

BIOMONITORING AND BIOMARKER RESPONSE OF FISH SMOKERS TO POLY AROMATIC HYDROCARBONS IN RIVERS STATE, NIGERIA

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Received: 22-11-2021

Accepted: 12-02-2022

ABSTRACT

A study was carried out on 40 volunteer fish smokers, 10 from each Station. Station 1 (Alakahia market), Station 2 (Choba Market), Station 3 (Choba main market) and Station 4 (Rumosi Market) all in Obio/akpor Local government of Rivers state, Nigeria. The study was carried out for a period of 12 weeks, to evaluate the health status of exposed individuals to poly aromatic hydrocarbons gotten from smokes, using haematological parameters and urine analysis. The cyanohaemoglobin method was used to determine haemoglobin (Hb), packed cell volume (PCV) was determined by micro haematocrit method, Red blood cell (RBC) was determined with the improved Neubauerhaemaocytometer, WBC was determined with the improved Neubauer counter, Neutrophils, lymphocytes and Eosinophils were determined on blood film stained with mayGrunwald-Giemsa stain while Gas Chromatography was used for urine samples. PCV from station 1, 2, 3 and 4 had a mean value of 38.33, 38, 33.33 and 35.00 respectively with significant difference ($P < 0.05$) occurring in station 3 when compared to the other stations and within stations 2, 3 and 4. Hb had a mean value of 12.8, 12.67, 11.13 and 11.67 in stations 1, 2, 3 and 4 respectively also with significant difference ($P < 0.05$) occurring in station 3 when compared to the other stations and within stations 2, 3 and 4. WBC had a mean value of 4.77, 6.23, 5.93 and 4.83 in stations 1, 2, 3 and 4 with no significant difference ($P > 0.05$), the RBC had a mean value of 4.40, 4.33, 3.60 and 3.90 in stations 1, 2, 3 and 4, Neutrophil had a mean value of 36.67, 46.0, 41.0 and 41.0 in stations 1, 2, 3 and 4. Stations 1, 2, 3 and 4 had a mean value of 58.33, 47.67, 57.67 and 57.33 for lymphocytes and 5.0, 9.33, 1.33 and 1.67 for Eosinophil. It was recorded that the different stations had no statistically significantly difference ($P > 0.05$) for RBC, Neutrophil, Lymphocyte and Eosinophil. The urine analysis revealed that station 1 had a total PAH of 0.1726, station 2 had a total of 0.058 while station 3 and 4 had a total of 0.088 respectively. The results reveal that the smokers have PAHs in their body with increases risk of Kidney disease, Anaemia, Bronchitis and other lung diseases.

INTRODUCTION

In most urban cities of Nigeria today, people are exposed to dangerous chemicals and other toxic compounds without any form of protection. This is caused due to occupational exposure or activities, occupations such as fish smoking is one of

such occupations and these smokers are not usually properly educated, so they are ignorant of the long term effects of incomplete combustion of carbonaceous materials on their health (Suchanova *et al.*, 2008). About 100 different chemicals are formed during the incomplete combustion of coal, oil, gas and garbage or other

organic substances such as tobacco or charbroiled meat (ATSDR, 1995). These compounds are known to be carcinogenic (Suchanova *et al.*, 2008). Human exposure to PAHs occurs in three main ways; Inhalation, dermal contact and consumption of contaminated food or diet (Farhadian *et al.*, 2011). Regarding to the generation of smoke, it has been showed that poplar wood generated the highest number and concentration of total and carcinogenic PAHs, while oak, cherry tree, beech samples were similarly less effective. Hard wood instead of soft wood have been recommended considering the amount of PAHs produced (Guillen *et al.*, 2000). Smoking of food especially fish is one of the most ancient technologies which has been used for years (Simko, 2002), the highest concentration of PAHs in smoked products such as smoked fish is obtained immediately after the smoking is done. The concentration decreases due to decomposition, triggered by light and interactions with other compounds also present in the environment (Simko, 1991). The actual levels of PAHs in smoked foods depends on several variables in the smoking process, including types of smoke generator, combustion temperature and the degree of smoking (Gracia-Falcon and Simal-Gandara, 2005). Wood smoke exposure causes a decrease in lung function and an increase in the severity of existing lung diseases, it has also been reported that wood smoke aggravates asthma, emphysema, pneumonia and bronchitis with long term exposure leading to arteriosclerosis, throat, lung and lymph system cancer (American Lung Association, 1991). Exposure of preschool children living in homes heated with wood

burning, stoves or in houses with open fireplaces yielded the following effects; decreased pulmonary lung function in young asthmatics (Koenig *et al.*, 1993), increased incidence of acute bronchitis and frequency of wheezing and coughing and also increased incidence duration and possible severity of acute respiratory infections (Collins *et al.*, 1990; Kammen *et al.*, 1998). PAHs are considered environmentally significant because of their potential toxicity to the higher organisms and resistance to microbial attack (Kanaly and Harayama, 2000). Some PAH are highly carcinogenic, genotoxic and cytotoxic (Boldrin *et al.*, 1993), PAHs and their derivatives have been recognized as the major culprit causing human lung cancer, anaemia, asthma, splenomegaly, bladder cancer and breast cancer etc, (Okana *et al.*, 2005; Booker and White, 2005; Hazra *et al.*, 2004; Miller *et al.*, 2005). Some major compounds found in PAHs include Benzo(a)pyrene and

Anthracene. Anthracene exhibit toxicity to fish, algae and show bioaccumulation in the food chain (Sutherland, 1992) while benzo(a)pyrene short term exposure can result in skin rash or eye irritation with redness and/or a burning sensation while long term exposure can lead to loss of colour, thinning of the skin, wart and bronchitis (EPA, 2004). Considering the health risk of smoking fish not just to the consumers of the smoked fish but also to the smokers themselves, this study is aimed at evaluating the health effects incurred by fish smokers using biomarkers in blood and urine analysis.

MATERIALS AND METHODS

Sample Collection: Samples were collected from 40 exposed subjects, 10 from each station. These exposed subjects are individuals that use fire wood to smoke fish and the study location was based on accessibility to the markets and availability of volunteers. Station 1 (Alakahia market), Station 2 (Choba Market), Station 3 (Choba main market) and Station 4 (Rumosi Market) all in Obio/akpor local government of Rivers state, Nigeria. A total of 5ml of venous blood was collected from 40 volunteers using sterilized syringes; urine samples were also collected for analysis. The samples were transported on ice to the laboratory and were processed within 2 h after collection.

Biochemical Analysis: The cyanohaemoglobin method was used to determine haemoglobin (Hb) by using diagnostic kits from Sigma diagnostics USA, and packed cell volume (PCV) was determined by micro haematocrit method. Red blood cell (RBC), and thrombocyte count were determined with the improved Neubauerhaemaocytometer according to Dacie and Lewis (1991). WBC was determined with the improved Neubauer counter, while differential counts as Neutrophils, lymphocytes and Eosinophils were determined on blood film stained with mayGrunwald-Giemsa stain (Miale, 1982). The urine was collected in an amber vials with a cap and a Teflon septum. Samples were preserved at pH<2.0 with sodium bisulphate, 10ml of the sample were removed and discharged through the septum with glass syringe. 3ml of n-pentane were then injected through the septum with a 5ml syringe. The sample was extracted for 2mins in a Vortex

apparatus. The vials were then opened and 1.5ml of the organic extract were placed into the vials and used for the GC analysis.

Method of Data Analysis: Data were analyzed using Tukey test at a level of 5% probability, using Assitat Software Version 7.7 en (2017).

RESULTS

The effects of PAH from Fish smoking on the haematological parameters of the fish smokers are shown in Table 1, for PCV station 1, 2, 3 and 4 had a mean value of 38.33, 38, 33.33 and 35.00 respectively with significant difference ($P<0.05$) occurring in station 3 when compared to the other stations and within stations 2,3 and 4. Hb had a mean value of 12.8, 12.67, 11.13 and 11.67 in stations 1, 2, 3 and 4 respectively also with significant difference ($P<0.05$) occurring in station 3 when compared to the other stations and within stations 2,3 and 4. WBC had a mean value of 4.77, 6.23, 5.93 and 4.83 in stations 1, 2, 3 and 4 with no significant difference ($P>0.05$), the RBC had a mean value of 4.40, 4.33, 3.60 and 3.90 in stations 1, 2, 3 and 4, Neutrophil had a mean value of 36.67, 46.0, 41.0 and 41.0 in stations 1, 2, 3 and 4. Stations 1, 2, 3 and 4 had a mean value of 58.33, 47.67, 57.67 and 57.33 for lymphocytes and 5.0, 9.33, 1.33 and 1.67 for Eosinophil. It was recorded that the different stations had no statistically significantly difference ($P>0.05$) for RBC, Neutrophil, Lymphocyte and Eosinophil. The urine analysis revealed that station 1 had a total PAH of 0.1726, station 2 had a total of 0.058 while station 3 and 4 had a total of 0.088 respectively.

Table 1. Effects of PAH from Fish smoking on the haematological parameters of the fish smokers

Stations	PCV (%)	Hb (g/dl)	WBC (mm ³)	RBC (10 ¹² /L)	L (%)	N (%)	E (%)
1	38.33±0.58 ^a	12.80±0.17 ^a	4.77±1.70 ^a	4.40±0.53 ^a	58.33±9.24 ^a	36.67±14.50 ^a	5.00±5.29 ^a
2	38.00±2.00 ^{ab}	12.67±0.65 ^{ab}	6.23±1.86 ^a	4.33±0.76 ^a	47.67±4.73 ^a	46.00±1.73 ^a	9.33±5.01 ^a
3	33.33±2.89 ^b	11.13±0.98 ^b	5.93±1.16 ^a	3.60±0.46 ^a	57.67±7.23 ^a	41.00±6.93 ^a	1.33±0.58 ^a
4	35.00±1.00 ^{ab}	11.67±0.35 ^{ab}	4.83±0.92 ^a	3.90±0.17 ^a	57.33±0.58 ^a	41.00±2.65 ^a	1.67±2.36 ^a

Key: PCV= Pack cell volume, Hb= Haemoglobin, WBC= White blood cell, RBC= Red blood cell, N= Neutrophil, L= Lymphocyte, E= Eosinophil,

^{a-b} Different letters in the same column indicate significance difference (p<0.05) within the stations

Reference range (Timzing et al., 2014)

RBCs × 10¹²/L: Male: 4.5-5.5; Female: 4.0 -5.0

HB: 13-18g/dl

PCV%: Male 40-54; Female 36-46

WBC: 4-11,000cells/mm³

Neutrophil (%): 40-75

Eosinophil (%): 1-6

Lymphocytes (%): 20-45

Table 2. Urine Analysis of Fish Smokers

PAH (mg/μL)	Station 1	Station 2	Station 3	Station 4
Acenaphthene	0.018±0.030	-	0.004±0.0031	0.004±0.0031
Acenaphthylene	0.021±0.017	0.005±0.005	0.015±0.024	0.015±0.024
Anthracene	0.025±0.017	0.003±0.004	0.003±0.004	0.003±0.004
Benzo(a)pyrene	0.001±0.0008	0.005±0.003	0.009±0.010	0.009±0.010
Benzo(b)flouranzthene	-	-	0.001±0.0006	0.001±0.0006
1,12-Benzoperylene	0.001±0.002	0.002±0.002	0.006±0.007	0.006±0.007
Chrysene	0.059±0.007	0.003±0.001	0.003±0.0008	0.003±0.0008
1,2,5,6Dibenzanthracene	0.0003±0.0005	-	0.007±0.008	0.007±0.0008
Fluoranthene	0.004±0.003	0.001±0.0007	0.004±0.003	0.004±0.003
Fluorene	0.0003±0.0005	0.003±0.002	0.017±0.022	0.017±0.022
Indeno(1,2,3)pyrene	0.005±0.0006	0.002±0.003	0.004±0.004	0.004±0.004
Naphthalene	0.003±0.005	0.001±0.002	0.004±0.003	0.004±0.003
Phenanthrene	-	0.001±0.002	0.007±0.003	0.007±0.003
Pyrene	0.035±0.044	0.032±0.042	0.004±0.005	0.004±0.005
Total PAHs	0.1726	0.058	0.088	0.088

DISCUSSION

The results from the haematological analysis carried out will be compared to the data of Timzinget *al.*, (2014) who compiled a comprehensive haematological reference range for normal Nigerian adults, considering the fact that haematological

parameters are influenced by various factors like ethnicity and religion (Subhashree, 2012). The PCV of all the stations were generally lower than the normal value of PCV (0.41 or 41%) for healthy Nigerian adults according to Obi *et al.* (1984), and among the stations, it was

station 3 that had the least value indicating that the smokers in that station are at a greater risk. Low PCV has been linked to anaemia and Booker and White (2005) also reported similar results due to exposure to benzo(a)pyrene which is a major component of PAHs. Pack cell volume measures the amount or percentage of blood that is composed of red blood cells (Charles, 2017), and also any factor that raise or increase the amount of other blood constituent have been reported to decrease PCV (Sedesse, 2011). One of the factors identified is an abnormal increase in white blood cells due to infection and diseases (Sedesse, 2011). Damages to the kidney can also lead to lower value of PCV considering that the kidney also plays a pivotal role in regulating the PCV (Dunn *et al.*, 2007). PAHs contains some compounds like benzo(b)fluoranthene which have been linked to kidney damage, these damages done to the kidney (leading to kidney diseases) also affects the red blood cell negatively by reducing the amount of red blood cell in circulation and this might be why the red blood cell analyzed was also low, the kidney is known to play a vital role in red blood cell production via erythropoietin hormone secreted by the peritubular capillary lining cells of the kidney (Adamson, 1996; NIDDK, 2014; AKF, 2018). When compared with the station, it was found that station 3 was the most adversely affected Hb, the liver plays a very important role in protein synthesis and any pathological changes on the liver will likely affect the Hb synthesis (Mehrnazet *et al.*, 2018). The results on PCV, HB and RBC is in agreement with Mehnazet *et al.*, (2018). The WBC also increased in the stations when compared to the reference

range of 4.4 – 4.8, this increase in WBC is an indicator of diseases, infection or inflammations in the body (Mayo Clinic, 2018b), the levels of lymphocyte also increased in all the stations with station 1 having the highest value, the values from the different stations were also higher than the reference range of 39.9 – 42.1 according to Timzinget *et al.*, (2014). Abnormally high lymphocyte have also been linked to inflammations and infections (Mayo Clinic, 2018a) and considering that wood smokes have been linked to bronchitis which is an inflammation of the bronchiole and respiratory infections (American Lung Association, 1991; Kammenet *et al.*, 1998) one can rightly expect such haematological changes. The neutrophil levels was also lower in the in all the stations compared to the reference range of 49.1 – 52.3, but station 1 was the least, This decrease in neutrophil leads to Neutropenia which weakens the immune system and increases a patients susceptibility to diseases. This decrease in the neutrophil level recorded in the stations has been linked to the presence of benzene (Qu, *et al.*, 2004), the results on HB, WBC, Neutrophil, and Lymphocytes also agree with Awodele *et al.*, (2015). The Eosinophil level was higher only in stations 1 and 2 when compared to the reference range of 1.32 – 1.91 with stations 3 and 4 being within the normal range, this abnormally high mean value is known be caused by infections (Mayo Clinic, 2018c). When we look at the haematological results generally, we can see that the fish smokers from the different stations all had a negative result showing that the smoking process has negative effect on them. The urine analysis done confirmed the presence of these PAHs in their body but it doesn't

truly ascertain the amount but only indicates the amount excreted. This means that more of the PAHs might still be in circulation in the blood and exerting its negative effect in the liver, kidney and other vulnerable organs. Station 1 had the highest amount of PAHs with a value of 0.1726 while station 2 had the least with 0.058.

CONCLUSION

Smoking of Fish exposes the smokers to PAHs and other toxic pollutants from the smokes and increases the risk of Anaemia and lung disease such as bronchitis as revealed by the haematological results. Therefore, it's important that a more environmentally healthy alternative of fish processing be considered, also proper sensitization of the market women should be carried out by the environmental health agencies and also the government on the health risks attached to fish smoking so as to discourage its practice.

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