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Original Article

Preliminary Investigation into Urinary 1-Hydroxypyrene as a Biomarker for Polycyclic Aromatic Hydrocarbons exposure among Charcoal Workers in Ogun and Oyo States, Nigeria

O.O. Olujimi [1,2,*], O.O. Ogunseye [2], K.O. Oladiran [1], S.D. Ajakore [1]

[1] Department of Environmental Management and Toxicology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[2] Department of Environmental Health Sciences, Faculty of Public Health, University of Ibadan, Ibadan, Nigeria

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ABSTRACT

Background: Urinary 1-hydroxypyrene (1-OHP) has been widely used as a biomarker of polycyclic aromatic hydrocarbons (PAHs) in occupationally exposed workers. The objective of this study is to investigate the concentration of urinary 1-OHP among charcoal workers as subjects and non-charcoal workers as controls.
Methods: Early morning urine samples were collected from 68 persons (25 charcoal workers in Igbo-Ora, 20 charcoal workers in Alabata, and 23 non-charcoal workers) who volunteered to participate in this study. 1-OHP determination in urine samples was carried out using high performance liquid chromatography after hydrolysis. Descriptive and inferential statistics were used for data analysis at $p < 0.05$.
Results: The mean urinary 1-OHP concentration (μmol/mol creatinine) among charcoal workers at Igbo-Ora and Alabata and non-charcoal workers were 2.22 ± 1.27, 1.32 ± 0.65, and 0.32 ± 0.26 ($p < 0.01$). There existed a relationship between respondent type and 1-OHP concentration. Charcoal workers were 3.14 times more at risk of having 1-OHP concentrations that exceed the American Conference of Governmental Industrial Hygienists guideline of 0.49 μmol/mol creatinine than non-charcoal workers (relative risk = 3.14, 95% confidence interval: 1.7–5.8, $p < 0.01$).
Conclusion: Charcoal workers are exposed to PAHs during charcoal production and are at risk of experiencing deleterious effects of PAH exposure. Routine air quality assessment should be carried out in communities where charcoal production takes place. Assessment of urinary 1-OHP concentration and use of personal protective equipment should also be encouraged among charcoal workers.
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1. Introduction

Most people living in rural areas and growing population in the urban centers in Nigeria today derive their energy from burning of biomass most especially charcoal [1,2]. Charcoal production consists mainly of two phases, i.e., wood cutting and pyrolysis in kilns [3]. However, the sequential phases involve cutting of wood, assembling it into cone-shaped piles, covering it with grasses and soil, and finally letting it burn until charcoal is produced [3,4]. Kiln sizes vary from one location to another and usually are between 15 m^3 and 90 m^3 [3–5]. Many of the tasks involved could be regarded as potentially hazardous. During the period of production, workers are exposed to polycyclic aromatic hydrocarbons (PAHs) from incomplete combustion of wood and noxious smoke for several hours per day [6]. Human beings can be exposed to PAHs from several sources, including (i) coke and gasworks, (ii) heavy petroleum distillates, (iii) burning or pyrolysis of organic materials, and or (iv) consumption of grilled food or inhalation of polluted air [7–9]. Charcoal production might take between 6 days and 14 days depending on the season, wood types, and stack [4].

PAHs are one of the major groups of carcinogens produced from the incomplete combustion of organic matter [8,9]. PAHs are known to trigger cancer in humans most especially in certain occupational settings and in cigarette smokers; therefore, International Agency for Research on Cancer has classified PAH exposure in these occupational settings as a Group 1 carcinogen [9,10]. Various biomarkers, including PAH-DNA adducts [11,12] and urinary metabolites of PAHs, have been used to assess human uptake and/or metabolic activation of PAHs [13–15]. Urinary 1-hydroxypyrene (1-OHP) has been widely used as a useful biomarker of PAHs exposure among occupationally exposed workers. 1-OHP is a urinary metabolite of the noncarcinogen

* Corresponding author. Department of Environmental Management and Toxicology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.
E-mail address: olujimio@funaab.edu.ng (O.O. Olujimi).

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pyrene, which always occurs in PAH mixtures that include carcinogens, such as benzo[a]pyrene with a half-life of 18–20 hours after initial inhalation [15,16].

Several studies have reported on 1-OHP as a biomarker of different groups of exposed workers [9,17,18], whereas studies on 1-OHP as an indicator of PAHs exposure among Nigeria workers are very scanty [19] and nonexisted for charcoal workers. Therefore, this study aimed to assess the levels of 1-OHP as an indicator for PAHs exposure of charcoal workers during pyrolysis and charcoal harvesting in Nigeria.

2. Materials and methods

2.1. Study design

This study adopted a cross-sectional comparative design which involved the assessment of urinary 1-OHP concentrations among charcoal workers as subjects and non-charcoal workers as controls.

2.2. Study locations

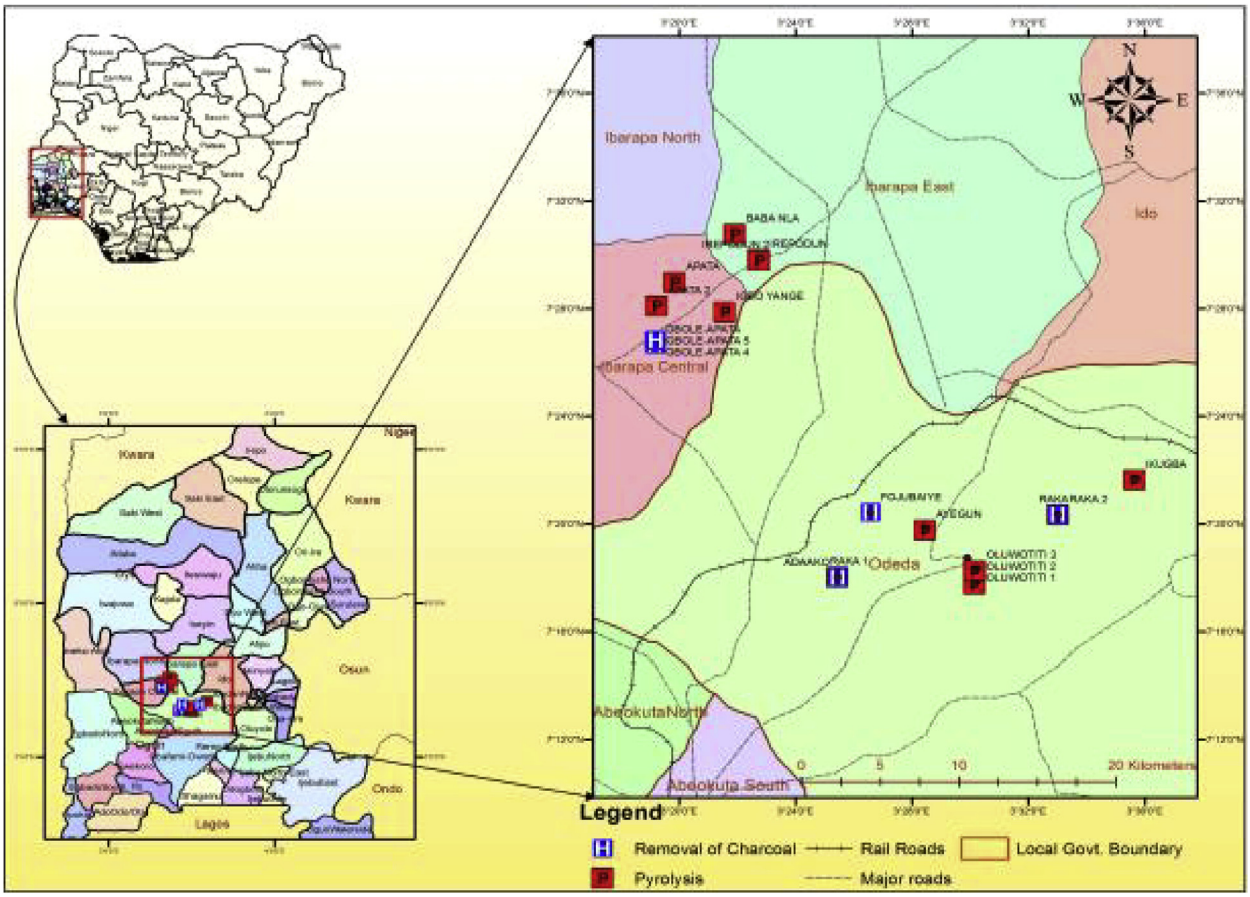
The study took place at Igbo-Ora and Alabata in Oyo and Ogun States, respectively Map 1. Igbo-Ora and Alabata are two of the major hubs of charcoal production in South Western Nigeria. Igbo-Ora is the headquarters of Ibarapa Central Local Government Area (LGA) of Oyo state, Nigeria. Igbo-Ora is located about 70 kilometers from Ibadan and has a population of about 60,000 people [20]. Most people are small-scale farmers, traders, and artisans. Alabata community is in Odeda LGA, with the headquarters at Odeda. It is one of the LGAs that constitute Ogun state. Odeda LGA is populated mainly by Egba ethnic group.

2.3. Study population

The participants selected for this study were charcoal workers and non-charcoal workers in Igbo-Ora and Alabata. Socio-demographic characteristics such as age, gender, educational status, marital status, religion, and tribe were collected from the participants using structured questionnaire. The participants signed the consent form after they were informed about the purpose of the study. Ethical approval for the study was obtained from the Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria. The ethical approval number was UI/EC/15/0167. A total of 68 persons (25 charcoal workers in Igbo-Ora, 20 charcoal workers in Alabata, and 23 non-charcoal workers) volunteered to participate in this study. Early morning urine samples (after charcoal pyrolysis and/or charcoal removal process) for the period of exposure were collected from charcoal workers and non-charcoal workers.

2.4. Urinary sampling and analysis

High performance liquid chromatography (HPLC)–grade acetonitrile was obtained from BDH chemical, UK. The metabolite, 1-hydroxypyrene, was obtained from Sigma–Aldrich, South Africa. Analysis was carried out using a Cecil 1100 series HPLC system at the Central Laboratory, University of Ibadan, Nigeria. Method of Choosong et al [18] was adopted with slight modification. Early morning urine samples (after charcoal pyrolysis and/or charcoal removal) were collected from all voluntary workers for the period of exposure. Early morning urine was used because the half-life of the parent congener benzo[a]pyrene is between 18 hours and 20 hours. An aliquot of 10 mL was separated into another tube to



Map 1. Showing sampling sites.

determine the urinary creatinine. The remaining spot urine sample was stored in a polypropylene tube and frozen at −20°C before sample preparation and analysis. A small portion of the urine sample (400 μL) was placed in an eppendorf tube. After adding acetate buffer (100 μL, 0.5M, pH 5) and β-glucuronidase (5 μL; 2000 unit), the sample was vortex mixed for about 30 seconds. The sample was then incubated at 37°C in a shaking bath for 16 hours (hydrolysis). Acetonitrile (700 μL) was added, and the sample was then vortexed for 10 seconds, centrifuged at 10,285 g, and incubated at 20°C for 10 minutes. Finally, a clear supernatant from the preparation was analyzed for 1-OHP using HPLC (Cecil 1100 Series). The prepared calibration concentration ranged from 5 ppb to 100 ppb, whereas the detection limit was calculated as three times the standard deviation of the lowest detectable concentration. It was calculated as 1.1 μgL^{-1}.

2.5. Statistical analysis

Data were entered and analyzed using statistical package for the social sciences (SPSS), version 20. Descriptive and inferential statistics were used in this study. Descriptive statistics was used to summarize data. Mean ± standard deviation and range were calculated for urinary 1-OHP (μmol/mol creatinine) of charcoal workers and non-charcoal workers. Inferential statistics was performed using *t* test, analysis of variance, and Chi-square test at 5% level of significance.

3. Results

3.1. Socio-demographic characteristics of respondents

Table 1 shows the socio-demographic characteristics of respondents. Most charcoal workers were within age 21–30 years (43.90 %), males (73.70 %), married (73.70 %), Christians (86 %), Tiv (64.9 %), and respondents with no education (35.10 %). The mean age of charcoal workers was 35.67 ± 10.47. Most non-charcoal workers were within age 31–40 years (38.60 %), males (71.90 %), married (56.10 %), Christians (63.20 %), and Yoruba (66.70 %) with tertiary education (36.8 %). The mean age of non-charcoal workers was 35.46 ± 12.82.

3.2. Urinary creatinine and 1-hydroxypyrene

Table 2 shows the range and mean of urinary 1-OHP concentrations among charcoal workers (subjects) and non-charcoal workers (controls). The creatinine level in subjects and controls ranged from 24.20 to 298.60 mg/dL and 9.70 to 300.00 mg/dL, respectively. The ranges of urinary 1-OHP concentration (μmol/mol creatinine) among charcoal workers at Igbo-Ora, charcoal workers at Alabata, and non-charcoal workers were 0.35–4.83, 0.34–3.23, and 0.01–0.79, respectively. The mean urinary 1-OHP concentration (μmol/mol creatinine) among charcoal workers at Igbo-Ora, charcoal workers at Alabata, and non-charcoal workers were 2.22 ± 1.27, 1.32 ± 0.65, and 0.32 ± 0.26, respectively. According to the American Conference of Governmental Industrial Hygienists (ACGIH) [21], the control exposure limit (CEL) is 0.49 μmol/mol creatinine. Most charcoal workers at Igbo-Ora (96.00 %) and Alabata (95.00 %) recorded 1-OHP concentrations above the ACGIH guideline, whereas most non-charcoal workers (69.60 %) recorded 1-OHP concentrations below the ACGIH guideline (Fig. 1).

Table 1
Socio-demographic characteristics of respondents

Socio-demographic characteristics	Subgroups	Percentage (%)	
		Subjects	Controls
Mean age of subjects 32.67 ± 10.47 Mean age of controls 35.46 ± 12.82	≤20 21–30 31–40 41–50 >50	10.5% 43.9% 31.6% 7.0% 7.0%	7.0% 35.1% 38.6% 5.3% 14.0%
Gender	Male Female	73.7% 26.3%	71.9% 28.1%
Educational status	No education Primary education Secondary education Tertiary education	35.1% 31.6% 31.5% 1.8%	17.5% 21.1% 24.6% 36.8%
Marital status	Married Single	73.7% 26.3%	56.1% 43.9%
Religion	Christianity Islam	86.0% 14.0%	63.2% 36.8%
Tribe	Yoruba Tiv Hausa Others	12.3% 64.9% — 22.8%	66.7% 21.1% 12.2 —

Table 2
Mean and range of 1-hydroxypyrene (μmol/mol creatinine) among respondents

Parameter	Charcoal workers (Igbo-Ora)	Charcoal workers (Alabata)	Non-charcoal workers (Controls)
Mean	2.22 ± 1.27	1.32 ± 0.65	0.32 ± 0.26
Range	0.35–4.83	0.34–3.23	0.01–0.79

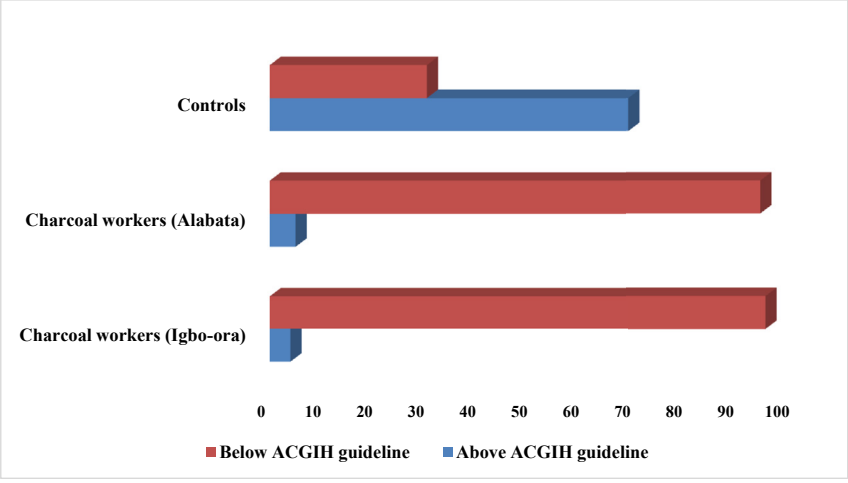


Fig. 1. Proportion of respondents with 1-hydroxypyrene levels below or above the ACGIH guideline.
ACGIH, American Conference of Governmental Industrial Hygienists.

3.3. Comparison of urinary 1-OHP concentrations across respondent types and risk assessment

Table 3 shows the comparison of urinary 1-OHP concentrations across respondent types. The result showed that there was a statistically significant difference between 1-OHP concentrations across subjects and controls ($p < 0.01$). Table 4 shows the relationship between respondent types and 1-OHP concentration. Chi-square test showed that there is a relationship between respondent type and urinary 1-OHP (Chi square 33.17, $p < 0.01$). The result also showed that charcoal workers (subjects) were 3.14 times more at risk of having 1-OHP concentrations that exceed the ACGIH guideline than non-charcoal workers (controls) (RR = 3.14, 95% confidence interval: 1.70–5.80, $p < 0.01$).

4. Discussion

The range of 1-OHP concentration for charcoal workers at Igbo-Ora and Alabata (subjects) and non-charcoal workers (controls) were 0.35–4.83, 0.34–3.23, and 0.01–0.79, respectively. The mean 1-OHP concentration for charcoal workers at Igbo-Ora and Alabata (subjects) and non-charcoal workers (controls) were 2.22 ± 1.27, 1.32 ± 0.65, and 0.32 ± 0.26, respectively. This result is similar to that reported in the study of Elovaara et al [22] and Wispriyono et al [23] where urinary 1-OHP concentrations were high among occupationally exposed subjects. The mean 1-OHP concentration among charcoal workers (1.82 ± 1.13) was higher than the ACGIH CEL of 0.49 μmol/mol creatinine [21]. The mean 1-OHP concentration among charcoal workers was about six times higher than that of non-charcoal workers and about four times higher than the ACGIH guideline of 0.49 μmol/mol creatinine. The result showed that most charcoal workers at Igbo-Ora and Alabata recorded 1-OHP concentrations above the ACGIH guideline. There was a statistically significant difference in 1-OHP concentrations across subjects and controls ($p < 0.01$). There existed a relationship between respondent type and urinary 1-OHP concentration while risk assessment indicated that charcoal workers (subjects) are 3.14 times more at risk of having 1-OHP concentrations that exceed the ACGIH guideline than non-charcoal workers (controls). This result is also in line with a previous study [13] where urinary 1-OHP concentrations were significantly higher in coke oven workers (5.46 μmol/mol creatinine) than those in controls (2.96 μmol/mol creatinine; $p < 0.01$). It has been reported that coke oven workers were 2.45 times more likely to have high levels of urinary 1-OHP (odds ratio = 2.45, $P < 0.05$) than controls [13]. This implies that charcoal workers are exposed to high levels of PAHs during charcoal production because PAHs are typical and abundant in fine smoke particles from biomass burning [18]. Hence, charcoal workers are susceptible to adverse effects of PAH exposure. PAH is a class of compounds considered to have human carcinogenic potential [15,22,24,25] and a capacity to trigger tumors [23]. PAHs have also been implicated in the development of cardiovascular diseases as they contribute to oxidative stress and enhanced progression of atherosclerosis and are associated with acute myocardial infarction [26]. The creatinine level in subjects and controls ranged from 24.2 mg/dL to 298.6 mg/dL and 9.7 mg/dL to 300 mg/dL, respectively. These values of urinary creatinine were within the normal range of 50–360 mg/dL [18]. In a study that assessed urinary 1-OHP among 68 exposed workers by Ifegwu et al [19], it was reported that certain occupations predispose individuals to significant exposures to PAHs.

It is however important to state that the values of 1-OHP reported in this study were based on the CEL guideline as provided by the ACGIH and not on the Biological Exposure Indices of the ACGIH released in 2017. Therefore, the assessment was control based and not health based due to exposure duration that is dependent on the work shift of the charcoal producers as against the recommended 40 hours per week in real industrial set-up.

In conclusion, the study assessed urinary 1-OHP concentrations among charcoal workers and non-charcoal workers in Igbo-Ora, Oyo State and Alabata, Ogun State. Urinary 1-OHP concentrations among charcoal workers were significantly higher than non-charcoal workers and the ACGIH CEL. A relationship exists between respondent types and urinary 1-OHP concentration, and charcoal workers are at higher risk of having 1-OHP concentrations above the ACGIH guideline. These findings established that there are public health implications for communities and individuals that commercially engage in charcoal production. Hence, charcoal workers are at risk of experiencing deleterious effects of PAH exposure. Routine air quality assessment should be carried out in communities where charcoal production takes place. Assessment of urinary 1-OHP concentration and use of personal protective equipment such as nose masks, hand gloves, eye goggles, and coveralls should also be encouraged among charcoal workers.

Table 3
Comparison of urinary 1-hydroxypyrene concentration (μmol/mol creatinine) across respondent types

Charcoal workers (Alabata)	Charcoal workers (Igbo-Ora)	Non-charcoal workers (controls)	*p*
1.32 ± 0.65	2.22 ± 1.27	0.32 ± 0.26	**0.000**
1.82 ± 1.13		0.32 ± 0.26	**0.000**

The *p* value is less than 0.01 ($p < 0.01$).

Table 4
Relationship between respondent types and urinary 1-hydroxypyrene concentration

Subjects		Level of urinary 1-OHP		
		Below guideline	Above guideline	Total
Respondent types	Igbo-Ora	1 (4.0%)	24 (96.0%)	25 (100.0%)
	Alabata	1 (5.0%)	19 (95.0%)	20 (100.0%)
	Controls	16 (69.6%)	7 (30.4%)	23 (100.0%)
Total		18 (26.5%)	50 (73.5%)	68 (100.0%)

Chi square 33.168
$p < 0.01$
1-OHP, 1-hydroxypyrene.

Conflicts of interest

Authors report no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.shaw.2017.12.004.

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