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Risk of Pulmonary-Reproductive Dysfunctions, Inflammation and Oxidative DNA Damage in Exposure to Polycyclic Aromatic Hydrocarbon in Cigarette Smokers

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Abstract

Background: Exposure to cigarette smoke has been associated with pulmonary and reproductive dysfunctions; inflammatory response, oxidative stress and oxidative DNA damage induced by polycyclic aromatic hydrocarbons (PAHs) present in cigarette smoke have been implicated in the pathogenesis of these disorders. The peak expiratory flow rate (PEFR), a biomarker of inflammation and oxidative DNA damage (8-hydroxy-2-deoxyguanosine (8-OHdG), tumor necrosis factor alpha (TNF-α)), reproductive hormones (testosterone (TST), luteinizing hormone (LH), follicle stimulating hormone (FSH)) cotinine and urinary PAH metabolite (1-hydroxypyrene (1-HOP)) were estimated in male active smokers.

Methods: One hundred men aged 20-47 years, comprising 50 active male smokers and 50 non-smokers, were randomly recruited into this comparative cross-sectional study. The PEFR was measured using a peak flow meter, serum levels of cotinine, FSH, LH, TST, TNF-α, and urine 8-OHdG by enzyme-linked immunosorbent assay and 1-HOP by high-performance liquid chromatography. Data analysis was done using a t-test and correlation analysis at $p \leq 0.05$.

Results: Smokers had significantly higher cotinine (49.73±31.76 versus 0.51±0.69 ng/ml, $p \leq 0.001$), 8-OHdG (16.34±12.10 versus 5.79±2.14 ng/ml, $p \leq 0.001$) and lower PEFR (309.20±56.05 versus 452.80±45.76 L/min, $p \leq 0.001$) and LH (5.75±2.06 versus 6.97±2.79 mIU/ml, $p=0.015$) compared to non-smokers. Duration of exposure to cigarette smoke correlated positively with cotinine ($r=0.937$, $p \leq 0.001$) and 1-HOP ($r=0.813$, $p \leq 0.001$) while cotinine correlated positively with 1-HOP ($r=0.863$, $p \leq 0.001$) only in smokers.

Conclusion: Reduced lung function and luteinizing hormone and concurrent increase in oxidative DNA damage associated with exposure to cigarette smoke may suggest the involvement of PAH-induced DNA damage in the development of pulmonary and reproductive impairment in smokers.

Keywords: Cigarette Smoke, Hormones, Lung, Oxidative Stress, Inflammation

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Introduction

Cigarette smoking still remains an enormous public health problem and is now the world's single leading

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↑What is "already known" in this topic:
- Exposure to cigarette smoke has been associated with multiple organ dysfunctions.
- Nicotine and polycyclic aromatic hydrocarbons present in cigarette smoke has been implicated
- Effects of nicotine and PAH exposure on indices of OS, inflammation and pulmono-reproductive functions in cigarette smokers is still uncertain.

→What this article adds:
- Cigarette smoking is associated with increased oxidative DNA damage and reduction in lung function and luteinizing hormone.
- Exposure to cigarette smoke and increasing duration of exposure is positively associated with increased urinary excretion of PAH metabolite; 1-hydroxypyrene.

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cause of several preventable diseases and premature deaths and has been described as a common risk factor for diabetes, cardiovascular disease, chronic respiratory disease, and cancer (1). Tobacco use has been reported to be responsible for 8 million deaths each year, with more than 7 million of these deaths accruing from direct tobacco use and 1.2 million from secondhand smoke (2). Adverse effects of cigarette smoking have been linked to the diverse effects of the complex mixture of chemical constituents of cigarette smoke (CS) on biological systems which can be influenced by age, sex, race, genetic variations, individual genetic susceptibility as well as variations in smoking pattern (3, 4).

Major toxic, mutagenic and carcinogenic constituents of CS include nicotine, CO, tar, N-nitrosamines, aromatic amines, cadmium, and polycyclic aromatic hydrocarbons (PAHs) (5). Among these, nicotine and PAHs are the most extensively studied, and their pathways of metabolic activation and detoxification have been well documented (6). Exposure to nicotine and PAHs in CS has been shown to be associated with adverse effects on multiple organs. Chronic exposure to nicotine and PAHs has been related to decreased lung function (decline in FEV_1/FVC) among adults in occupational settings (7). PAHs have been linked to impairment of the reproductive system. Exposure to PAHs has been shown to cause disruptive endocrine activity and contributes to decreased testosterone and increased luteinizing hormone (LH) concentrations (8). Exposure to CS has also been implicated in the modulation of both innate and adaptive immunity either by attenuation of defensive immunity or exacerbation of pathologic immune responses through the induction of both inflammatory and anti-inflammatory mediators (9). Pathologic mechanisms both specific and non-specific, employed by the different components of CS to effect adverse health complications commonly involve OS, oxidative DNA damage, genetic mutations and vasomotor dysfunction (4).

Despite public health warnings on the dangers of CS, a greater proportion of young people are increasingly engaging in this unhealthy social behavior with reckless abandon. This has led to an increased incidence of smoking-related illnesses in the population especially among smokers. Association of exposure to nicotine and PAHs in CS and the development of chronic diseases have been documented. However, studies on this association is inadequate compared to the burden of the disease in the area of study, more so its combined effects on immune, pulmonary and reproductive functions of smokers. Evaluation of biomarkers that may predict the organ and systemic damage associated with exposure to PAHs in CS may be important in identifying individuals at risk of development of these disorders and implementing appropriate preventive and remediation measures to avert undesirable health outcomes. This study, therefore, assessed the peak expiratory flow rate, urine creatinine, PAH metabolite (1-hydroxypyrene) and biomarker of oxidative DNA damage and inflammation (8-hydroxy-2-deoxygnanosine & tumour necrosis factor-alpha) in active smokers and the effect of duration of exposure to CS on the levels of these indices.

Methods

Design of study

This comparative cross-sectional study was carried out in Calabar, Nigeria between March and September 2019. The study population was recruited after written informed consent and after approval from the health ethical committee of the state ministry of health. All ethical principles guiding the conduct of medical research involving human subjects were observed as outlined in the Helsinki declaration of 1975 and more recent revisions.

Selection of subjects

One hundred apparently healthy men aged 20-47 years living within the study area were enrolled in the study. The subjects enrolled in the study were regrouped according to their smoking history into:

- Active smokers comprise 50 individuals who smoke >3 cigarettes per day for the past 1 year and above,
- Non-smokers comprise 50 individuals who are never been smokers and have not been exposed to secondhand smoke (10).

Inclusion criteria: Male subjects who are apparently healthy and have been first hand smokers for at least one year prior to the time of this study.

Exclusion criteria: Those who were former smokers, with history of alcohol, drug abuse or substance addiction, individuals with any form of chronic organ or systemic illness or prolonged medication.

Socio-demographic information, health status, family and medical history and life style habits were obtained using a semi-structured questionnaire while blood pressure and anthropometric indices were obtained using standard methods.

The sample size was obtained using the formula by Daniel and Cross (11); $n = Z^2P\ (1 - P)/d^2$ (where Z = standard normal deviation at 95% confidence interval = 1.96; P = estimated prevalence of lung cancer; d = precision limit 5% = 0.05) with a global prevalence of lung cancer of 2.8% (12) and 10% attrition ratio giving a total of 100 participants (50 test participants and 50 controls).

Sample collection

Whole blood samples and spot urine samples were collected from all subjects of the study into a plain container for blood samples and a universal plastic container for urine samples respectively and transported to the laboratory in a cold box. The blood samples were spun at room temperature at 500g for 10 minutes. Sera were collected for the estimation of hormones (follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (TST)), a biomarker of inflammation (tumor necrosis factor alpha (TNF-α)) and cotinine while spot urine was used for the estimation of PAH metabolite (1-hydroxypyrene (1-HOP)), creatinine and marker of oxidative DNA damage (8-hydroxy-2-deoxyguanosine (8-OHdG).

Laboratory Methods

Estimation of Peak Expiratory Flow Rate (PEFR)

The peak expiratory flow rate was determined using a hand-held peak flow meter which measures the volume of

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air forcefully expelled from the lungs in one quick exhalation and is a reliable marker of airway function (13).

Estimation of Cotinine

Cotinine levels were measured using a competitive enzyme-linked immunosorbent assay method using a test kit obtained from Calbiotech, California, USA. The cotinine in the sample competes with a cotinine enzyme conjugate for the antibody binding site on the ELISA microwell. Unbound components were washed off, followed by the addition of substrate which resulted in the formation of a colored complex whose color intensity is inversely proportional to the concentration of cotinine in the sample (14).

Estimation of urine 1-hydroxypyrene

The estimation of urinary 1-HOP was done using high-performance liquid chromatography (HPLC) as previously described. The concentration of urinary 1-HOP was adjusted to urinary creatinine excretion (µg/g creatinine) to correct for urine flow rate (15).

Estimation of urine Creatinine

Creatinine in the sample, in an alkaline medium reacts with picric acid to form a yellow complex whose intensity at 520 nm is in direct proportion to creatinine levels in the sample (16).

Estimation of 8-hydroxy-2-deoxyguanosine (8-OHdG).

The 8-OHdG concentration was estimated based on the competitive ELISA technique. A competitive reaction occurs between 8-OHdG in the sample and 8-OHdG enzyme conjugate for antibody binding site on the microwell plate. The addition of a substrate solution leads to color development, the intensity of which is inversely related to 8-OHdG in the sample, which was read from a standard curve (17).

Assay of tumor necrosis factor alpha (TNF-α)

The TNF- α was estimated using the ELISA method which involves competition between TNF- α in the sample and TNF- α labeled conjugate for a limited number of antibody binding sites on a microtiter plate. After washing, a substrate solution was added and the intensity of the colour complex formed is proportional to TNF- α levels in the sample (18).

Hormonal assays

The reproductive hormones; FSH, LH, and testosterone were analyzed based on the ELISA

method using a test kit procured from Calbiotech, California, USA as previously described (19-21).

Data analysis

Analysis of data was done using SPSS version 20.0, and results were presented as mean ± standard deviation. Comparison of mean variables between groups was done using the t-test while associations between variables were determined using Pearson correlation analysis at a probability level of $P<0.05$.

Results

The comparison of age, BMI, cotinine, 1-HOP, 8-OHdG, TNF-α, PEFR, FSH, LH, TST, and urine creatinine in smokers and non-smokers. Smokers had lower BMI, PEFR, LH ($p\leq0.001$, $p\leq0.001$, $p=0.015$) and urine creatinine and higher cotinine and 8-OHdG compared to non-smokers ($p\leq0.001$, $p\leq0.001$). Other indices studied were not significantly different between the 2 groups ($p>0.05$) (Table 1).

The correlation plot of cotinine with the duration of exposure to cigarette smoke in smokers was depicted in Figure 1. A positive correlation was observed between cotinine and duration of exposure to cigarette smoke (r=0.937, $p\leq0.001$).

Figure 2 shows the correlation of 1-HOP with the duration of exposure to cigarette smoke in smokers studied. 1-HOP correlated positively with the duration of exposure to cigarette smoke (r=0.813, $p\leq0.001$).

The correlation of cotinine with 1-HOP in smokers is shown in Figure 3. A significant positive correlation was observed between cotinine and 1-HOP (r=0.863, $p\leq0.001$) in smokers studied

Table 1. Comparison of Age, BMI, cotinine, 1-hydroxypyrene, indices of oxidative DNA damage and inflammation, lung and reproductive hormones in Smokers and Non-Smokers

Parameter	Smokers n=50	Non-Smokers n=50	*P*-value
Age (years)	26.46±4.95	27.30±3.75	0.342
BMI (kg/m^2)	20.78±1.91	22.41±2.14	<0.001*
Cotinine (ng/ml)	49.73±31.76	0.51±0.69	<0.001*
1-HOP (µg/g Cr $^{-3}$)	1.68±1.77	1.44±1.86	0.517
8-OHdG (ng/ml)	16.34±12.10	5.79±2.14	<0.001*
TNF-α (pg/mL)	27.12±20.42	25.12±19.20	0.615
PEFR (L/min)	309.20 ± 56.05	452.80 ± 45.71	<0.001*
FSH (mIU/ml)	4.71±1.96	5.44±3.54	0.202
LH (mIU/ml)	5.75±2.06	6.97±2.79	0.015*
TST (ng/ml)	7.19±2.51	7.98±1.89	0.083
uCr (mg/L)	255.88 ± 30.42	268.98 ± 21.97	0.015*

Result presented as Mean ±SD; * = Significant at P ≤ 0.05; BMI = Body mass index; 1-HOP= 1-hydroxy Pyrene, 8-OHdG=8hydroxy-2-deoxyguanosine, TNF-α=tumour necrosis factor alpha, PEFR=peak expiratory flow rate, FSH=follicle stimulating hormone, LH=luteinizing hormone, TST=testosterone, uCr=urine creatinine.

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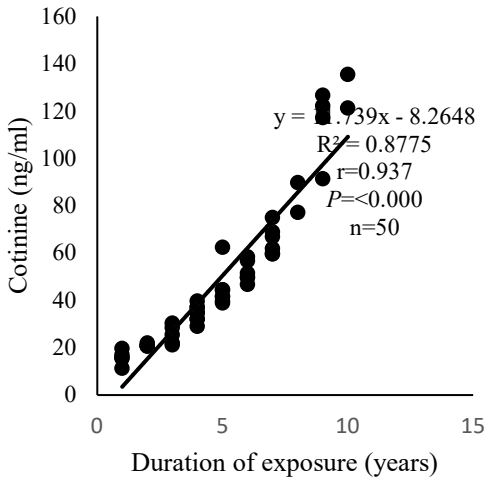

Fig. 1. Correlation plot of cotinine against duration of exposure in smokers

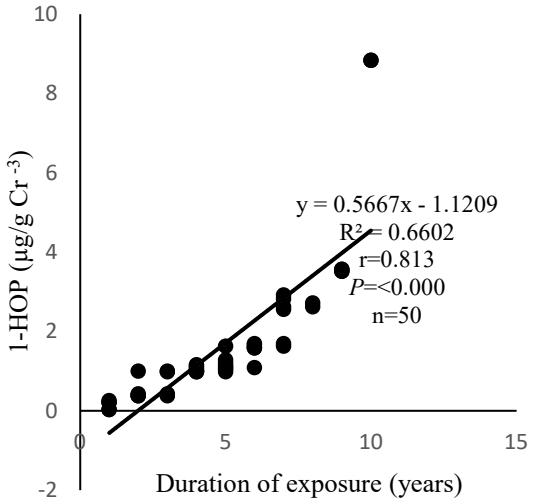

Fig. 2. Correlation plot of 1-HOP against duration of exposure in smokers

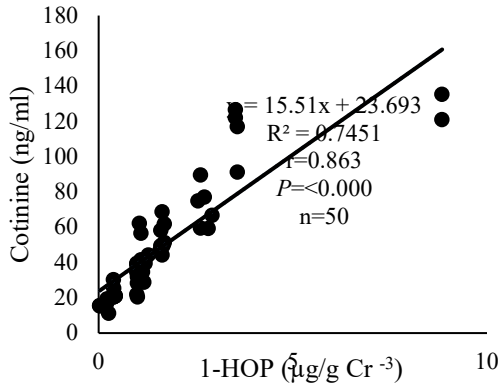

Fig. 3. Correlation plot of cotinine against 1-HOP in smokers

Discussion

Adverse health outcome associated with CS has been linked to the specific yet differential effects of the various toxic components of CS on vital organs and systems in humans. The association between exposure to PAHs in CS and the development of chronic organ dysfunctions have been documented. This study assessed the lung function, reproductive hormones, biomarkers of inflammation and oxidative DNA, cotinine and urinary PAH metabolite in male active smokers.

In this study, the cotinine levels of smokers were significantly higher than non-smokers studied. Higher cotinine levels in smokers are as a result of their exposure to nicotine present in CS. Cotinine is the major metabolite of in vivo nicotine metabolism and has been consistently utilized as a reliable index of exposure to tobacco smoke because of its persistence in body fluids and readily available method of analysis (22, 23). Consistent with our observations, active smokers have been shown to have higher levels of cotinine when compared to their passive and non-smoking counterparts (24). A positive correlation was observed between cotinine levels and 1-hydroxypyrene in smokers in this present study. Cigarette smoke has been shown to be a significant source of PAHs exposure and urinary 1-OHP levels have been significantly correlated with the amounts of cigarettes smoked (25). This implies that the higher the exposure to CS and consequently PAH in CS as determined by cotinine levels, the higher the levels of 1-HOP and vice- versa. Positive associations between secondhand smoke exposure, measured as serum cotinine and urinary concentrations of nine PAH biomarkers have also been demonstrated. Exposure to nicotine in CS has been implicated in exaggerated inflammation, tobacco addiction, vascular system defects, reproductive impairment and teratogenicity (22).

The urine level of 1-hydroxypyrene in smokers was not significantly higher than non-smokers studied. A similar observation has been made between total urinary PAH metabolite in active smokers versus non-smokers. Besides, smoking yields of PAH compounds have been shown to vary considerably based on the type of cigarette, genetic variability and individual differences in PAH metabolizing enzymes and smoking pattern (26). However, CS has been shown to induce a 16% increase in urinary 1-HOP excretion and active smokers have been reported to have higher urine levels of 1-HOP than non-smokers (27, 28).

Active smokers had lower BMI compared to the non-smoking population of the study. Lower BMI observed in smokers may be related to nicotine-induced reduction in appetite, enhanced thermogenesis, reduced absorption and calorie storage and modulation of leptin biosynthesis (29, 30). In line with our observations, cigarette smoking has been shown to be inversely related to BMI and weight gain with smoking cessation (29, 31).

Higher levels of 8-OHdG level were observed in smokers than in non-smokers. Exposure to chemical constituents of cigarette smoker as nicotine and PAH has been shown to increase ROS levels and decreases total antioxidant capacity leading to oxidative stress, lipid peroxidation, and interference with DNA repair capacity that eventually induce oxidative DNA damage which plays a significant role in CS-induced carcinogenesis (32). 8-OHdG

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which is a repair product of oxidized guanine in DNA, has been consistently used as a reliable marker of cellular and generalized oxidative DNA damage in humans (33). Thus higher 8-OHdG levels observed in smokers may be the result of higher CS-induced oxidative DNA. A previous study has demonstrated a positive relationship between 8-OHdG and the number of cigarettes consumed per day multiplied by the number of years (Brinkman index) which may imply that the level of oxidative DNA damage in smokers may be a function of the level of exposure to CS in a lifetime (29). Significantly increased levels of 8-OHdG have also been reported in the urine of active smokers when compared to passive and non-smokers (29, 32). Oxidative DNA damage has been implicated as the major pathologic mechanism of all smoking-related diseases and other conditions such as, aging, neurodegenerative and cardiovascular diseases (29, 34).

Smokers in this study recorded lower PEFR compared to their non-smoking counterparts and this is in agreement with the findings of other studies (35, 36). Lower PEFR in smokers may be attributed to CS-induced irritation of the respiratory tract leading to hypertrophy of the mucosal cells, increased secretion of mucus and formation of mucosal plugs (35). PAH-induced inflammation and OS coupled with nicotine-induced angiogenesis in the respiratory tract associated with exposure to CS may be responsible for bronchial constriction and increased airway resistance, loss of lung elastic recoil, delayed forced expiration, ciliary movement, mucociliary clearance and further clogging up of the airways (35). Studies of pulmonary function among smokers have demonstrated significant impairment of almost all indices of lung function (PEFR, FVC and FEV1) especially those indicating airway obstructions compared to non-smokers (35, 36, 37).

The TNF-α levels of smokers though higher than non-smokers, were not statistically significant. This is in agreement with the findings of a previous work which also demonstrated comparable levels of TNF-α in bronchoalveolar lavage fluid in healthy smokers and non-smokers (38). However, elevated TNF-α levels have been reported in exhaled breath condensate in male active smokers compared to non-smokers which may be indicative of CS-induced inflammatory process in the lungs (39). CS has been shown to promote the accumulation of immune cells and inflammation in the airways by inducing the secretion of pro-inflammatory cytokines as IL-1, TNF-α and IL-6 (4). Inflammatory response to CS could be influenced by gender and ethnicity while elevated levels of TNF-α have been demonstrated in a number of pulmonary disorders (38, 40).

Lower levels of LH were observed in smokers relative to non-smokers studied. Lower LH in smokers may be related to PAH and nicotine-induced alteration in the homeostasis of the hypothalamic-pituitary axis leading to stimulation of secretion of cortisol, oxytocin, vasopressin and growth hormones which inhibit secretion of prolactin and LH (5). A previous study had demonstrated higher estradiol, lower LH, FSH and prolactin and comparable levels of testosterone in smokers compared to non-smokers (41). In the hypothalamic-pituitary-gonadal axis, an increase in FSH and LH levels is associated with an anticipated increase in testosterone and inhibin B which in turn, inhibit LH and FSH levels through negative feedback mechanisms. However, contrary to our findings, higher levels of LH were demonstrated in current smokers who consumed 5 or more cigarettes per day compared with nonsmokers. A decrease in both male and female fertility has been associated with tobacco consumption (42).

Conclusion

The finding of reduced peak expiratory flow rate and luteinizing hormone in conjunction with increased oxidative DNA damage in active smokers may suggest that PAH-induced oxidative DNA damage may be the underlying mechanism in the development of cigarette smoke-related multiple organ dysfunctions.

Conflict of Interests

The authors declare that they have no competing interests.

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