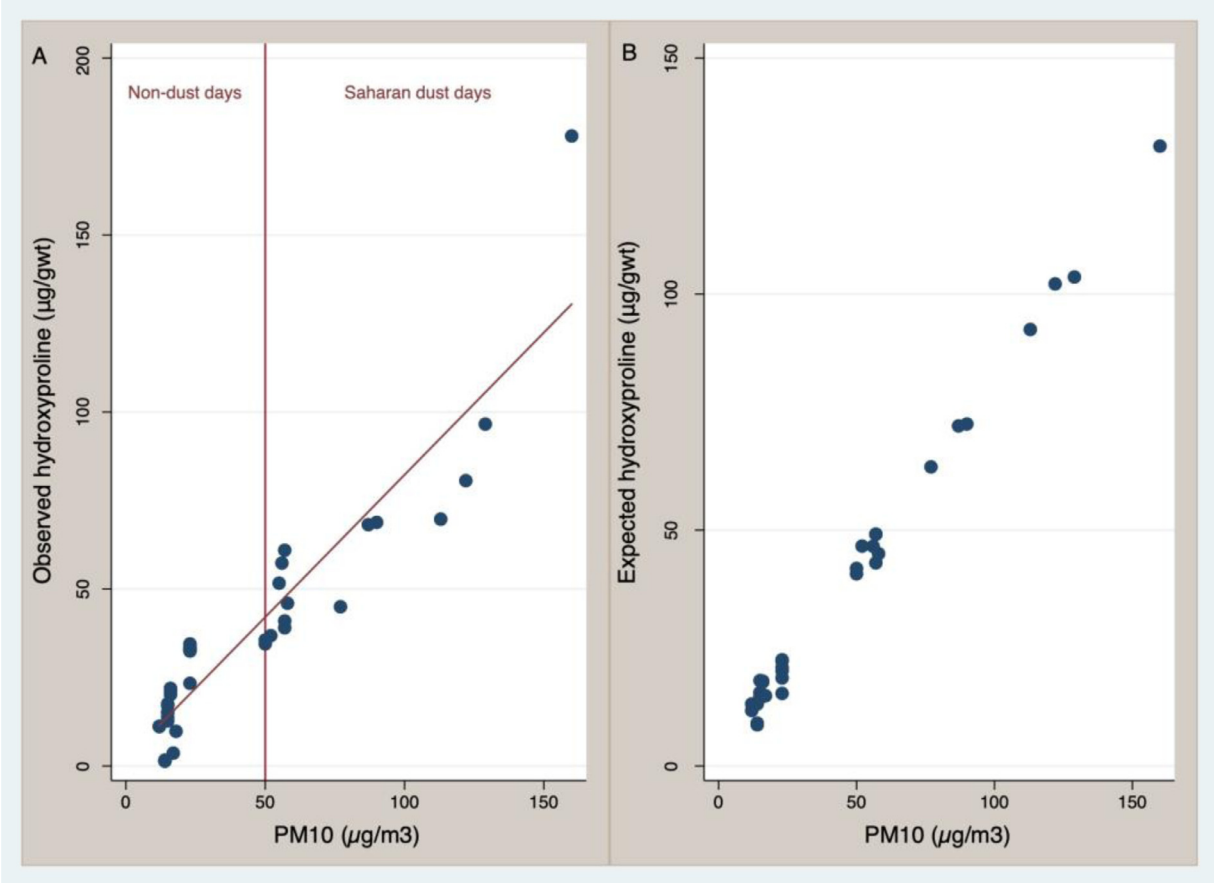

Fig 5. **A**: Observed values of hydroxyproline in the sputum of patients vs PM_{10} concentrations in ambient air. Samples collected during Saharan dust days are differentiated from those collected during nondust days. Red line is the univariate linear regression model. **B**: Predicted or expected concentrations of hydroxyproline in the sputum vs PM_{10} by controlling for all variables of the maximum model (SO_2, NO_2, NO and O_3). Data are a total of 38 sputum samples. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Every year, 700−1400 Tg (10^{12} grams) of desert dust are emitted in North Africa, prompting events of high PM concentrations and poor air quality in the region, but also in the Atlantic and in Southern Europe.[21] Particle size plays a key role on dust impact on health. Particles larger than 10 μm are not breathable. Thus, they can only damage external organs−mostly causing skin and eye irritations, conjunctivitis and enhanced susceptibility to ocular infection. Inhalable particles (smaller than 10 μm, i.e.PM_{10}) enter into the respiratory tract and are, thus, associated with airway inflammation.[13,21] In our study, we found that an increase of 1 $\mu g/m^3$ of desert dust in the PM_{10} fraction is associated with an increase of 3.84 pg/gwt of TGF-β1 and of 0.80 μg/gwt of hydroxyproline in sputum. We found a strong relationship between these variables. Surprisingly, almost 90% of the variability of the TGF-β1 and hydroxyproline in sputum is explained or can be predicted with the increases in PM_{10} concentrations due to desert dust. This suggests that individual comorbidities play an irrelevant role on the levels of TGF-β1 or hydroxyproline.

Despite extensive investigations, it is unclear how Saharan dust events leading to high PM_{10} levels, can initiate adverse cardiovascular responses. It has been claimed from animal experiments that PM evoke particularly intense inflammatory infiltrates in the airway.[22−24] Studies in rat tracheal explants found that exposure to PM may induce inflammation in the airway wall.[22,23] Pirela et al.[23] have shown that an injection of PM into mouse trachea induces inflammatory reactions in mouse lung tissue and increases pro-inflammatory cytokine levels in alveolar lavage fluid. Recently, Hu et al [24] demonstrated that the levels of pro-fibrosis cytokine, TGF-β1, were increased by prolonged exposure to PM. Four week exposure to PM in an animal model of established lung fibrosis not only exacerbated lung fibrosis, but also induced an acute inflammatory reaction.

Table 3. Maximum model and reduced model for the relationship between PM_{10} and hydroxyproline

Maximum model			
Variable	Coefficient	95% Confidence interval	*P*
PM_{10} $\mu g/m^3$	0.80	0.67–0.92	< 0.001
SO_2 $\mu g/m^3$	−0.27	−2.22–1.69	0.78
NO_2 $\mu g/m^3$	−0.05	−1.96–1.86	0.96
NO $\mu g/m^3$	−0.83	−8.24–6.58	0.82
O_3 $\mu g/m^3$	−0.20	−0.62–0.23	0.36

Model performance	R^2	$R^2_{adjusted}$	AIC	BIC
	85.97%	83.64%	295.61	305.11

Reduced model			
Variable	Coefficient	95% Confidence Interval	*P*
PM_{10}	0.80	0.69–0.91	<0.001

Model performance	R^2	$R^2_{adjusted}$	AIC	BIC
	85.68%	85.28%	303.01	306.28

NO, nitrogen monoxide; NO_2, nitrogen dioxide; O_3, ozone; PM_{10}, particulate matter with an aerodynamic diameter smaller than 10 microns; SO_2, sulphur dioxide.

Oxidative stress is the pre-eminent pathophysiological factor for the adverse vascular health effects of air pollution, being well documented in the lungs.[25,26] The lungs' endogenous defenses prevent the systemic penetration of PM. Depletion of low molecular weight antioxidants such as ascorbate, glutathione, and tocopherol with subsequent depletion of reduced cofactors such as nicotinamide adenine dinucleotide phosphate may result in potentiation of oxidative stress.[25] Likewise, the dust inhalation may damage airway protective mucosae, rendering individuals susceptible to endotoxins, leading to secondary toxicity.[25,26]

There are several strengths in this study. First, our study is the first one that analyzes these associations in humans. Second, all our patients live in the Canary Islands, a territory close to the Saharan Desert, the greatest source of desert dust of the world.[19] This allowed us to evaluate the association of PM_{10} derived from natural sources under the worst possible conditions. Finally, the strict selection of patients excluding smokers and patients with lung diseases should make individual comorbidities irrelevant. Despite these strengths, the study has also limitations that should be acknowledged. The number of participants recruited in the study was relatively small. We do not analyze the cellularity of the sputum samples. Moreover, we do not taken samples of the Saharan dust for chemical analysis. The relationship that we found, between PM_{10} and TGF-β1, does not necessarily imply a cause-effect relationship. First, because $PM_{2,5}$ and $PM_{2,5-10}$ had to be excluded of the model due to extremely high levels of collinearity. Second, because we cannot rule out the existence of other unknown or unmeasured variables that can be related with these PM and may be the true cause of the relationship. Anyway, due the exclusion of patients with lung comorbidities and the finding that the 90% of the variability of the TGF-β1 and hydroxyproline in sputum is explained by PM_{10} concentrations, Saharan dust events seem responsible for the different levels of TGF-β1 and hydroxyproline in sputum.

In summary, results of our pilot study suggest that high PM_{10} episodes due to Saharan events are associated with intense inflammatory reaction in the airway mucosae. The effects are statistically significant, and the pattern of results is coherent and consistent. Undoubtedly, the data deriving from of this study require further investigations with a larger sample size and repeated measures. Understanding how desert dust exposure interacts with specific tissues and cell populations at the molecular level could shed light on other health-damaging effects of dust exposure.

ACKNOWLEDGMENTS

All authors have read the journal's authorship agreement and the manuscript has been reviewed by and approved by all named authors.

We thank to (i) the Giovanni Earth data system of NASA for providing MERRA-2 dust modeling and (ii) the Department of Environment of the Government of the Canary Islands for providing air quality data. Analyses and visualizations used in this article were produced with the Giovanni online data system, developed and maintained by the NASA GES DISC. This study was performed within the frame of the project VARDUST-SAL (PGC 2018-099166-B-I00), funded by the Ministry of Science, Research and Innovation of Spain, the Research State Agency of Spain and the European Regional Development Fund (ERDF). JLD is awarded with a posdoc contract "Agustín de Bethencourt," funded by the ProgramaFomento de Transferencia del Conocimiento of the Cabildo de Tenerife.

Conflicts of Interest: All authors have read the journal's policy on conflicts of interest and declare no conflict of interest.

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