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Hematological Profile of Employees of Petroleum Stations Exposed to Fuel in Yaounde

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ABSTRACT

The automobile industry is experiencing a considerable boom today with the consequence of an exponential increase in filling stations. The number of these stations is estimated at 574 in Cameroon with about 100 in Yaounde. The benzene rich fuel they pump out constitutes a serious public health problem. The objective of this study was to evaluate the impact of fuel on the hematological parameters of people working in filling stations exposed to gasoline in Yaounde. A cross sectional study was carried out between April and August 2018 on 6 filling station attendants in Yaounde. A blood sample was collected from a total of 160 participants (80 of whom were exposed and 80 not exposed) in order to run a full blood count. Most of parameters of the Full Blood Count(FBC) showed a significant difference in the study population relative to the control population. There was a significant increase in RBCs (P=0.03), monocyte (P=0.02), HTE (P=0.03) and MCV (P=0.02) compared to the control population. We had a significant decrease of PNE (P=0.01).We noticed significant variations in certain hematological parameters which necessitates a regular control for proper health monitoring. Further studies would enable us to relate these different FBC values to personal exposure levels in order to make conclusions about the importance of the impact of benzene on the hematological parameters.

Keywords: *Fuel, exposure, hemogram, hemotoxicity.*

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INTRODUCTION:

Gasoline is the generic term for petroleum fuel or petrol.. It is a complex volatile, and inflammable mixture of saturated or unsaturated hydrocarbons[1]. It is mainly used for internal combustion engines and also as an industrial solvent, a thinner and as a decorative agent. The characteristics of this complex mixture varry and depend on the origin of the crude oil, differences in processing techniques and blends, season to season changes, and the additives required to meet particular performance specifications[1], [2]. Most of the toxological effects of gasoline are attributed to some specific volatile components of gasofline like benzene, toluene, xylene and ethlene. [3], [4].Occupational exposure to gasoline is mainly throughthe reapiratory routes and to a lesser extent through the transcutaneous routes[5], [6]. Consequently, exposures in high concentrations over short periods or in low concentrations over prolonged periods have been associated with a series of hematological abnormalities and a high risk of developing cancers[1]. Studies have shown that exposure to gasoline might lead to the permanenet suppression of the bone marrow functions followed by a reduction in the formation of blood cells in a condition called aplastic Anemaia. Benzene, one of the volatile components of petrol is a known carcinogen primarily affecting the hematopoetic sytem. Systemic exposure to benzene can lead to acute or chronic clinical disorders, of the cardiovascular, neurological, Liver, respiratory, renal, gastrointestinal, dermatological, immunological, metabolic and allergic reactions. Benzene is metabolised in the liver by cytochrome p4502E1 (CYP2E1) through the benzene oxide intermediate to its primary metabolite phenol. It is further metabolised to by CYP2E1 to hydroquinone (HQ). the resulting HQ is then transported to the bone marrow where it is oxidized to benzochinones, which subsequently release reactive oxygen species (ROS) damaging hematopoietic cells, as such, chronic exposure to benzene is associated with hematological effects like Acute Myeloid Leukemia (AML), chronic myeloid leukemia, acute lymphoblastic leukemia, and Aplastic anemia myelodydplastic syndrome[7], [8].The use of these hydrocarbon fuels in numerous activities especially in the ever growing automobile industry, has sollicited their high demand especially in the transport sector, reason for the sudden uprise in the number of filling stations.According to the website of the 'Média Energie' in 2018, work-related exposition to these hydocarbon fuels is increasing exponentially due the appearance of petroleum product distribution stations which grew 64.7% in seven years, from 526 in 2011 to 814 in 2018, according to government sources[9]. However, knowing that about 110 million people are exposed to gasoline constituents in the course of refueling at self-service gasoline stations [10],per week, accruing to approximately 100 min/year. During refueling, total hydrocarbon concentrations in the air fall within the range of 20-200 ppm by volume [11].

Investigations geared towards bringing out the effects of Petroleum products on the body can be done by carrying out various biological analyses amongst which is the full blood count [12]. As such, it is imperative to study the heamatological profile of personnes regularly exposed to this petroleum products in filling stations in the city of Yaounde inorder to be able to analyse the presentation of the cells therein.

METHODS:

Participants and Full blood count characteristics

This study was carrried out between April and September 2018, after ethical clearance was obtained form the national ethical committee N_0 : 2017/07/476/CE/CNERSH/SP. A total of 160 subjects were recruited, 80 exposed and80 unexposed. The exposed participants were sampled based on a technique of non probablistic method of convenience. They fulfilled the criteria of inclusion which were: having worked in a filling station for at least 10 months, no distinction with regards to gender, aged at least 21 years and having consented to participate in this study.

Participantspresenting factors that may influence the results namely; an inflammatory syndrome; Consuming dietary supplements or treatments affecting hematopoietic physiology at the time of collection; a blood transfusion less than three months old; a blood donation less than three months old; a known chronic disease, in connection with hematological physiology (sickle cell disease, Hodgkin's disease ...), smoking and pregnant women, were excluded.

Blood sampling was done for each individual on tripotassic EDTA (ethylene diamine tetraacetic) tubes. The completion of a digital hemogram by the MS-H652 automatic blood counter and manual smear stained with MGG (May-Grunwald Giemsa), as well as a manual count of reticulocytes for specific cases of anemia was performed on smear stained brilliant cresyl blue . The variables of the study were socio-demographic characteristics and the set of parameters of the hemogram as represented in the table below.

Study Variables	
Socio-demographic	Hemogram parameters
Age	Leukocytes (PNN, PNE, Monocytes, Lymphocytes)
Sex	Hematocrit
	Red Blood Cells
	Red Blood Cell const (MCV, MCHT, MCHC, HTE)

MCV: mean cell volume; **MCHT**: mean of corpuscular hemoglobin tensor; **MCHC**: mean of corpuscular haemoglobin concentration; HTE:hematocrit; PNN: neutrophil polynuclear; PNE: eosinophilia polynuclear

Statistical analysis

Statistical analysis were done using the SPSS 20.0 software. Continious data was presented as mean±SD. The mean values of two groups were compared by Student's t- testfor independent variables. A p value <0.05 was considered statistically significant.

RESULTS:

The study included a sample of 80 exposed employees of Yaoundé city fuel-fillingstations and 80 non-fuel subjects. This group of individuals allowed us to assert or refute the link between the level of fuel exposure and the various parameters of the blood count by group comparison.

Demographical Characteristics of the study groups

Data analysis showed that more than a third of the agents in this study were 21 to 30 years old,46.66% of the participants were aged 31 to 40, and those over 40 years old were the least represented (16.67%) (figure 1). In general, eight out of ten service station agents, or 80% were men, while20% of them were women (figure 2).

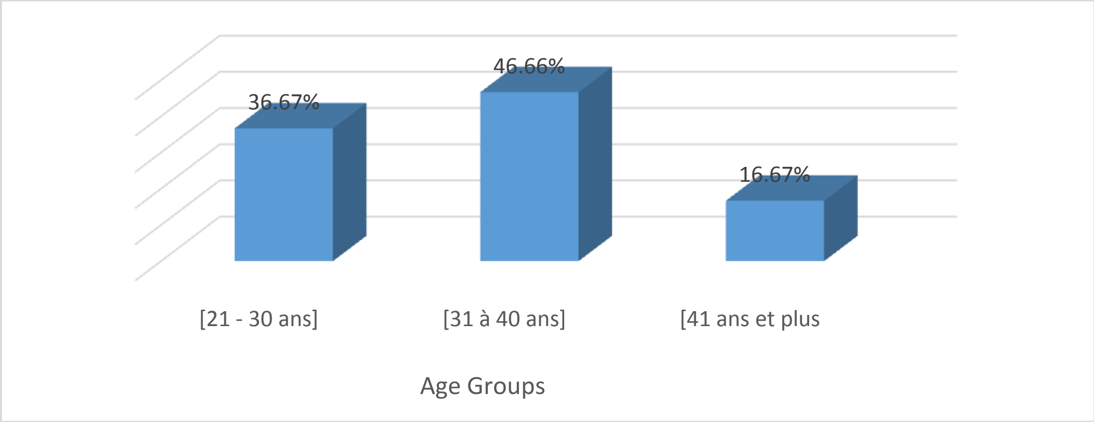

Figure 1: Distribution of employees of petroleum stations according to age groups

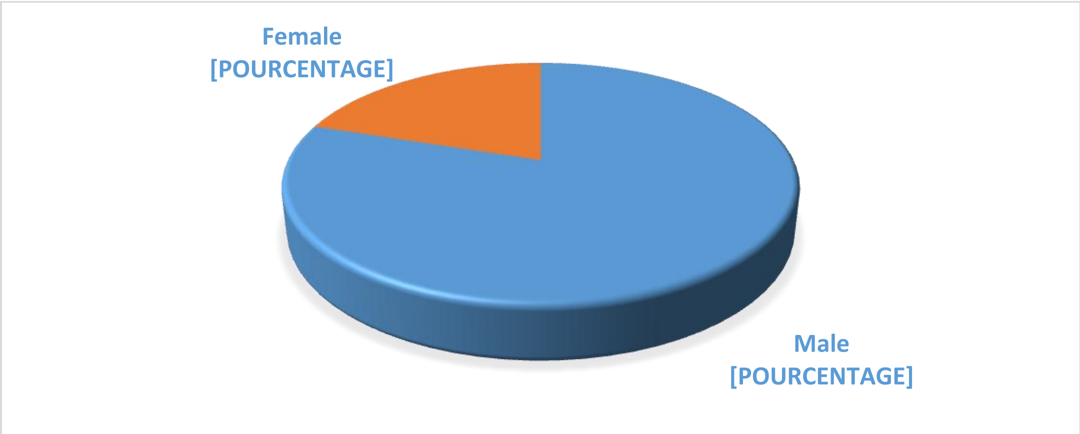

Figure 2: Distribution of employees of petroleum stations according to sex

Half of the people surveyed in our study had less than 10 hours of work a day. However, it should be noted that the minimum daily working time is 8 hours and the maximum duration is 13 hours. In our sample, from the point of view of professional experience, people employed in service stations for more than 10 years were the most represented (43.34%) followed by those who worked from 5 to 10 years in station (two-thirds) (figure 3).

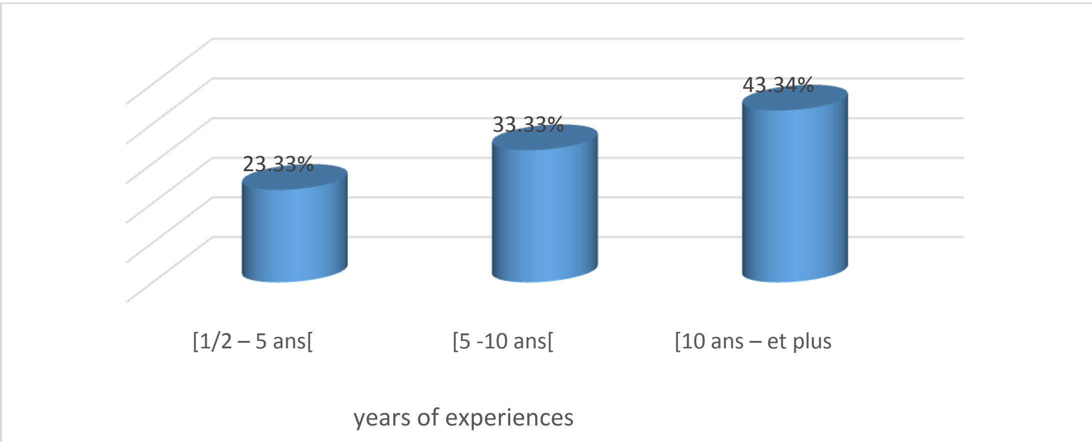

Figure 3: Distribution of employees of petroleum stations according to years of experience

Relationship between red blood cells, red blood cells constant, hematocritand study populations

The analysis of red blood cells showed that the average number of RBCs was significantly higher in exposed participants compared to unexposed participants (4.7 x 10^6 ± 0.1 vs 4.4 x10^6 ± 0.1) per mm^3,with a p.value of 0.03 (Table 1). Average values of mean cell volume (MCV) and hematocrit (HTE) were significantly elevated in exposed compared to unexposed individuals with respectively (88.1 ± 1.7 Vs 83.4 ± 1.1, p = 0.02) and (40.5 ± 0.9 Vs 37.9 ± 0.8, p = 0.03). On the other hand, the average values of the mean hemoglobin corpuscular tensor (MCHT) were higher in the exposed than in the unexposed (29.6 ± 0.7 Vs 29.2 ± 0.6; p=0.74), but the difference was not statistically significant. However, mean values of mean hemoglobin concentration (MCHC) were lower in exposed individuals than in unexposed persons (33.6 ± 0.4 Vs 34 ± 0.5) with p.value 0.57 (Table 2).

Tableau 1: Average number of red blood cells per study population

	exposed		***unexposed***		***P. value***
	Mean	*Standard deviation*	*Mean*	*Standard deviation*	
Number of red blood cells	*4,7*	*0,1*	*4,4*	*0,1*	***0,03***

Tableau 2: Mean values of red blood cells constant and hematocrit according to the study populations

	Exposed		***unexposed***		***P. value***
	Mean	*Standard deviation*	*Mean*	*Standard deviation*	
MCV	*88,1*	*1,7*	*83,4*	*1,1*	***0,02***
MCHT	*29,6*	*0,7*	*29,2*	*0,6*	***0,74***
MCHC	*33,6*	*0,4*	*34*	*0,5*	***0,57***
HTE	*40,5*	*0,9*	*37,9*	*0,8*	***0,03***

MCV: mean cell volume; **MCHT**: mean of corpuscular hemoglobin tensor; **MCHC**: mean of corpuscular haemoglobin concentration; HTE:hematocrit

Relation between haemoglobin and study populations

The study showed that more than 3/4 of the employees, ie 76.67%, had a hemoglobin within the reference range. The hemoglobin in the participants ranged from 9 to 17,4g/1. The mean value of hemoglobin was higher in exposed persons than in unexposed persons (13.8 ± 0.4 Vs 13.2 ± 0.3, p = 0.29)(Table 3).

Tableau 3: Mean value of haemoglobin according to the study population

	Exposed		***Unexposed***		***P. value***
	Mean	*Standard deviation*	*Mean*	*Standard deviation*	
value of haemoglobin	*13,8*	*0,4*	*13,2*	*0,3*	***0,29***

Relation between plateletsand study populations

The number of platelets in the participants varried from 102 to 401 × 10^3 per mm^3 of blood. The average platelet count was higher in the unexposed than in the exposed (209.9 ± 11.7 Vs 223.4 ± 13.5, p = 0.45) participants (Table 4).

Tableau 4: Mean number of platelets according to the study Population

	Exposed		***Unexposed***		***P. value***
	Standard deviation	***Mean***	***Standard deviation***	***Mean***	
Number of platelets	*209,9*	*11,7*	*223,4*	*13,5*	***0,45***

Study of leucocyte lines in the study populations

The minimum number of white blood cells in the participants was 3.11 x 10^3 / mm^3 blood and the maximum number was 8.7 x 10^3 / mm^3. The mean eosinophilic polynuclear (PNE) value wassignificantly higher in the unexposed than in the exposed (205.1 ± 30 Vs 104.7 ± 21.6, p = 0.01) participants. Also, the average monocyte value was significanlty elevated in exposed compared to unexposed participants (435 ± 29.1 Vs 348.7 ± 20.6, p = 0.02). However, mean values of neutrophils (NNP) and lymphocytes were higher in the unexposed than in the exposed individuals with respectively (2277.1 ± 143.5 Vs 1846.2 ± 147.4) per mm^3 and (2361.3 ± 132.6 Vs 2223.7 ± 119.1, p = 0.44) (Table 5).

Tableau 5: Mean value of leucocyte lines according to the study populations

	Exposed		*Unexposed*		***P. value***
	Standard deviation	*Mean*	*Standard deviation*	*Mean*	
PNN	*1846,2*	*147,4*	*2277,1*	*143,5*	***0,08***
PNE	*104,7*	*21,6*	*205,1*	*30*	***0,01***
Monocytes	*435*	*29,1*	*348,7*	*20,6*	***0,02***
Lymphocytes	*2223,7*	*119,1*	*2361,3*	*132,6*	***0,44***

PNN: neutrophil polynuclear; PNE: eosinophilia polynuclear

Morphological abnormalities of blood parameters in the study populations

The observed red blood cell abnormalities are: roll red blood cells, hypochromia, anisocytosis, microcytosis and macrocytosis. It appeared that in unexposed subjects, there was a homogeneous distribution of hypochromia and microcytosis of 13%. As for the subjects exposed, the red blood cell abnormality most commonly identified was hypochromia (10%), (Table 4). We also hadplatelet aggregation, platelet anisocytosis and macrothrombocytosis. Platelet clusters were the most observed abnormalities in the two populations, ie 6.7% in the unexposed subjects and 20% in the exposed patients (Figure 4).

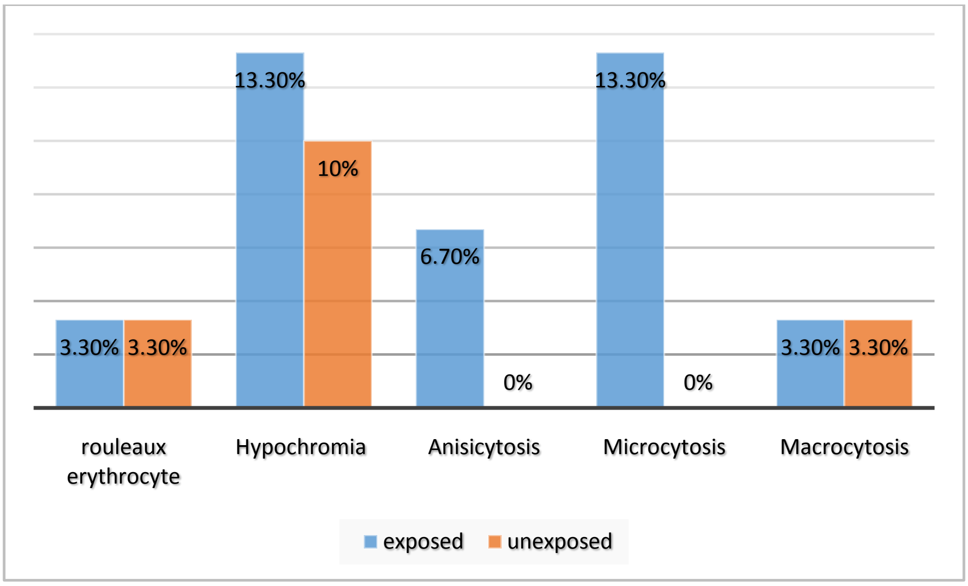

Figure 4: Distribution of red blood cells anomalies according to study populations

The only abnormality seen in the leukocyte lineage was granulocytic hyposegmentation, which was generally observed in two subjects, one exposed and unexposed individual (Figure 5).

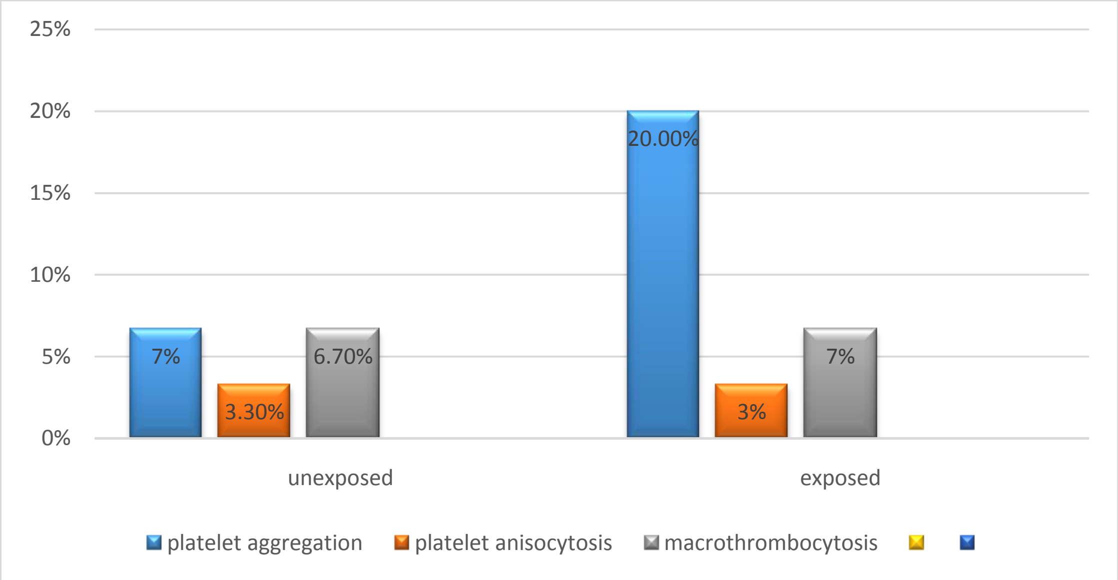

Figure 5: Distribution of platelets anomalies according to study populations

DISCUSSION:

The overall objective of this study was to determine the relationship between the level of fuel exposure and blood cell parameters. For this purpose, a sample of 80 employees of service stations without distinction of sex, nor of activity station and exercising for at least 6 months in a continuous way was integrated in the study. A blood count was therefore performed by the HORIBA ABX Micros 60 1.7.0 automated Counter on all blood samples. However, it was considered important to associate with this blood count, a reticulocyte count in order to characterize cases of anemia as presented by the epidemiological study of reticulocytes and solvents [13]. In addition, a population of 80 subjects not exposed to fuel was also included in the study. This was done in order to better understand the link between the level of exposure and the parameters of the blood count following information presented by other related studies [14] on the profile of persons exposed or not to atmospheric pollution due to road traffic in Cotonou.

The study showed that the majority of service station employees is male, with 80% men versus 20% women. These results are consistent with the reality that the profession is more male-dominated than female. This result is also close to Cardoso et al's 1995 epidemiological study of reticulocytes and solvents [13]. From the general observation, it appears that the value of the various parameters of the hemogram depends neither on the seniority nor the daily work duration. This clarification was also apparent in the previously cited study in which the dose-response relationship was therefore discarded. This result could be explained by exploiting the exposure indicators described by [15] to characterize the target population as having uncertain protection and possible exposure to fuel.

With regard to red blood cells, the change in the average number according to the type of population was statistically significant. The highest mean number wasobserved in exposed patients, ie 4.7×10^6 / mm^3 v 4.4×10^6 / mm^3 ; P = 0.03. This was contraryto the results from a study done by HINSON which showed a non significant difference [14].

This result could be explained by the fact that the population of the study was predominantly male while that of the non-exposed is predominantly female, taking into accout the fact that the reference values of the red blood cells vary according to the gender [16]. In addition, the excitatory action of benzene, the most incriminated in the toxicity of fuels on the bone marrow, explain this result [17].

Like red blood cells, the erythrocyte constant like VGM and hematocrit depend on exposure to fuel. However, mean VGM values were higher in the exposed (88.1 ± 1.7 fL v 83.4 ± 1.1 fL, P = 0.02) and the mean hematocrit (40.5 ± 0, 9 v 37.9 ± 0.8%, P = 0.03) were statistically significant. This would seem to be in line with the red blood cell result previously cited for similar reasons. In general, although low frequency cases of anemia were observed in both exposed and non-exposed individuals. For both populations, the number of reticulocytes was considerably greater than 20000 G/L of blood, thus describing regenerative anemias. This result corroborates that of Cardoso, which, however, showed an increase in the number of reticulocytes in the exposed subjects [13].

The average platelet count is higher in the unexposed than in the exposed (209.9 ± 11.7 Vs 223.4 ± 13.5) patients with a value of 0.45. many studies have found similar results where platelets are higher in petroleum unexposed group than exposed but the differences were not significant [8], [16], [17].The changes observed for exposure groupings may be due to age, exposure (service) period. Most of the parameters suggest that both age and exposure period have strong impacts in defning the patterns of variations observed in the haematological indices among the oil workers. He implications of these variations in the haematological profiles are great for the oil workers, given the wide array of conditions or circumstances that could precipitate such deviations from normal [16].

As for white blood cells, it appears that the average number of PNEs was statistically different according to the type of population, as well as that of monocytes with respectively P = 0.01 and P = 0.02. These results seem to be similar to those of an INVS's study on the technical elements of occupational exposure to petroleum fuels and solvents [18], the only difference being that they did not observe significant variations in monocytes.In view of these results, the hypothesis of the link between fuel exposure and blood cell parameters can not be ruled out. As in the previous study, the phenomenon justifying these results could not be elucidated. Regarding the qualitative aspect of the figured elements, although identified, the morphological abnormalities in the two types of populations could not be associated with exposure to fuel. This study was not without limit. For example, we have the size of the population that is small. We have the male-to-female ratio and the age for matching which were not respected.

STRENGTHS AND LIMITATIONS

The exponential increase of petroleum stations in Cameroon [6] brings us to the question of whether fuel have no effect on health, especially since people directly in contact are in no way protected. From this observation, our study was born which was to evaluate the impact of fuel on the hematological parameters of station agents. it appears that protective measures must be taken to ensure the safety of these agents at work and also that the Ministry of Labor and Health must work in synergy to ensure the respect of their security. This first study conducted in Cameroon on the effect of fuel on health will open the eyes of Cameroonians and even readers who will be careful when working with petroleum derivatives.

In addition, a study on the entire territory would be more representative. likewise, a cohort should promote the individual follow-up of each agent to show the true involvement of the fuel. it would be important also to:

- ✓ Perform, in addition to the blood count, additional biological tests to detect the presence in the body of toxic substances related to chronic exposure to fuel;
- ✓ Carry out atmospheric measurements of the substances involved in the toxicity of the fuel at service stations.

CONCLUSION:

Ultimately, this study aimed globally to establish the profile of the blood count of people chronically exposed to fuel in some filling stations in the city of Yaounde. The study was conducted with 80 employees at six filling stations. For the sake of comparison, 80 subjects not chronically exposed to fuel were integrated in the study.With reference to the specific objectives of the study,the profile of the target population hemogram was presented. Statistical analyzes thus showed a statistically significant association between exposure and variation of parameters such as: PNEs, monocytes, MCV, HTE, RBCs. This study may, however, serve as a pilot study for a cohort in which a follow-up of the blood count of filling station employees will be carried out in order to better understand the link between exposure and blood count parameters.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Ethical Committee of the Catholic University of Central Africa and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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