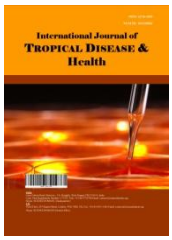

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Tumour Necrotic Factor Alpha level and Some Biochemical Parameters as a Measure of Health Risks Due to Exposure to Liquefied Petroleum Gas on Vendors in Calabar, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AUS, ENS and ERO did the laboratory analysis, literature review and reading, authors NAAC and EMH were the supervisors, author YD did the statistical analysis, authors AEU, NLL and UCP administered questionnaires, took anthropometric measurements, blood sample collection and separation. All authors read and approved the final manuscript.

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ABSTRACT

Background: The human body is constantly exposed to chemicals and other substances injurious to health from the environments, foods etc. These substances are taken into the body either consciously or unconsciously through the mouth, nose, and skin which often lead to oxidative

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stress, gene mutation, direct injury on cells and tissue, alteration of immune system etc, resulting in cancer development and other chronic and acute health challenges and death. This study is aimed at assessing the health risks due to chronic exposure to non-combusted Liquefied Petroleum Gas (LPG) and appraising the potential inflammatory and liver problem among its vendors who did not wear Personal Protective Equipments (PPE).

Methods: Forty-one apparently healthy Liquefied Petroleum Gas sellers and Forty-one apparently healthy non Liquefied Petroleum Gas sellers/user (control), aged 18 to 50 years were recruited for this study. The Body Mass Index (BMI) was determined by taking the ratio of weights in Kg to the square of heights in meters. Tumor Necrosis Factor Alpha (TNF-α) was estimated by Enzyme Linked Immunosorbent Assay (ELISA), 2-butanol was estimated using High Performance Liquid Chromatography (HPLC), ALT and AST were estimated using Colorimetric method. Data were analyzed using Student's t-test and Pearson's correlation at $p<0.05$.

Results: From the results obtained, AST, ALT and BMI did not show significant variation but TNF-α and 2-butanol showed a significant variation compared to control. TNF-α and 2-butanol showed a significant positive correlation among the exposed group. The duration of exposure does not appear to cause a significant difference in the levels of the biochemical parameters.

Conclusion: This study therefore showed that exposure to Liquefied Petroleum Gas has no effect on anthropometric and liver parameter but appears to cause significant elevation of TNF-α and 2-butanol, meaning that sellers may be predisposed to inflammation. It is therefore recommended that vendors of Liquefied Petroleum Gas (LPG) always wear their PPE to avoid deleterious effect on their health.

Keywords: TNF-α, 2-butanol; inflammation; occupational exposure; pollution; health hazards; public health.

ABBREVIATIONS

LPG : Liquefied Petroleum Gas
BM : Body Mass Index
TNF-α : Tumor Necrosis Factor Alpha
AST : Aspartate Amino Transferase
ALT : Alanine Amino Transferase
Pg/ml : Picograms per Milliliter
Mg/L : Milligrams per Liter
IU/L : International Units per Litre
HPLC : High Performance Liquid Chromatography

1. INTRODUCTION

The human body is constantly exposed to chemicals and other substances injurious to health from the environments, foods etc. These substances are taken into the body either consciously or unconsciously through the mouth, nose, and skin and often lead to oxidative stress, gene mutation, direct injury on cells and tissue, alteration of immune system etc, resulting in cancer development and other chronic and acute health challenges and death [1-3].

The demand for energy has continued to be on the increase, not only in Nigeria but all over the world due to increasing population, desire for better standard of living and growth of agricultural and manufacturing industries. The increasing demand for energy and the need to use environmentally friendly and safe fuel for cooking, heating, and drying food has made cooking gas (Liquefied Petroleum Gas) usage very common. The major reoccurring effect of the increasing demand for energy is the air pollution [1-3]. "According to the WHO, household air pollution is responsible for 7.7% of global mortality or 4.3 million deaths, mostly in Asia and Sub-Saharan Africa" [4]. "The hazardous effect of air pollution is the reason behind the increasing campaign to shift from the environmentally, ecosystem and health less friendly forms of fuel to more friendly ones amidst the growing energy demand worldwide. It is stated that about three billion people worldwide who continuously rely on solid fuels, cooking and heating on open fires or traditional stoves are exposed to high levels of health-damaging pollutants including small particulate matter and carbon monoxide, sometimes exceeding accepted guideline values by a factor of 20" [5,6]. "In 2010, household air pollution from solid fuels was reported as the third leading risk factor for global disease burden, contributing to 4.3%. Health problems linked to both indoors and outdoors air pollution include lung cancer, ischaemic heart disease, acute lower respiratory infections in children under five years, asthma and chronic obstructive pulmonary disease in adults" [6,7]. "The overarching principle of the 2014 WHO guidelines on indoor

and even outdoor air quality is that there is no "acceptable" level of air pollution, and even the lowest levels of air pollution are harmful to human health. It is reported that the so called cleaner burning solid fuel cook stoves cannot achieve the WHO annual intermittent air quality target-1 (AQT-1) for particulate matter, set at 35 µg/m3 for PM2.5 (particulate matter less than 2.5 microns in aerodynamic diameter). Therefore, in order to reach the AQT-1 for PM2.5 in areas with persistent high background levels of PM2.5, where household air pollution (HAP) causes increase outdoor (ambient) air pollution, community-level adoption of clean cooking technologies is encouraged" [6,7]. "It is therefore stated that the Liquefied Petroleum Gas (LPG) which is said to be clean- burning, efficient, versatile and portable fuel, produced from crude oil refining appeared to be the answer to fuel shifting and is one of several pathways to meeting the objective of universal access to clean cooking and heating solutions by 2030; one of the three pillars of the UN Sustainable Energy for All (SE4All) initiative" [6,7].

"LPG is currently used predominantly by more than upper half of the income groups in low and lower-middle-income countries and especially urban and suburban households. In 2016, consumption of LPG reached 500,000 Metric Tonnes (MT) in Nigeria" [8]. "However, in 2018, 2019 and 2020 it rose to 635,452, 840,594.37 and over 1million MT respectively, although this is still far short of the World Bank estimated market potential for the country which, as far back as 2004, was 3.2Million MT Per Annum" [8]. The consumption targeted by the Nigeria Gas Policy is to reach 5million MT by the end of 2022 [8]. This shows steady and much more expected increases in consumption of the commodity and hence a rise in the exposure of its vendors to the commodity. Again, and more people other than the current vendors are expected to be exposed to it as more people join the trade as the market for the commodity is expanding.

The Federal Government of Nigeria's resolve to deepen LPG penetration in Nigeria is aimed to create ''healthier life'' for Nigerians [8]. This leaves the researchers with the interest to find out if LPG is very safe for human health especially when exposed to the non-combusted form as it is expected, hence meeting the need of the government in creating 'healthier life' for its citizens.

"Although, the by-products of incomplete combustion of LPG which contains a considerable level of Polycyclic Aromatic Hydrocarbons (PAHS), Oxides of Nitrogen (NOx), Carbon Monoxide (CO), and other compounds have been studied and thought to cause undesirable health outcomes" [9-11], very limited studies in the literature has made an attempt to assess the inflammatory and liver function parameters and 2-butanol (a metabolite of butane which is a major component of LPG) as a means of understudying the possible undesirable health effects due to exposure to non-combusted LPG.

Some studies assessed the effects of combustion by-products of cooking gas used in indoor environments and found that exposure to such fuel is associated with negative health effects including pulmonary functions reduction [12-16], while others reported similar potential health effects with non-combusted form but failed to assess the parameters assessed in this present study [17-20].

Therefore, borrowing a leaf from the above studies, this study seeks to identify the effects of chronic exposure to non-combusted LPG on the prevalence of liver disease, inflammation, the possibility of developing cancer and the effect of duration of exposure on these health indices through the analysis of these parameters.

"Tumor Necrosis Factor Alpha (TNF-α) is a cell signaling protein (cytokine) involved in inflammation and is one of the cytokines that make up acute phase reaction and is a marker of early inflammation. The primary role of TNF-α is in regulation of immune cells. TNF-α has been implicated in cancer and a variety of chronic diseases. The release of TNF-α is also triggered by inflammatory stimuli such as air pollutants. TNF-α production arises from numerous cell types of the lungs and other organs, including epithelium, endothelium, activated macrophages and monocytes, and probably also smooth muscle [21-24] hence its relevance in this study".

"The LP Gas mix that is specified for the Nigerian domestic market for cooking is one that is butane rich, that is, a 70/30 or 75/25 butane/propane mix" [25]. "Most reports of butane intoxication are from cases of abuse or suicide attempts. The predominant effects observed in abuse cases are central nervous system (CNS), liver, cardiac and lung effects. Acute exposure to high level butane causes asphyxia and slight anesthesia. It can

also cause dizziness, headache, in-coordination and narcosis. Extremely high concentrations can cause death by displacement of oxygen from the lungs. Case studies also reveal that serious brain damage and underdeveloped organs can occur in fetuses in case of high single exposures during the week 27 or 30 of pregnancy" [26]. Sugie et al. [27] reported "three cases of sudden death due to inhalation of portable cooking stove fuel (case 1), cigarette lighter fuel (case 2), and liquefied petroleum gas (LPG) (case 3). Specimens of blood, urine, stomach contents, brain, heart, lung, liver, kidney, and fat were collected and analyzed for propylene, propane, isobutane, and n-butane". n-Butane and propane were the major substance found in these tissues [27]. From the above, it is clear that butane is the major component of LPG used as cooking gas in Nigeria and that when LPG is inhaled either acutely or chronically, butane could be traceable in the body tissues either as intact butane or as metabolites (eg. 2-butanol). 2-butanol appears to be the most stable and analyzable metabolite of butane, therefore the need to study how the body manages butane by evaluating the levels of 2-butanol in the blood sample of the exposed group cannot be overemphasized.

2. METHODS

2.1 Study Design

A case control design was used in the study. The range of the sizes of cylinders refilled was from 4kg to 70kg. The amount sold per participant each day was obtained from their record books over the period of two months. The average of this was 950kg and was taken to indirectly represent the daily LPG exposure since it was not possible to directly determine the amount escaping into the ambient air. A total of eighty two subjects which consisted of forty one apparently healthy LPG (cooking gas) sellers and forty one apparently healthy none gas sellers/users (control) and who were all residents of Calabar, Cross River State, Nigeria were used for the study. The inclusion criteria for the exposed group were: residence in Calabar, age ranging from 18 to 50 years, At least one year exposure to LPG and selling for at least 6 hours daily, and apparently healthy. The inclusion criteria for the control were: residence in Calabar, age ranging from 18 to 50 years, apparently healthy and no exposure to LPG. Well structured questionnaires were administered randomly to the participants to obtain information on age, medical history, physical lifestyle, family history, drug usage, occupation and duration on the job. The purpose and nature of the research was explained to the participants and written consent was obtained.

2.2 Laboratory Methods

2.2.1 Determination of tumor necrosis factor alpha

Tumor Necrosis Factor Alpha was determined using Enzyme Linked Immunosorbent Assay (ELISA) kits obtained from Elabscience, USA and following the manufacturer's protocol [28].

2.2.2 Determination of 2-butanol

2-butanol level was estimated using Waters 616 & 626 High Performance Liquid Chromatography (HPLC) manufactured by Waters Corporation Milford, USA following the standard procedures [29,30].

Determination of Alanine Amino Transferase and Aspartate Amino Transferase was done using proprietary reagent made by Randox Laboratories Limited United Kingdom [31].

2.3 Data Analysis

The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive statistics was used to explore the characteristics of the subjects considered while independent t-test was to study the difference in the means of cooking gas sellers and non-sellers. Pearson Correlation was used for correlation analysis. For determination of significance difference, P-value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Description of Study of Participants' Anthropometric Parameters

Table 1 describes the participants' anthropometric parameters. Eighty two participants took part in the study, 41 were cooking gas sellers exposed to LPG and 41 non sellers, not exposed to LPG. The mean of weight, height and BMI of sellers were 66.98±7.79, 1.66±0.07, and 24.07±2.69 respectively and that of control were 64.83±7.26, 1.64±0.07, 23.66±3.04 respectively. This shows no significant difference in the anthropometric parameters.

Table 1. Participants' anthropometric parameters

Parameters	Sellers			Non sellers			T value	P value
	N	Mean	SD	N	Mean	SD		
Weight(kg)	41	66.98	7.79	41	64.83	7.26	1.29	0.2
Height(cm)	41	1.66	0.07	41	1.64	0.07	0.94	0.35
BMI (kg/m^2)	41	24.07	2.69	41	23.66	3.04	0.66	0.51

*SD = standard deviation. *Significant at .05 level; **Significant at .01 level*

3.2 Biochemical Parameters Assessment

The results of the findings of biochemical parameters assessment as indicated in Table 2 showed that the means of TNF-α, 2-butanol, ALTand AST were higher in the seller group (TNF- α : 73.97±83.27; 2-butanol: 2.32±1.48; ALT: 21.11±7.82; AST: 21.61±4.99) except AST as compared with the non sellers group (TNF-α: 41.56±29.16; 2-butanol: 0.57±.67; ALT: 21.08±6.88; AST: 21.83±5.35). The assessment shows a significant higher variation in the TNF- α and 2-butanol of the sellers (73.97±83.27 and 2.324±1.48 respectively) at less than 5% significance level compared to control (41.56±29.16 and 0.57±0.67 respectively).

3.3 Correlation between Some Biochemical Parameters among the Exposed Group

Table 3 revealed a significant positive correlation between TNF-α and 2-butanol with correlation coefficient (Pearson Correlation) of 0.32. This implies that any increase in one will most likely lead to corresponding increase in the other.

Table 2. Biochemical parameters assessment among liquefied gas vendors

Parameters	Seller			Non seller			T value	P value
	N	Mean	SD	N	Mean	SD		
TNF-α (Pg/ml)	41	73.97	83.27	41	41.56	29.16	2.35	.023**
2-butanol (Mg/L)	41	2.32	1.48	41	0.57	0.67	6.90	<0.001**
ALT (IU/L)	41	21.11	7.82	41	21.08	6.87	0.06	0.07
AST (IU/L)	41	21.60	4.99	41	21.83	5.34	-0.19	0.85

*TNF-α = Tumor Necrotic Factor Alpha, ALT = Alanine Amino Transferase, AST= Aspartate Amino Transferase, SD = Standard Deviation, *Significant at .05 level; **Significant at .01 level*

Table 3. Correlation between selected biochemical parameters among LPG vendors

Parameters		TNF-α (Pg/ml)	2-butanol (Mg/L)
TNF-α (Pg/ml)	Pearson Correlation	1	0.32*
	Sig. (2-tailed)		0.04
	N	41	41
2-butanol (Mg/L)	Pearson Correlation	0.32*	1
	Sig. (2-tailed)	0.04	
	N	41	41

**. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed)*

Table 4. Biochemical Parameters Assessment among LPG Vendors Based on Duration of Exposure

Parameters	1-3 years			Above 3 years			T value	P value
	N	Mean	SD	N	Mean	SD		
TNF-α (Pg/ml)	24	54.38	40.13	17	101.76	116.86	-1.61	0.13
2-butanol (Mg/L)	24	2.04	0.96	17	2.76	1.99	39	0.13
ALT (IU/L)	24	18.58	5.72	17	24.88	8.96	-2.55	0.06
AST (IU/L)	24	20.63	4.16	17	22.88	5.36	-1.52	0.14

*TNF-α = Tumor Necrotic Factor Alpha , ALT = Alanine Amino Transferase, AST= Aspartate Amino Transferase , SD = Standard Deviation, *Significant at .05 level; **Significant at .01 level*

3.4 Assessment of Biochemical Parameters among LPG Sellers Based on Duration of Exposure

Table 4 shows the biochemical parameters compared between two classes of years of exposure. The mean of 1-3years exposure for TNF-α, 2-butanol, ALT and AST were 54.38±40.13, 2.04 ±0.96, 18.58±5.72, 20.63±4.16 respectively and Table 4, 3 years were 101.76±116.86, 2.76±1.99, 24.88±8.96 and 22.88±5.36 respectively. There is no significant variation in their means when compared among the duration of exposure.

4. DISCUSSION

"Acute inhalation of liquefied petroleum gas (LPG) has been observed to be associated with death through respiratory system attacks" **[**32]. This study evaluated the potential effect of chronic exposure to LPG on liver health and immune system and also assessed the metabolite of butane, a component of butane. The findings from this study show that LPG sellers and non sellers/users have comparable BMI with values within the normal range. These findings agree with 33 and 34 who worked on similar subjects. The findings in this study could be inferred that chronic exposure to LPG is not likely to cause increased or decrease BMI.

In this study, tumour necrosis factor alpha (TNF-α) was significantly higher in the LPG sellers than control. Although there is scarcity of data on TNF-α levels among LPG exposed group, data exists on the over expression and production of TNF-α in lung cells and tissues following pollution and injury and 35 stated that TNF-α is implicated as a key cytokine in many inflammatory lung diseases. In a study in these subjects, pulmonary symptoms and poor functions were reported [1]. The significant elevation of TNF-α in this study in addition to the evidence of its involvement in aetio-pathogenesis of lung disease as previously studied, suggests that LPG sellers may be predisposed to inflammation which may be responsible for the poor pulmonary symptoms and functions as observed [33-36].

In this current study, a significantly higher level of 2-butanol in liquefied petroleum gas sellers than control was observed. There is insufficient information on the levels of 2-butanol in LPG sellers. This finding will open up researches in this area. 36 stated that detection of metabolites of butane, 2-butanol, 2-butanone in blood sample was an indicator of butane exposure or abuse. Increased 2-butanol seems to have influence on TNF-α, judging from the significant positive correlation both of them have as observed in this study.

Liver function parameters such as Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) did not show a significant variation among LPG sellers and control. This may mean that there is no observable liver damage as a result of LPG exposure among these subjects. This disagrees with 20 which observed remarkable differences in these parameters among these subjects.

The duration of exposure does not seem to show a significant variation on the levels of the biochemical parameters as observed by the comparable means that exist among the groups of duration of exposure. There is a dearth of information on the effect of duration of exposure to this commodity on vendors.

One of the major implications of this study is the creation of awareness on the hazardous health effects of continuous unprotected exposure to non-combusted LPG on vendors which include prevalence inflammatory parameter and 2-butanol. In addition, the study further provides evidence that could help in designing and implementing policy to protect and promote health of LPG vendors. The study is able to establish that chronic exposure to LPG could be a major source of inflammation among vendors but failed to identify the particular organ(s) involved.

5. CONCLUSION

The study is observes that even though LPG is established to be a safer source of energy compared to biomas and other traditional ways of cooking, it and the metabolites of its components such as 2-butanol may be hazardous to health just like every other air pollutants if the vendors did not protect themselves with appropriate Personal Protective Equipments [PPEs]. It may have the potential to cause allergic and inflammatory responses and diseases and if not checked, may have the potential to cause cancer. The liver appears not to be affected greatly in this study even though it is the organ where metabolism of substances including LPG is taking place. This may not mean that LPG and its metabolites does not affect the liver at all, but

may be that the duration of exposure is not enough to cause a noticeable changes in these subjects or due to the fact that liver undergoes constant regeneration.

ETHICS APPROVAL AND CONSENT

Ethical approval was sought and obtained from the Health Research and Ethics committee of the Cross River State Ministry of Health, Nigeria. The purpose and nature of the research was explained to the participants and written consent was obtained.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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