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Research Article

Altered Pulmonary Indices and Total Anti-Oxidant Status in Subjects Occupationally Exposed to Chemical Dust

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Abstract

Toxic effect of inhaled industrial dust is becoming topical in the development of respiratory diseases, this work examined alteration in Pulmonary Function Indices (PFI) in relation to induction of cytokines (IL1 and TNF-α) and their effect on the Total Antioxidant Status (TAS) in one hundred and twenty adult subjects working in a pharmaceutical industry. Respiratory function was determined using Spirometry, cytokines were estimated using a sandwich ELISA method and TAS was estimated based on principle of Ferric Reducing Antioxidant Power (FRAP). Using percentage FEV1/FVC ratio, 31.2% (39) of subjects had Normal Respiratory function (NR); 24.8% (31) had Mild Restriction (MRs); 24% (30) had Moderate Restriction (MR); 10.4% (13) had Moderately Severe Restriction (MSR); 5.6% (7) had Severe Restriction (SR); 2.4% (3) had Mild Obstruction (MO) while 0.8% (1) had Obstruction combined with Restriction (OR). Mean TAS was 0.64±0.29 and 1.07±0.44 in test and controls (NR), mean TNF-α was 3.9–14.50 and 3.9-84.75 in test and controls respectively while Mean IL1 was 3.9-128.75 and 3.9-55.20 in test and control subjects. Aside from 20% of subjects showing various kinds of abnormal pulmonary dysfunctions, volume of expirable (FEV1) and reserve (FVC) capacity of the lungs in subjects with normal PFI (controls) were higher while the FEV1/FVC% which was significantly different in those with abnormal PFI correlated with the significantly low level of TAS observed in subjects with abnormal PFI. However, the low TAS correlated significantly with the increased level of proinflammatory cytokine (IL1) indicating increased inflammatory activity in subjects with abnormal PFI.

Key Words: Pulmonary function indices, total antioxidant status, Cytokines, spirometry

INTRODUCTION

Environmental pollutants have been implicated in a number of disease including cancer, liver, and respiratory diseases. There is a growing concern about respiratory diseases especially amongst subjects that are occupationally exposed to various types of industrial dust, chemicals, metals and other pollutants. This is informed by the increase in incidence of respiratory disease including, Chronic Obstructive Pulmonary Disease (COPD). Numerous epidemiological studies have shown the contribution of ambient pollutants to the development of health disorders (Anderson et al., 2012). Recent studies also demonstrated that exposure to particulate matter correlates with human health risks (Kim et al., 2015). Furthermore, the specialized cancer agency of the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) reported an increased risk of lung cancer as levels of exposure to particulate matter increases (Loomis et al., 2013). Ambient pollutants are now the third leading contributor to disability-adjusted life years associated with chronic respiratory disease (Lim et al., 2010).

Respiratory disease is a major cause of mortality and morbidity worldwide. In most developing countries including Nigeria, the burden of respiratory disease is largely unknown; however, the burden of infectious and non-infectious respiratory disease appears to be on the increase (Akanbi 2009). This is because the respiratory tract is vulnerable to occupational hazards (Harrington and Gill, 1987), which include exposure to organic and inorganic dusts generated from various sources including industries. Respiratory diseases include COPD, Asthma, Emphysema and others. Globally, as of 2010, COPD affected approximately 329 million people (4.8% of the population) and is slightly more common in men than women (Vos et al, 2010). This is as compared to 64 million being affected in 2004 (WHO, November 2012).

Chronic Obstructive Pulmonary Diseases (COPD): COPD is a type of obstructive lung disease in which chronic incompletely reversible poor airflow (airflow limitation) and inability to breathe out fully (air trapping) exist. The poor airflow is the result of breakdown of lung tissue (known as emphysema). Other respiratory diseases include small airways disease known as Obstructive Bronchiolitis which often develops as a significant and chronic inflammatory response to inhaled irritants (Decramer, Janssens and Miravitlles (2012).

Emphysema: This is also a chronic medical disorder of the lungs in which the air sacs are dilated or enlarged and lack flexibility, so that breathing is impaired and infection sometimes occurs while Asthma is a disease of the respiratory system, sometimes caused by allergies, with symptoms

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including coughing, sudden difficulty in breathing, and a tight feeling in the chest.

Pulmonary Function Indices remain some of the readily available tools/data employed in the diagnosis of chronic obstructive airways diseases especially in the vulnerable group. The indices include Forced Expiratory Volume (FEV1), Forced Expiratory Capacity (FVC) and Pulmonary Expiratory Function Rate (PEFR) as parameters to determine and establish a differential in the incidence of chronic airways diseases. The ratio of FEV1 /FVC expressed as % is usually diagnostic of the presence or otherwise of pulmonary disease. This measurement is performed with a Spirometer. Spirometry measures the amount of airflow obstruction present and could be carried out before and after the use of a bronchodialator-a medication to open up the airways.

Two main components are measured to make the diagnosis:

i. The Forced Expiratory Volume in one second (FEV1), which is the greatest volume of air that can be breathed out in the first second of a breath
ii. The Forced Vital Capacity (FVC), which is the greatest volume of air that can be breathed out in a single large breath.

Normally, 75-80% of the FVC comes out in the first second and a FEV1/FVC ratio of less than 70% in someone with symptoms of COPD defines a person as having the disease.

Particulate matter is continuously affected by both stationary (power plants, industries, incinerators, and residential heating) and mobile sources (road traffic) (Bilos et al., 2001). One major source of particulate matter is chemical dust, which is common in most industries or workplaces where chemicals are engineered or manufactured (Robert, 2011). Essentially, intense and prolonged exposure to workplace dusts, chemicals and fumes increase the risk of COPD (Devereux and Graham, 2006). Workplace exposures are believed to be the cause in 10-20% of cases (Laine and Christine, 2009). In the United States they are believed to be related to more than 30% of cases among those who have never smoked and probably represent a greater risk in countries without sufficient regulations (Vestbo, Jørgen, 2013). However, various adverse respiratory effects associated with chemical dust may be related to the production of pro-inflammatory cytokines, the type of which may depend on the composition of the particulate matter (Hetland et al., 2005).

Cytokines are small, secreted proteins that control immune responses. Within the lung, they can control host responses to injuries or infection, resulting in clearance of the insult, repair of lung tissue, and return to homeostasis. Problems can arise when this response is over exuberant and/or dysregulated. In such cases, chronic and repeated inflammatory reactions and cytokine production can be established, leading to airway remodelling and fibrosis with unintended, maladaptive consequences (Sergei 2003).

Oxidative stress is also believed to play an important role in the systemic manifestations of COPD (Langen, 2003). Considerable evidence has emerged in recent years implicating a central role for oxygen free radicals in the initiation of cellular injury that leads to the development of several lung diseases (Kehrer et al., 1993). It is well established that oxygen free radicals and their metabolites-collectively called reactive oxygen species (ROS)-can induce direct cell injury, which may trigger a cascade of radical reactions promoting the disease process (Rahman 1996).

Despite the fact that most studies linked chemical dust with health defect, not all studies, however, have established an association between particulate matter and respiratory disorders (Atkinson et al., 2014). Differences in the findings between studies may be attributable to the complexity of dealing with the individual variability of the studies and the disparity in the composition of particulate matter (Burnett et al., 1995). To date, few studies have investigated the inflammatory responses caused by particulate matter from chemical dust. Particularly in Nigeria, many studies have investigated effect of dusts from sand, cement and carpentry on pulmonary functions, but only few have investigated effect of chemical dust from pharmaceutical industry on the pulmonary functions of pharmaceutical worker. Hence, this research aims to explore the impact of chemical dust on pulmonary functions.

MATERIALS AND METHODS

One hundred and twenty adults (aged 28-52years) occupationally exposed routinely to chemicals (cases) while fifty adults (controls, age matched) working in non-industrial setting were recruited for this study. Amongst the 120 cases, those with Normal PFI also served as controls.They were screened: (a) for their pulmonary function using spirometry (b) total antioxidant and cytokine levels were analysed in blood using ELISA techniques based on standard laboratory procedure.

Pulmonary function tests

For the pulmonary function, the following indices of PFI were measured using a Spirometer (Spirolab III manufactured by Medical International Research, Italy):

FEV1 (Forced Expiratory Volume): Volume of air released in 1 second by the lungs

FVC (Forced Volume Vital Capacity): Measures the approximate volume of air retained in the lungs.

FEV1/FVC Ratio (%): Percentage of this volume that is approximately released during every process of inhalation and exhalation.

PEF (Peak expiratory flow): Maximum flow speed achieved per minutes

From the PFI evaluated using a spirometer, participants/cases could be classified as (a) Normal Spirometry (b)Mild Restriction (c) Mild Obstruction (d) Possibly mild Obstruction (e) Possibly Moderate Restriction (f) Possibly Moderate Severe Restriction or(g) Possibly Severe Restriction (PSR)

Determination of IL-1 and TNF-α

Both IL-1 and TNF-α were determined using a sandwich ELISA kit (manufactured by Cayman Chemical Company, USA) based on double-antibody 'sandwich' technique according to manufacturer's instructions.

Determination of Total Antioxidant Capacity (TAS)

Total Anti oxidant capacity was determined using the principle Ferric Reducing Antioxidant Power (FRAP) as described by Harma, (2003).

Statistical Analysis: TAS and cytokine levels were analysed and correlated with the various Respiratory indices and significant values established at $P \leq 0.05$.

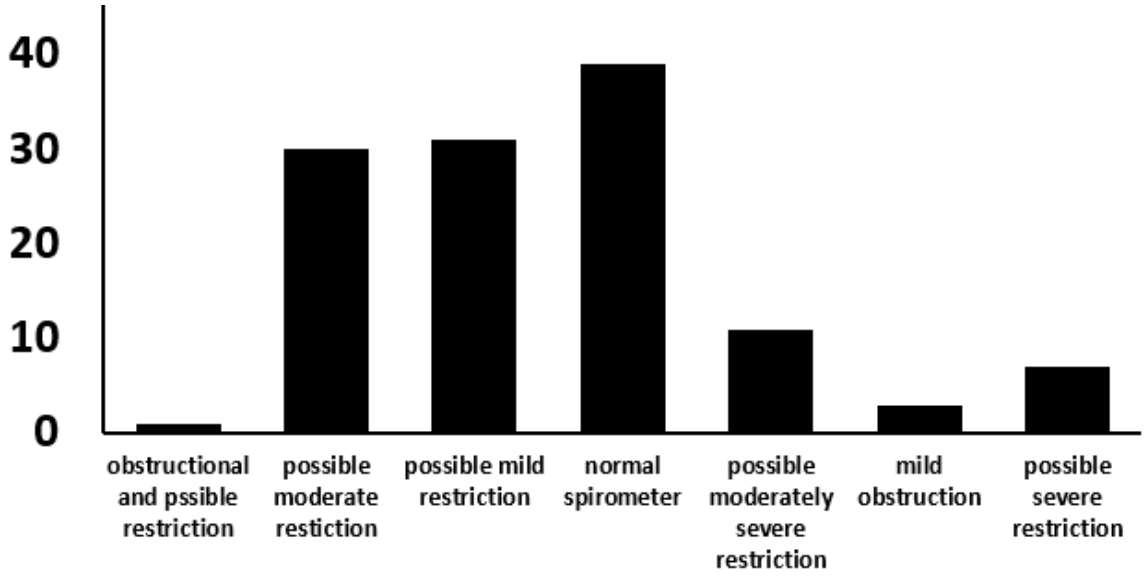

Figure 1
Summary of respiratory profile of participants

RESULTS

For clinical differentiation, exposed subjects with possible moderate and possible mild restrictions were grouped along with those with NS as having normal respiratory function. All the other categories of PFI were considered abnormal. Hence, 80% of the subjects had normal PFI while 20% had abnormal PFI.

From the PFI evaluated using a spirometer, participants/cases could be classified as: (a) Normal Spirometry (about 32%) (b) Mild Restriction (25.4%) (c) Mild Obstruction (24.6%) (d) Possibly Mild Obstruction (9.0%) (e) Possibly Moderate Restriction (5.7% (f) Possibly Moderate Severe Restriction (2.5%) and (g) Possibly Severe Restriction (0.8%))

Table 2:
Summary of Results of TAS, IL1 and TNF-α in the subjects

Variables	*Exposed*	*Unexposed*	*t*	*p*
TAS (µmol/l)	0.64 ±0.29	1.07 ±0.44	-4.810	0.000*
IL1-α	4.2 (3.9-128.75)	3.9 (3.9-55.20)	-1.200	0.230
TNF-α	3.9 (3.9-14.50)	4.10 (3.9-14.75)	-1.240	0.125

Clinically, subjects with mild restriction /obstruction will be asymptomatic; they are therefore usually classified with those showing normal spirometry. Hence, about 82% of participants in this study could be said to exhibit normal spirometry in spite of their exposure to chemical dust at work. However, correlation analysis shows a significant negative correlation between TAS and IL1, an indication of significant concurrent reduction in the two parameters due to the exposure (Table 4).

TNFα also correlated positively and significantly with peak expiratory flow volume of the lungs; an indication of a direct effect of the two parameters in dust exposure. Significant correlation between PEF and TNF-α in exposed group may indicate that changes in PEF could be reflective of pulmonary inflammation or heightened responsiveness.

Tables 2 and 3are the summary of ANOVA results of the Total antioxidant status (TAS), IL1 and TNF-α of cases and controls. Although there were no significant differences in the cytokine levels in cases and controls, TAS was significantly lower in exposed pharmaceutical workers compared to unexposed healthy individual.(p=0.000).

Table 3:
Summary of Correlation of PFI with TAS, IL1 and TNF- α

Variables	*NS*	*PMR*	*PMSR*	*F*	*P*
TAS (µmol/l)	0.55 ±0.24	0.54 ±0.32	0.51 ±0.29	1.520	0.224
FVC	3.63 ±0.71	3.00 ±0.47	2.40 ±0.46	11.995	0.009*
FEV_1	3.22 ±0.67	2.84 ±0.49	2.33 ±0.45	6.063	0.016*
FEV_1/FVC	88.62 ±6.38	94.55 ±6.22	96.79 ±5.26	5.395	0.014*
PEF	8.14 ±2.05	8.43 ±2.37	9.03 ±2.87	0.323	0.733
FEF	4.15 ±1.08	4.41 ±1.75	4.85 ±1.94	0.442	0.653
IL1	19.96	23.33	24.36	0.811	0.670
TNF-α	21.58	22.35	24.71	0.330	0.850

When the TAS values were compared with the various indices of pulmonary function using ANOVA in participants with normal, possible mild restriction and possible moderate severe restriction based on spirometry analysis, FEV1, FVC and by implication FEV1/FVC were found to be significantly different in cases compared to control. However, there was no significant difference between the cytokines and TAS in the two groups. This may be an indication of significant alteration

of the TAS in participants occupationally exposed to chemical dust in comparison to the non-exposed controls.

Table 4 :
Correlation of TAS with cytokines in exposed and unexposed

	Exposed	***Unexposed (r, p)***
(r, p)		
TAS/IL1	0.149,0.336	-0.469,0.037*
TAS/TNF	-0.052,0.738	-0.397,0.083

Table 5:
Correlation of TAS,IL1 and TNF with pulmonary functions in exposed

	TAS (r, p)	***IL1-α (r,p)***	***TNF-α (r,p)***
FVC	0.053,0.734	-0.158,0.305	0.105,0.498
FEV1	0.130,0.398	-0.105,0.497	0.119,0.440
FEV1/FVC	0.148,0.398	0.181,0.239	0.065,0.673
PEF	0.274,0.072	0.246,0.107	0.373,0.013*
FEF	0.151,0.329	0.283,0.062	0.237,0.121

DISCUSSION

A prescription is an order that is written by the physician or a Respiratory diseases are common amongst industrial workers especially in industries generating or producing dusty materials and more commonly where standard work ethics is not enforced; this is because the lungs are the route of entry for noxious particles and gases (Mohemid 2015). Amongst the respiratory diseases are COPD, asthma, emphysema and many others; COPD is most prominent of these diseases. Many factors have been linked as facilitating the development of COPD among which is smoking, exposure and inhalation of pollutants etc. In tracing the etiology of COPD, occupational exposure to dust and chemicals remains one of the most veritable sources of contacts. Hence exposure to dust like iron-dust in iron-smelting industries, fine metal granules as in motor and body repair factories, chemicals and various organic and inorganic dust particles as in pharmaceutical industries, smoking (passive and active) which exposes people to some toxic metals and a host of other vocations are of paramount importance. The common feature of this inhalation process is generation of inflammatory responses.

Inhalation of particle dust has been linked to initiation of inflammatory processes which usually manifests by increased proliferation of pro-inflammatory cytokines like TNF-α and IL1 (Borm et al 2002), thus observation of increased TNF-α and IL1 in subjects with abnormal PFI relative to the control and those with NS in this study may be a clear manifestation of inflammatory processes. That this may be the pathophysiology of cytokines proliferation has been reported by previous workers (Borm et al 2002).

From the results of pulmonary function analysis using spirometry conducted on the participants, 80% had Normal spirometry and 20% had a type of Pulmonary Obstruction or the other.

Spirometry essentially monitors inflow and outflow of air along with evaluating the capacity and capability of the lung to retain/store air in its lobules. In doing this FEV_1 (Forced expiratory volume) indicates the volume of air released in 1 seconds by the lungs which is an evaluation of breathing out process. Also, FVC measures the approximate volume of air retained in the lungs while the ratio of FEV_1/FVC (%) indicates the percentage of this volume that is approximately released during every process of inhalation and exhalation.
In this study, reduced FEV_1 was observed in all the participants showing abnormal Pulmonary Functions as compared to the control group among the subjects. The reduced FEV_1 in test subjects may be due to various degree of obstruction of their airways which would also be seen by the increase in the inflammatory cytokines in this group relative to the value of cytokines in the control. Moreover, given the localization and effects of chemical dust on lung tissue, the data here strongly suggests that chemical dust could be responsible, at least in part, for the pulmonary function reduction (FEV_1) and inflammatory cytokines response observed in pharmaceutical workers.

The first line of defence and clearance against exposure to chemical metals is mounted primarily by alveolar macrophages (Dorger et al 2002). When alveolar macrophage becomes activated by invading pathogens and particles, mostly from chemical dust, they release cytokines (Dorger et al 2002). This may form the basis for the findings seen in the cytokine levels of the pharmaceutical workers reported in this study. That test participants in this study exhibited reduced pulmonary function indices is not different from a study by Johncy et al, who found a statistically significant decrease in lung function indicators, including FEV1 and FVC, in sweepers exposed to road dust as compared to controls (Johncy 2013).

The observed association of reduced FEV_1 with chemical dust is consistent with previous studies showing an association between reduced FEV1 or FVC and exposure chemicals as seen in smelters (Dennekamp 2007). Cement factory workers are also exposed to a mixture of different chemical component have been linked to respiratory function deficits (AbuDhaise 1997).

However, IL_1 andnot TNF-α (which are both proinflammatory cytokines) only positively correlated significantly with TAS. It may then be inferred that the restriction/obstruction of the airways occurs due to inflammation and scarring within them. This contributes to the inability to breathe out fully causing low oxygen levels and, eventually, accumulation of CO_2in the blood due to poor gas exchange. The endothelium which is major determinant of vascular tone (blood flow), leucocyte and thrombocyte adhesion, and smooth muscle cell will start proliferating. This accentuates the synthesis and bioactivity of vasodilators such as NO (nitric oxide). prostacyclin and endothelium derived. An alteration in the redox balance in ECs leads to increased O^{2-} (superoxide anion) production and oxidative stress

TNF-α is a known proinflammatory cytokine usually produced by macrophages in response to particle invasion; hence the observed correlation between PEF and TNF-α in the test group corroborate the increased inflammatory process which may be the pathophysiology of the observed obstruction in the test participants. This is reinforced by the study of Schins and Borm (Schins 1995), Kim et al (Kim 1999) and Lee et al (Lee 2010) who variously reported significant increase in the TNF-α of silica exposed workers.

Though this study did not find a significant difference in the mean TNF-α between test and control groups, the pro-inflammatory cytokine TNF-α is considered to be an important mediator in inflammation (Saber 2005). Saber et al suggested

that TNF was not required for the induction of inflammation by chemical dust. Significant correlation between PEF and TNF-α in exposed group may indicate that changes in PEF could be reflective of pulmonary inflammation or heightened responsiveness. This also supports the need for regular spirometry test for pharmaceutical workers and others exposed routinely to particulate dust to stem the increasing incidence of respiratory diseases.

In this study, TAS was significantly lower in exposed pharmaceutical workers compared to unexposed healthy individual. This supports previous studies which reported reduced plasma antioxidant status in workers exposed to ozone (O_3) (Bocci *et al.*, 1998).

In conclusion, this study demonstrated oxidative stress (reduced antioxidant capacity) and altered pulmonary function in workers exposed to pharmaceutical dust. Hence, regular spirometry test and antioxidant supplementation is highly recommended for pharmaceutical industry workers to ameliorate the adverse effects.It has been recognized that this study is subject to a number of limitations. For example, it cannot be excluded that exposures to other unexamined pollutants may have also affected FEV1 or FVC levels. Also it cannot be excluded that false negative findings due to the relatively small sample size, further studies are needed to better clarify the role of inflammation in the relation of lung function to particulate matter and elemental component exposure

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