

Air Pollution and Sex Hormone Levels among Mechanics in Brazzaville (Republic of Congo)

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Abstract

Air pollution is today a real concern across the world, responsible for around 7 million deaths per year around the world. It is currently involved in the appearance and worsening of several non-transmissible pathologies, making it an environmental, health, and societal problem. The objective of this work was to evaluate the effects of short-term exposure to air pollution on the level of sex hormones in mechanics. We conducted a cross-sectional, analytical, prospective case-control study in Brazzaville on 228 patients (76 cases and 152 controls) from June 2020 to December 2022, a period of 30 months. This study made it possible to evaluate, on the one hand, the level of sexual hormones in men exposed to automobile pollution according to the automated method (ELFA) on mini vidas and, on the other hand, the quality of the air by a colorimetric, punctual method on a Dräger tube coupled with a Dräger accuro pump. The results obtained made it possible to establish a statistically significant link between automobile pollutants (CO, CO₂, NO₂, SO₂) and the increase in LH and FSH on the one hand and the decrease in testosterone on the other hand. We also noted that the concentrations of automotive pollutants during this study were all above the standards required by the WHO. Exposure to automobile pollutants affects the hypothalamic-pituitary axis and therefore disrupts the level of sex hormones.

Keywords

Exposure, Automobile Pollutants, Level, Sex Hormone

1. Introduction

Now recognised throughout the world, the effects of air pollution on human health are well-accepted concepts. Moreover, in 2021, the WHO estimated that air pollution is responsible for around 7 million premature deaths each year and leads to the loss of hundreds of millions of years of healthy life worldwide. This disease burden falls mainly on low- and middle-income countries [1]. Several epidemiological, clinical, and toxicological studies have shown an association between the appearance of non-communicable diseases and short- or long-term exposure to air pollution [2]. So-called air pollution has been associated with respiratory and cardiovascular pathologies, including myocardial infarction, heart failure, high blood pressure, and strokes [3] [4] [5]. At the same time, epidemiological studies have described the involvement of air pollution in neurodevelopmental disorders and neurodegenerative diseases such as Parkinson's disease and Alzheimer's, as well as in bronchial, bladder, and breast cancers [6] [7] [8].

However, previous studies have demonstrated that certain components of PM_{2.5}, particles with an aerodynamic diameter less than 2.5 µm, inhaled into the respiratory tract can enter the human circulatory system by crossing the pulmonary blood barrier and can cause adverse effects in multiple systems, including the reproductive system [9]. The impact of air pollution on the reproductive system has become both a health and societal problem, affecting both men and women. However, most of these studies seem to support a negative impact of air pollutants on human reproduction, particularly with precocious puberty, adverse pregnancy outcomes, and increased miscarriage rates [10] [11]. This negative impact is also observed on foetal development, hence premature births and pregnancy complications [12] [13] [14]. This work aims to evaluate the effects of short-term exposure to air pollution on the level of sex hormones among mechanics in the city of Brazzaville.

2. Methods

This study was approved after review by the Health Sciences Research Ethics Committee (HSREC). Informed consent was obtained from participants before enrollment in the study.

2.1. Description of the Study Site and Type

This study was done in Brazzaville, the political capital of the Congo, which extends from north to south over an area of 264 km². It represents the most populous city in the country, with 2,552,813 inhabitants in 2021. It has nine districts with a demographic density of 9670 inhabitants per km². Our analyses were carried out within the garages for air analyses and at the National Public Health Laboratory in the reproduction unit for semen analysis. This is an analytical, prospective cross-sectional study between two groups, an exposed group G1 and a control group G2, ranging from June 2020 to December 2022, *i.e.*, 30

months.

2.2. Sampling and Eligibility Criteria

A total of 228 patients, all male, including 76 patients from group G1 (mechanics) and 152 patients from group G2, as well as 27 automobile garages, constituted our sample. The sampling was obtained by simple random sampling, going door to door. Before obtaining informed consent, all participants were informed of the objectives of the study. Patients eligible for this study had for the G1 group, an age between 18 and 50 years old, had been practicing for at least one year, and consented to the study. For the G2 group, the patients were all coming, aged between 18 and 50 years, lived a certain distance from garages and major avenues, were non-smokers, had no known fertility problems, and consented to the study.

2.3. Data Collection

2.3.1. Epidemiological Investigation

After obtaining informed consent, a standardised questionnaire on a survey sheet made it possible to collect socio-demographic data, medical or surgical history, lifestyle habits of each participant, and the different rates of sex hormones (testosterone, FSH, LH, and prolactin). The anonymity of each participant was guaranteed by the allocation of a code and an identification number.

2.3.2. Biological Investigation

It was carried out at the National Public Health Laboratory and concerned the hormonal dosage of testosterone, LH, FSH, and prolactin.

A vacuum blood sample was taken at the elbow crease from a dry tube, making it possible to obtain a serum after centrifugation at 4000 revolutions per minute. This serum then allowed the measurement of the different hormones (total testosterone, prolactin, LH, and FSH). These different assays were carried out by the enzyme-linked fluorescent assay (ELFA) method, automated with a device from the manufacturer, Rayto Chamaray (mini Vidas). All stages of the test, as well as the temperature, were controlled by the automaton.

Principles of the method

ELFA is a solid-phase enzyme-linked immunosorbent assay method that allows the detection and quantification of specific proteins in biological samples in the presence of an enzyme (alkaline phosphatase) and a fluorogenic substrate (4-methyl lumbelliferyl).

Procedure

To do this, each reagent kit consisted of a disposable device called the solid phase receptacle (SPR) for testing as well as a pipetting device. For any analysis, the sample enters and exits the SPR for a specified time; it is then transferred to a well containing a specific antibody (anti-LH, anti-FSH, anti-PRL, anti-TES) conjugated to an enzyme (alkaline phosphatase). The sample/conjugate mixture enters and exits the SPR, and the specific protein (the 4 hormones) will then bind

to the SPR-coated antibodies and conjugate to form a sandwich. Washes will remove unbound conjugates while a substrate flows through the SRP. The enzyme remaining on the walls of the SPR catalyzes the conversion of the substrate into the fluorescent product (4-methylumbelliferone). The fluorescence intensity is proportional to the protein concentrations of LH, FSH, and testosterone PRL present in the sample, and the results are automatically printed.

2.4. Environmental Investigation

Through different automobile garages in the districts of Brazzaville, air samples were taken, 4 garages were selected for each district, and 4 samples were taken per automobile garage during the two seasons and over the course of two years (2020 and 2022), *i.e.*, a total of 144 samples for the 9 districts. The quantitative evaluation of the concentrations of gaseous automobile pollutants (NO₂, SO₂, CO, and CO₂) in the ambient air at the garage level was carried out using the point method using a measurement system on the reagent tubes of Dräger. This work being a continuation, the description of the methodology used for this investigation aimed at analysing the air is detailed in the previous publication [15].

2.5. Statistical Analysis

The data has been processed using Excel 2016 software (Microsoft Corporation, USA). This tool was used for the statistical analysis and design of graphs. The Census and Survey Processing System (CSPro) 4.1 software was also used for data entry. The Chi-squared test and Fisher were used for the comparison of qualitative variables and the student for the comparison of quantitative variables.

3. Results

3.1. Sample

Our sample consisted of 228 subjects (76 cases and 152 controls) for the year 2020; we recorded 4 lost to follow-up during the year 2022, thus reducing the sample size to 224 subjects (74 cases and 150 controls) or one case for two controls (Table 1).

The largest numbers of our subjects were recorded in Poto-Poto and Ouénzé among both cases and controls (Table 2).

In 2020 and 2022, the most representative age group obtained was that of 36 to 46 years, followed by 26 to 35 years for both cases and controls, with an average

Table 1. Distribution of the study sample.

	2020		2022		Lost
	n	%	n	%	
Cases	76	33.3	74	33.0	2
Controls	152	66.7	150	67.0	2
Total	228	100	224	100	4

age of 37 ± 7 years for the year 2020 and 38 ± 8 years for the year 2022 (**Table 3** and **Table 4**).

3.2. Identification of Risk Factors Associated with Respondents

Concerning the risk factors, only alcohol consumption was statistically significant both in 2020 and in 2022, at the respective rates of 11.8% among cases and 28.3% among controls for the year 2020, compared to 12.2% among cases and 28.7% among controls for the year 2022 (**Table 5**).

3.3. Hormone Levels

In this study, we noted a statistically significant decrease in testosterone to 11% for cases and 3% for controls in 2020, compared to 27% for cases and 7% for controls in 2022 (**Table 6**).

In this study, we noted a statistically significant increase in FSH at 8% in cases and 1% in controls for the year 2020, compared to 28% in cases and 5% in controls for the year 2022 (**Table 7**).

Table 2. Distribution of the sample by district.

District	2020		2022		Lost	
	Cases	Controls	Cases	Controls	Cases	Controls
Makélékélé	7	8	7	8	0	0
	9%	5%	9%	5%		
Baongo	6	8	6	8	0	0
	8%	5%	8%	5%		
Poto-Poto	17	37	17	36	0	1
	22%	24%	23%	24%		
Moungali	10	14	9	14	1	0
	13%	9%	12%	9%		
Ouenzé	16	19	16	18	0	1
	21%	13%	22%	12%		
Talangäi	9	17	9	17	0	0
	12%	11%	12%	11%		
M'filou	5	20	5	20	0	0
	7%	13%	7%	13%		
Madibou	2	15	2	15	0	0
	3%	10%	3%	10%		
D'jiri	4	14	3	14	1	0
	5%	9%	4%	9%		
Total	76	152	74	150	2	2

Table 3. Distribution of the sample by age group.

Age per year	2020		2022	
	Cases	Controls	Cases	Controls
[18 - 25]	9 12%	4 3%	8 11%	3 2%
[26 - 35]	25 33%	53 35%	23 31%	48 32%
[36 - 45]	31 41%	72 47%	28 38%	71 47%
[46 and over]	11 14%	23 15%	15 20%	28 19%
Total	76	152	74	150

Table 4. Distribution of the sample by contribution to the average ages.

	2020				2022			
	Sample	Cases	Controls	Test of student	Sample	Cases	Controls	Test of student
Nombre of observations	228	76	152		224	74	150	
Average age	37	36	37	$t = -1.623$; $p = 0.106$	38	37	38	$t = -0.978$; $p = 0.329$
Standard deviation	7	8	7		7	8	7	

Table 5. Distribution of risk factors.

Risk Factors	2020			2022		P-value
	Cases	Controls	P-value	Cases	Controls	
Varicocele treated or not						
Yes	1 1.3%	5 3.3%	0.380	0 0.0%	5 3.3%	0.112
No	75 98.7%	147 96.7%		74 100.0%	145 96.7%	
Cryptorchidism						
Yes	1 1.3%	1 0.7%	0.616	1 1.4%	1 0.7%	0.608
No	75 98.7%	151 99.3%		73 98.6%	149 99.3%	

Continued**Excessive use of condoms**

Yes	0	0		0	0	
	0%	0%	0.360	0%	0%	0.512
No	76	152		74	150	
	100.0%	100.0%		100.0%	100.0%	

Sexually transmitted infection

Yes	10	14		9	14	
	13.2%	9.2%	0.360	12.2%	9.3%	0.512
No	66	138		65	136	
	86.8%	90.8%		87.8%	90.7%	

Bilharzia

Yes	10	0		0	0	
	0.0%	0.0%		0.0%	0.0%	
No	0	0		0	0	
	0.0%	0.0%		0.0%	0.0%	

Obesity

Yes	16	18		13	18	
	21.1%	11.8%	0.066	17.6%	12.0%	0.256
No	60	134		61	132	
	78.9%	88.2%		82.4%	88.0%	

Alcohol consumption

Yes	67	109		65	107	
	88.2%	71.7%	0.005	87.8%	71.3%	0.006
No	9	43		9	43	
	11.8%	28.3%		12.2%	28.7%	

Tobacco consumption

Yes	1	1		1	1	
	1.3%	0.7%	0.616	1.4%	0.7%	0.608
No	75	151		73	149	
	98.7%	99.3%		98.6%	99.3%	

Others (Surgeries, history, family, Cancer)

Yes	0	0		0	0	
	0.0%	0.0%		0.0%	0.0%	
No	76	152		74	150	
	100.0%	100.0%		100.0%	100.0%	
Total	76	152		74	150	

We also noted a statistically significant increase in LH of 13% for cases and 4% for controls in 2020, compared to 31% for cases and 3% for controls in 2022 (Table 8).

Table 6. Distribution of testosterone levels.

	2020		P-value	2022		P-value
	Cases	Controls		Cases	Controls	
Testosterone						
Normal: 4 - 12 ng/mL	68	147	0.026	54	140	0.001
	89%	97%		73%	93%	
Abnormal: <4 ng/mL	8	5		20	10	
	11%	3%		27%	7%	
Abnormal: >12 ng/mL	0%	0%		0%	0%	

Table 7. Distribution of FSH levels.

	2020		Khi-deux	2022		Khi-deux
	Cases	Controls		Cas	Controls	
FSH						
Normal: 1.7 - 12 mUI/mL	70	151	(P-value = 0.003)	53	143	(P-value = 0.000)
	92%	99%		72%	95%	
Abnormal: >12 mUI/mL	6	1		21	7	
	8%	1%		28%	5%	
Abnormal: <1.7 mUI/mL	0%	0%		0%	0%	
	13%	4%		31%	3%	
	100%	99%		97%	99%	

Table 8. Distribution of LH levels.

	2020		P-value	2022		P-value
	Cases	Controls		Cases	Controls	
LH						
Normal: 1.5 - 12 mUI/mL	66	146	0,01	51	145	0.000
	87%	96%		69%	97%	
Abnormal: >12 UI/L	10	6		23	5	
	13%	4%		31%	3%	
Abnormal: <1.5 UI/L	0%	0%		0%	0%	
	100%	99%		97%	99%	

In this study, no significant difference was noted in prolactin in either 2020 or 2022 (**Table 9**).

3.4. Correlation between Environmental Parameters and the Proportion of Hormones in Cases

It was found in this study that automobile pollutants (NO₂, CO, CO₂ and SO₂) significantly influenced testosterone, LH, and FSH; the correlation coefficient is close to 1, with a p-value of 0.05. However, our data did not detect a link between prolactin and different automobile pollutants. Furthermore, with the exception of LH, no link has been established between the different hormones and the concentration of NO₂ (**Table 10**).

3.5. Concentrations of Pollutants in Ambient Air

The results of the environmental investigation were previously published. The concentrations of the different automobile pollutants (CO, CO₂, NO₂, and SO₂) measured in the different garages were all higher than WHO air quality standards, with peaks in Poto-Poto and Ouénzé [15].

Table 9. Distribution of prolactin levels.

	2020		P-value	2022		P-value
	Cases	Controls		Cases	Controls	
Prolactin						
Normal:	76	151	0.479	72	148	0.467
<17 ng/mL	100%	99%		97%	99%	
Abnormal:	0	1		2	2	
>17 g/mL	0%	1%		3%	1%	

Table 10. Correlation between environmental parameters and the proportion of hormones in cases.

Proportion of abnormal cases of hormonal markers		environmental parameters			
		CO ₂	CO	NO ₂	SO ₂
Testosterone	correlation coefficient	0.98	0.84	0.92	0.62
	P-value	0.000	0.004	0.0004	0.073
FSH	correlation coefficient	0.69	0.89	0.86	0.40
	P-value	0.0400	0.0018	0.0029	0.2884
LH	correlation coefficient	0.83	0.79	0.91	0.71
	P-value	0.0006	0.0119	0.0006	0.0321
Prolactin	correlation coefficient	0.38	0.17	0.07	0.55
	P-value	0.3171	0.66	0.85	0.12

4. Discussion

Today, hormonal dosage is an important element in the diagnosis and evaluation of male infertility. The objective of this work is to evaluate the level of sex hormones in subjects exposed to automobile pollution. This study involved 36 garages across the 9 districts of Brazzaville, *i.e.*, 4 garages per district, as well as 76 subjects exposed to automobile pollutants (mechanics) and 152 control subjects for the year 2020, compared to 74 subjects exposed to automobile pollutants (mechanics) and 150 control subjects for the year 2022. During this study, we recorded four (04) lost to follow-up (*i.e.*, two from group G1 and two from group G2) for reasons of mobility or death. Also, the largest number of our sample (21% in 2020 and 22% in 2022) for the G1 groups and (24% in both 2020 and 2022) for the G2 groups were recruited in Poto-Poto. This is explained by the fact that it was in Poto-Poto where people were most interested and receptive to information on the dangers posed by ambient air pollution and therefore more able and motivated to participate in this study.

The results related to age from this study show an average age of 36 ± 8 years for the G1 group and 38 ± 7 years for the G2 group, with extremes of 18 to 50 years. The majority of our subjects, *i.e.*, 38% of the G1 group in 2020 and 31% in 2022 compared to 47% of the G2 group, were made up of subjects in the age group of 36 to 45 years old, followed by that of 26 to 35 years old. These results could be justified by the fact that the African population is young and the said profession requires good health and physical endurance. These results are similar to those of Frikh who found an average age of 39 years for both the exposed groups and the control groups [16].

In this study, risk factors were sought in order to avoid confusion when interpreting the results. This study reveals that alcohol consumption was the only statistically significant risk factor in both cases (11.8%) and controls (28.3%) for the year 2020, compared to 12.2% in cases and 28.7% in controls for the year 2022. These results are in agreement with those of Concordéli *et al.*, who noted a deleterious effect of alcohol on fertility and parameters of sperm [17]. Similarly, Najapour *et al.*, revealed that lifestyle habits (alcohol, tobacco, STIs, etc.) have a negative effect on sperm quality [18]. This result can be explained by the fact that the congolese have integrated alcohol consumption into their daily lifestyle, unaware that this promotes the overproduction of reactive oxygen species, which will potentiate the effects of stress on health in general and on the reproductive system in particular.

In this study, we noted significant increases in FSH at 8% for cases and 1% for controls in 2020, compared to 28% for cases and 5% for controls in 2022. As for LH, we noted significant increases of 13% for cases and 4% for controls in 2020, compared to 31% for cases and 3% for controls in 2022. However, no significant differences were reported for prolactin during this time interval. Our results are in agreement with those found by Julie Carre and Anawalt, who found significantly higher levels of reproductive hormones, FSH, and LH in traffic police of-

ficiers exposed to air pollutants compared to the control population [19] [20]. Similarly, Zheng revealed the existence of a strong link between exposure to air pollution and the level of sex hormones [9]. The study demonstrated that it is important to control ambient air pollution exposure to reduce effects on the reproductive health of men.

At the same time, Radwan reported a negative association between low testosterone levels and exposure to certain air pollutants, such as PM₁₀, PM_{2.5}, CO, and Nox [21]. These results could be explained by the fact that air pollutants act on the hypothalamic-pituitary axis by inhibiting the secretion of GnRH, which would cause a reduction of available pituitary hormones. It can also interfere with the normal hormonal functioning of an individual by attaching to a hormonal receptor with a key-lock model, replacing the endogenous hormone, and blocking its physiological action [22]. Likewise, a pollutant could block the production, transport, and degradation mechanisms of the endogenous hormone and thus modify their serum levels in the blood.

5. Conclusion

This study, one of the first done in Congo, was part of a transversal, analytical study with prospective data between two groups (cases and controls). Its objective was to evaluate the short-term effects of automobile pollution on the levels of sexual hormones among mechanics in Brazzaville. An average age of 36 ± 8 years was observed for the cases and 38 ± 7 years for the controls, with extremes of 18 to 50 years, and the age group going from 36 to 45 years was the most representative. This study also reveals that alcohol consumption was the only statistically significant risk factor. The concentrations of air pollutants observed in the nine (09) districts of Brazzaville were all higher than the 2005 and 2021 WHO air quality standards. Additionally, we noted a significant drop in testosterone and an increase in LH and FSH among mechanics.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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