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Biochemical Correlates of Alcohol Abuse among Adults with Non-Communicable Diseases in Niger Delta, Nigeria

Faith Ajiebabhio Ogbole[1*] and Osuagwu Uchechukwu Obinna[1]

[1]Biochemistry Unit, Department of Chemical Sciences, University of Africa Toru – Orua, Bayelsa State, Nigeria

ABSTRACT

Background: Niger Delta, Nigeria is renowned for the traditional production of alcoholic beverages. However, limited information exist on the drinking patterns of adults with non-communicable diseases (NCDs) in this region. The present study investigated the prevalence and patterns of alcohol abuse among adults with NCDs in Niger Delta. The sociodemographic variables associated with habitual alcohol consumption (HAC) as well as differences in hematocrit and interleukin-2 gene expression levels between habitual and non-habitual alcohol consumers with NCDs were also evaluated.

Methods: Randomly selected two hundred and ninety-seventy adults with clinically confirmed diabetes and high blood pressure from randomly selected communities in Niger Delta were evaluated for alcohol abuse and sociodemographic variables associated with HAC using a structured questionnaire. Haematocrit and IL-2 gene expression levels were measured using a microhematocrit reader and real-time PCR machine respectively.

Results: Prevalence of current alcohol consumption among adults with NCDs was 90.9%. Patterns of alcohol abuse found were HAC-66.67%, binge drinking-10% and alcohol dependence-25%. Sex, age, drinking setting, and affordability were significantly associated with HAC. The average standard drink (alcohol units) consumed weekly by each habitual consumer with NCDs was 22.79±6.39. The brands of alcohol consumed were spirits-50%, palm wine-37%, beer-11% and fruit wine-2%. Compared with non-habitual consumers with NCDs, a significant upregulation in IL-2 gene expression followed by a reduction in haematocrit levels was found among habitual consumers with NCDs.

Conclusions: A high prevalence of HAC was found among adults with NCDs in the present study. This information may have implications in the management of NCDs.

Keywords: Niger Delta, habitual alcohol consumption, hematocrit, IL-2 gene expression, NCDs

1.0 Introduction

Non-communicable diseases are diseases that cannot be transmitted from one person to another. They include cardiovascular diseases, stroke, cancer, diabetes and chronic respiratory diseases [1]. According to World Health Organization, the rise in deaths from non-communicable diseases in Africa is alarming with 50% to 88% of deaths in seven African island countries and 100,000 to 450,000 annual deaths in African`s most populous countries [2]. In Nigeria, the most populous country in Africa [3], non-communicable diseases accounted for 27% of deaths in 2019 [4]. Previous studies have reported a very high prevalence of non-communicable diseases among adults in the Niger Delta region of Nigeria [5, 6, 7].

Alcohol is a major risk factor for non-communicable diseases [8]. It is also a toxic and psychoactive substance [9]. Alcohol addiction defined as the number of days spent consuming alcohol is a major problem globally [9] especially in Africa [10]. Alcohol abuse among secondary school students [11] and undergraduate students [12] in Nigeria have been previously reported. Despite the fact that the Niger Delta region of Nigeria is home to the production of alcohol, yet current studies and investigations on the contributions of alcohol consumption to non-communicable diseases have been greatly neglected in Niger Delta, Nigeria.

Alcohol production is an age long traditional occupation in Niger Delta, Nigeria [13]. The tropical rain forest that make up the Niger Delta vegetation is filled with naturally growing Raffia palm trees [14, 15] from which alcohol is locally produced from the sap that drains from the stems of palm trees following an incision on the stems. This process is referred to as palm wine tapping in Niger Delta [13, 16, 17]. The tapped palm wine which is whitish in appearance is usually consumed in that form or may be further distilled into the Nigeria`s palm wine spirit (local spirit) called “ogogoro” [13, 16, 17]. Ogogoro is also known by other common names such as “Kai-kai” and “push-me-I-push-you” in Nigeria and as Akpeteshie in Ghana [13, 16, 17, 18]. Although drinking below 14 units of alcohol weekly was initially regarded as safe for men and women`s health [19], however a recent 2024 report by WHO stated that no level of alcohol consumption is safe for human health [20].

Alcoholic beverages such as spirits (40 – 60% alcohol by volume [19]), palm wine (5% alcohol by volume [21]), beer (5 – 7% alcohol by volume [19]) and wine (12% alcohol by volume [19]) are commonly consumed in Nigeria [22]. However, information on the brands of alcoholic beverages most often consumed by subpopulations of adults with non-communicable disease is not available in literature.

Also, although a high prevalence of non-communicable diseases (NCDs) has been previously reported in Niger Delta, Nigeria [5,6,7], however, no previous research has evaluated the prevalence of alcohol consumption, pattern of alcohol abuse, hematological and gene expression profile of habitual alcohol consumers with non-communicable diseases. These information are needed for effective management non-communicable diseases among alcoholics. Therefore, the present study investigated the prevalence and patterns of alcohol abuse among adults with NCDs in Niger Delta, Nigeria. The sociodemographic variables associated with habitual alcohol consumption (HAC) as well as differences in hematocrit and interleukin-2 gene expression levels between habitual and non-habitual alcohol consumers with NCDs were also evaluated.

2. Materials and methods

2.1 Study location

The study location was Niger Delta Nigeria. One State, Bayelsa State was randomly selected in Niger Delta for the present study. Bayelsa State is centrally located in Niger Delta, Nigeria [23]. The State is located on latitude 4.7719^0N and longitude 6.0699^0E [24]. Bayelsa State is found in the heart of tropical rainforest where it houses the national forest with various species of palm trees [25, 26]. Various communities in Bayelsa State produce local spirits through the distillation of palm wine (fermented sap tapped from Raffia palm trees) [27]. Sagbama LGA was randomly selected out of the eight LGA in Bayelsa State and four communities were randomly selected out of Sagbama LGA for the present study. From each community, study participants were randomly selected. Thus, the present study employed a descriptive cross-sectional study design with multistage random sampling [28].

2.2 Sample size determination

Sample size was determined using the Cochran formular below as previously described by Charan [29].

$$n_0 = \frac{Z^2pq}{e^2}$$

Z-value (1.96) was obtained from Z-table at 95% confidence interval; n_0 was the sample size; e (0.05) was the desired level of precision (i.e. the margin of error)]; P [0.75 (75%)]; was the estimated proportion of the population consuming alcohol in Bayelsa State and was obtained from the findings of a previous pilot study; while q (0.25) was 1 – P. Therefore n_0 was equal to $(1.96)^2$ x 0.75 x 0.25 / $(0.05)^2$ = 3.842 x 0.188 / 0.0025 = 0.72 / 0.0025 = 288. Assuming a non-response rate of 3%, the sample size was increased to 297.

2.3 Ethical approval and informed consent

Ethical approval for the present study was obtained from Bayelsa State Ministry of Health Research Ethics Committee under Sagbama L.G.A Primary Health Care. Informed consent was obtained from each participant before the commencement of the study and participants were assured of confidentiality.

2.4 Inclusion and exclusion criteria

Two forms of non-communicable diseases, diabetes mellitus and hypertension [1, 2, 4], previously reported to be highly prevalent in Bayelsa State [5, 6, 7] were evaluated in the present study. Adults aged 18 years and above with clinically confirmed diabetes and high blood pressure where included in the study. Individuals younger than 18 years and those without non-communicable diseases where excluded from the study.

2.5 Study groups

Following the determination of the prevalence of current alcohol consumption and various patterns of alcohol abuse, the study population was divided into two groups: habitual alcohol consumers and non-habitual consumers.

2.6 Questionnaire and definition of operational measures of variables

The questionnaire for the present study was adapted from the Health Survey for England 2021 Questionnaires [19], the WHO Global Observatory Data on alcohol and health-related indicators [30] and the World Health Organization STEPwise approach to NCDs risk factor surveillance (STEPS) questionnaire [31]. Participants were asked to self-report their alcohol intake upon assessment by writing their answers on the questionnaire and alcohol consumption was reported in terms of standard drinks (number of alcohol units) consumed [19, 30, 31].

Table 1 Definition of operational measures of variables

Variable	Definition
Lifetime alcohol use	ever consumed alcohol other than a few sip at least once in a lifetime [19, 30, 31].
Current alcohol use	consumed a standard drink of alcohol at least once in the past year [19, 30, 31].
HAC	consumed at least one standard drink of alcohol weekly in the past 30 days [19, 30, 31].
Binge drinking	consumed at least 6 standard drinks of alcohol in a sitting in the past 30 days [19, 30, 31].
Alcohol dependence	current drinkers who have a very hard time giving up alcohol consumption [19, 30, 31].
Brand of alcohol consumed	self-reported type of alcoholic beverage (palm wine, beer, wine, or spirit) consumed [19, 30, 31].
Alcohol by volume (ABV)	strength (concentration) of pure alcohol in a drink expressed as percentage per volume. ABV was obtained from the label on the container of the brand alcohol reportedly consumed by study participants [19].
Volume of alcohol	self-reported volume of alcoholic beverage consumed, whether consumed in cans, bottles or sachets [19].
A standard drink (1 alcohol unit)	a drink that contains 8 ml or 10 g of pure alcohol regardless of the size of the container (glass, bottle, can) or type of alcohol (beer, wine, spirit) [19].
Formular for standard drink (alcohol unit)	ABV (%) x volume of alcohol consumed in litres [19]

For example the standard drink (alcohol unit) in a 60cl (0.6L) bottle of beer with an ABV of 5.2% = 5.2 x 0.6 = 3.12.

HAC: habitual alcohol consumption; ABV: alcohol by volume.

2.7 Blood sample collection

Blood sample was collected from the median cubical vein on the arm of each participant with the use of vacutainer needle and EDTA vacuum tubes. Collected blood was transferred into an ice park and transported to the laboratory for interleukin-2 (IL-2) mRNA gene expression and hematocrit levels analysis. Blood collection and handling was carried out according to the Centers for Disease Control and Prevention blood specimen collection for molecular diagnosis. [32].

2.8 Extraction of total RNA from the blood

Total RNA was extracted from fresh human blood samples within 24 hours of collection following the manufacturer`s protocol for total RNA minikit (Geneaid, New Taipei city, Taiwan) as previously described [6].

2.9. Gel electrophoresis of purified RNA

Exactly 100 ml of 1X Tris Acetate EDTA buffer (TAE) was measured into a conical flask and 0.7g of agarose powder was added into the buffer and mixed by swirling. The mixture was allowed to boil using a microwave and was allowed to clear while boiling (2-3 minutes of high heat) before removing it from microwave. The gel was allowed to cool to 60^0C by leaving it at room temperature for few minutes until it was no longer hot to touch (the gel was not allowed to solidify while cooling). Exactly 5 µl of ethidium bromide (10 mg/ml) was added to the gel before pouring it unto the gel tray in order to ensure visualization of the RNA fragments during electrophoresis. Ethidium bromide is a suspected carcinogen so it was handled with care. The base of the gel tray was sealed with little quantity of gel dispensed from a pipette. The gel was poured into the tray, allowed to solidify and combs inserted. The gel was allowed to solidfy for approximately twenty minutes. The RNA sample was mixed with 6x blue RNA loading dye (in a ratio of 5:1 that is 10 µl of RNA sample to 2 µl of loading dye). The mixture of RNA sample and loading dye (10 µl) was loaded into the gel wells created by the comb (one sample per well). Electrophoresis gel was run at 100 volts for 1 hour. The gel was transferred unto a UV transilluminator and visualized to assess the quality of RNA purified [33].

2.10 Complementary DNA (cDNA) synthesis

The purified RNA was reverse transcribed to cDNA following the manufacturer`s protocol for Fire Script cDNA synthesis kit (Solis BioDyne, Tartu Estonia). Briefly, 5 µl of Solisbiodyne Firescript cDNA synthesis kit (containing 0.5 µl of Oligo(dT) primer (G), 0.5 µl random hexamer primer (H), 0.5 µl dNTP mix (I), I µl reverse transcriptase (RT), 2 µl of 10x reaction buffer (J), and 0.5 µl riboGrip RNase inhibitor) and 15 µl of RNA template were dispensed into a 0.2 ml PCR tube. Spin, vortex, spin of all reagents and RNA template were carried out before dispensing. After dispensing, spin, vortex,

spin of the reaction mixture in the 0.2 ml PCR tube was carried out before loading unto the thermal cycler. The conventional cDNA PCR run; primer annealing at 25^0C for 10 min, reverse transcription at 48^0C for 15 min, inactivation at 85^0C for 5 min and hold at 4 ^{0}C was run for cDNA synthesis.

2.11. Real time PCR analysis for the measurement of IL-2 gene expression

The gene expression level of the inflammation marker, IL-2, was measured using real time PCR method. Each target and reference gene were amplified using a pair of primers. 5x HOT FIREPol Evagreen qPCR Supermix (Solis BioDyne, Tartu, Estonia) was used for gene expression analysis following the manufacturer`s protocol. The reaction was carried out in an Applied Biosystem StepOne Plus real-time polymerase chain reaction system (Thermo Fisher Scientific, California, USA). Custom designed primers synthesized by Genewiz (Genewiz, South Plainfield, New Jersey), were used to amplify the mRNA of the target gene of interest (IL-2). Amplification cycle threshold (Ct) was normalized by the mRNA expression of the reference gene (GAPDH gene). The cycling protocol were: Initial activation at 95^0C for 12 minutes, followed by 40 cycles of denaturation at 95^0C for 15 seconds, annealing at 53^0C for 30 seconds, elongation at 72^0C for 30 seconds. The reaction mixture contained 4 µl of PCR supermix, 1 µl primer mix, 4 µl of cDNA template, and 11 µl of water. The assay was run in duplicates using water as negative control (negative control was used to ascertain that the setup was free from contamination). IL-2 gene expression was reported as normalized fold change in the gene expression of habitual alcohol consumers relative to control (non-habitual consumers). IL-2 gene expression (relative quantification) was analyzed using $2^{-\Delta\Delta CT}$ ($2^{\text{-delta delta threshold cycle}}$) method [34] on the StepOne™ Software version 2.3 installed in the PCR machine.

Table 2 Primer sequence

Gene	Primer sequence	Reference
Interleukin-2	Forward 5'AAGAATCCCAAACTAACCAGGAT3'	[5, 6, 7]
Reverse	5'TCTAGACATGAAGATGTTTCAGTTCTC3`	
GAPDH	Forward 5'-GTCTCCTCTGACTTCAA-3'	[5, 6, 7]
	Reverse 5'ACCACCCTGTTGCTGTA-3`	

2.12 Measurement of hematocrit

The micro haematocrit method was used for measurement of haematocrit as previously described [35, 36]. Each capillary tube was filled with blood and the two ends of the tube were sealed with clay sealant. Centrifugation was carried out at a rate of 12000 revolution per minutes for 4 to 5 minutes. After centrifugation, the centrifuged tube was placed into the tube slot on the microhematocrit reader card and was adjusted until the 0% line on the card reader aligned with the interface of sealant and packed red cell. Then the percentage height of the packed red cell column was read.

2.13 Data analysis

SPSS software version 24 (SPSS Inc., Chicago, Illinois, USA) was used for data analysis. Results of descriptive statistics were presented as percentages and as mean ± standard deviation. Binary logistics regression was carried out to assess significant association of the sociodemographic variables with habitual alcohol consumption. Two-tailed independent t-tests was used to evaluate significant differences between means. In all cases, p value at <0.05 was considered statistically significant [37, 38].

3.0 Results

3.1 Prevalence of current alcohol consumption and different patterns of alcohol abuse

Figure 1, shows that a high prevalence of current drinking in the last 12 months was found. Also, as shown in Figure 1, a good proportion of the study population never drank alcohol in the past 12 months. Three major patterns of alcohol abuse was found. They were habitual alcohol consumption, binge drinking and alcohol dependence.

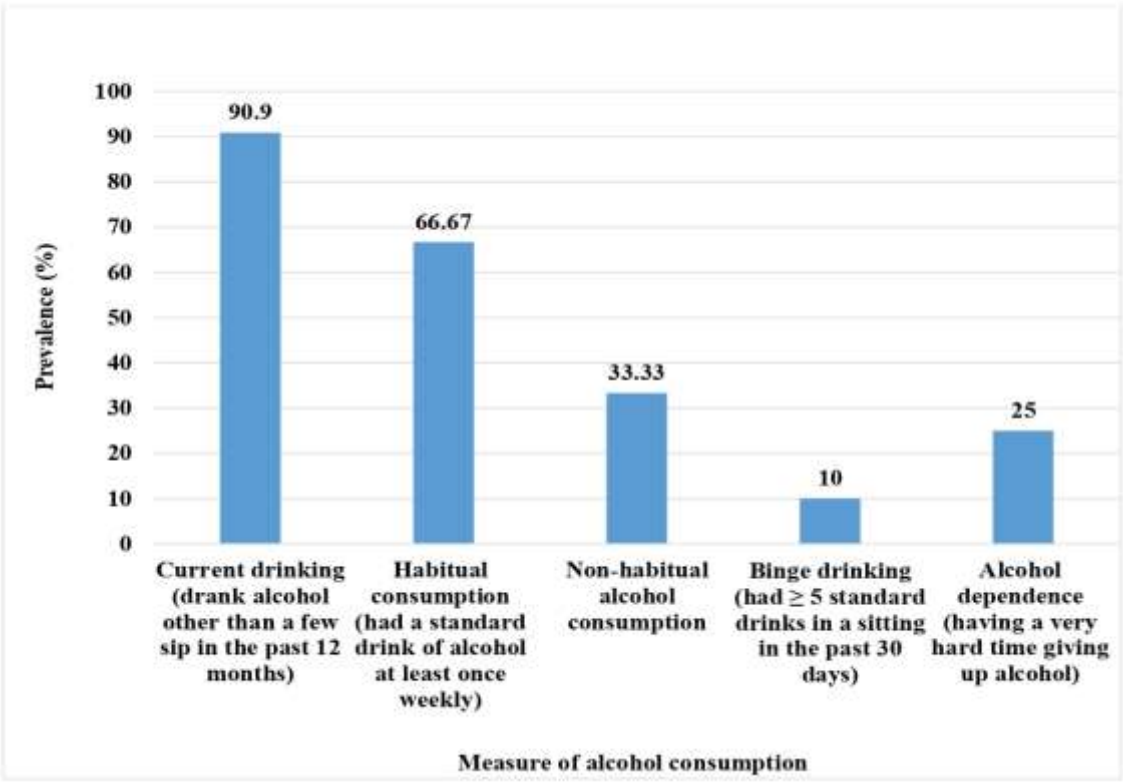

Figure 1 Prevalence of current drinking and different patterns of alcohol abuse among adults with non-communicable diseases in Bayelsa State, Niger Delta

3.2 Brands of alcohol consumed among habitual consumers with NCDs

As shown in Figure 2, spirit was the most consumed brand of alcohol among adults with NCDs, while fruit wine was the least.

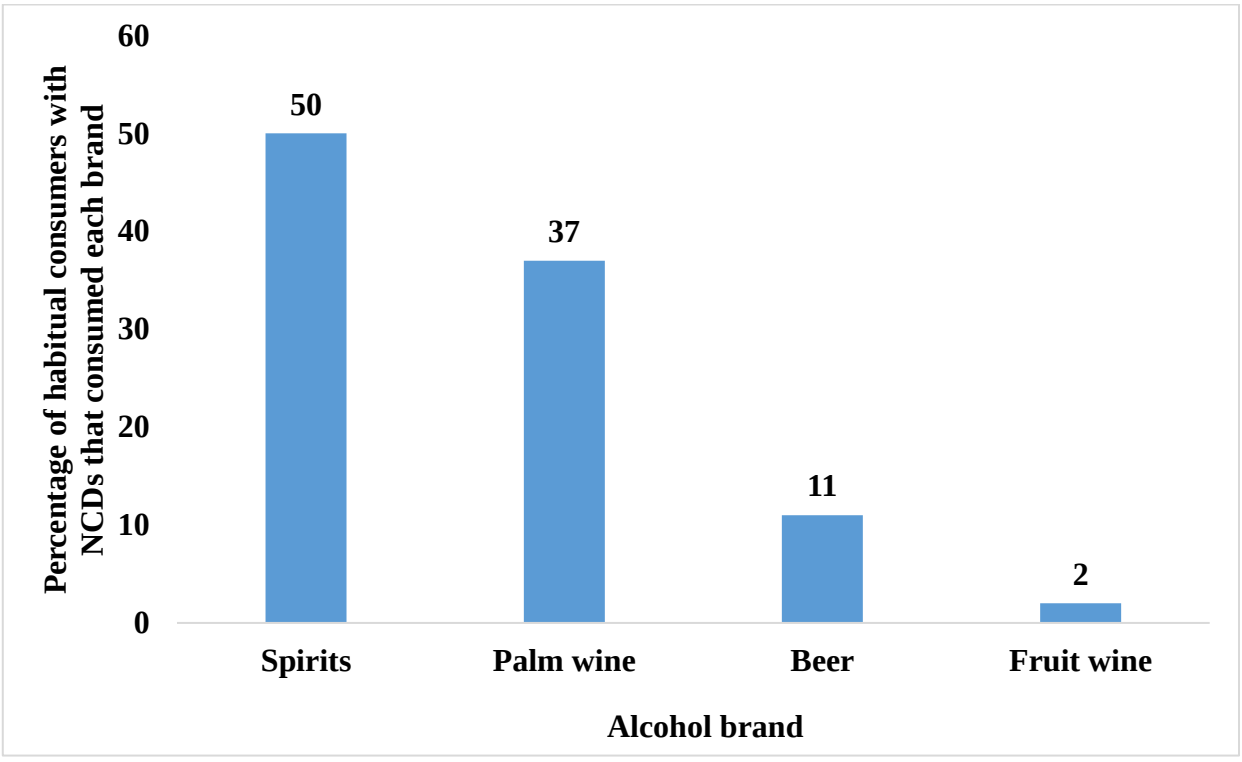

Figure 2. Brands of alcohol consumed by habitual consumers with NCDs

3.3 Sociodemographic variables associated with habitual alcohol consumption

As presented in Table 3, sex (p = .018), age (p = .017), drinking setting (p = .015) and affordability (p = .001) were associated with habitual alcohol consumption (HAC). Education (p = .974) and occupation (p < .319) were not associated with habitual alcohol consumption.

Table 3. Association of sociodemographic variables with habitual alcohol consumption

Sociodemographic	**Non-HAC**	**HAC**		
Variable	**N**	**N**	***P*-value**	**Odd ratio**
Sex			**.018**	**1.797**
Male	40	111		
Female	60	69		
Total	90	180		
Age (years)			**.017**	**.697**
18 – 25	18	34		
>25 – 35	43	57		
35 – 50	26	50		
>50	3	39		
Total	90	180		
Drinking setting			**.015**	**1.795**
Indoor	9	1		
Outdoor	12	3		
Both indoor & outdoor	69	176		
Total	90	180		
Occupation			**.319**	**1.092**
Farming / Fishing	31	90		
Civil service	23	11		
Crafts / SMEs	17	38		
Jobless	19	41		
Total	90	180		
Education			**.974**	**.995**
None	24	46		
Primary	40	76		
Secondary	17	40		
Tertiary	9	18		
Total	90	180		
Level of Affordability			**.001**	**1.500**
50 ml sachet of spirits (40% ABV)	42	90		

50 ml Palm wine		
(5% ABV)	14	67
600 ml of beer		
(5 – 8% ABV)	27	19
Red wine		
(10 – 12% ABV)	7	4
Total	90	180

ABV: Alcohol percentage by volume; SMEs: small medium enterprises; HAC: habitual alcohol consumption

3.4 Average and total standard drinks (alcohol units) consumed weekly among habitual consumers with NCDs

As shown in Table 4, the average and total standard drinks (number of alcohol unit) consumed weekly among habitual consumers with NCDs were 22.79±6.39 and 4102 respectively. Also as shown in Table 2, a significant difference in average standard drink consumed was found between males and females. Furthermore, Table 4 shows that the brand with the highest average and total standard drink consumed was spirit.

Table 4. Total and average alcohol units consumed weekly among habitual consumers with NCDs

Variable	**Total alcohol units/week**	**Average alcohol units per week**
Brand of alcohol		
Spirits	1924	25.01 ± 6.73^{b}
Palm wine	1676	21.38 ± 6.00^{a}
Beer	419	$22.05 \pm 5.56^{a, b}$
Red wine	83	$20.75 \pm 3.20^{a,b}$
Total	4102	22.79 ± 6.39
Sex		
Male	2624	23.64 ± 6.45^{a}
Female	1478	21.42 ± 6.10^{b}
Total	4102	22.79 ± 6.39

Values with different superscripts are significantly different.

3.5 Association of habitual alcohol consumption with hematocrit level

As shown in Figure 3, a significantly ($p < 0.001$) lower mean hematocrit (23.23 ± 5.90 %) was found among habitual consumers of alcohol with non-communicable disease compared with non-habitual consumers with non-communicable disease (44.58 ± 3.98%).

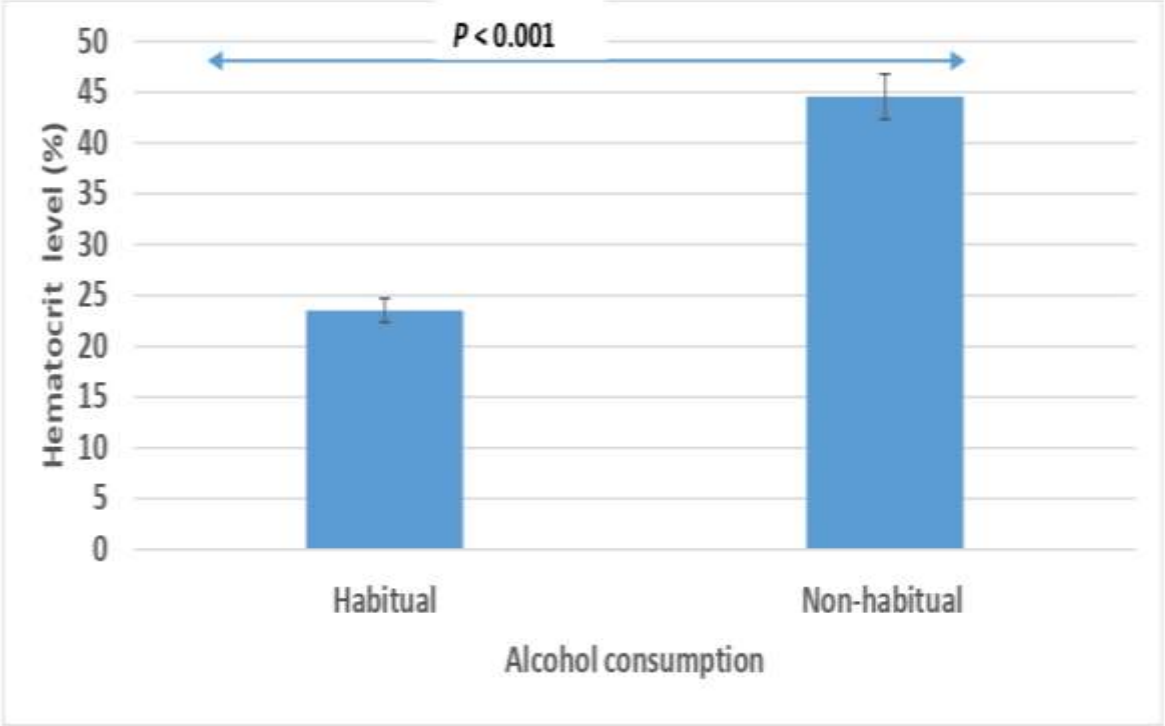

Figure 3 Relationship between habitual alcohol consumption and hematocrit level among individuals with non-communicable diseases in Bayelsa State, Nigeria. Results are presented as mean hematocrit level ± standard error of mean.

3.6 Gene expression of IL-2 among habitual consumers of alcohol with non-communicable diseases in Bayelsa State, Niger Delta

Figure 4 shows that interleukin-2 gene expression level was significantly higher ($P < 0.05$) among habitual consumers of alcohol with non-communicable diseases compared with non-habitual consumers with non-communicable diseases.

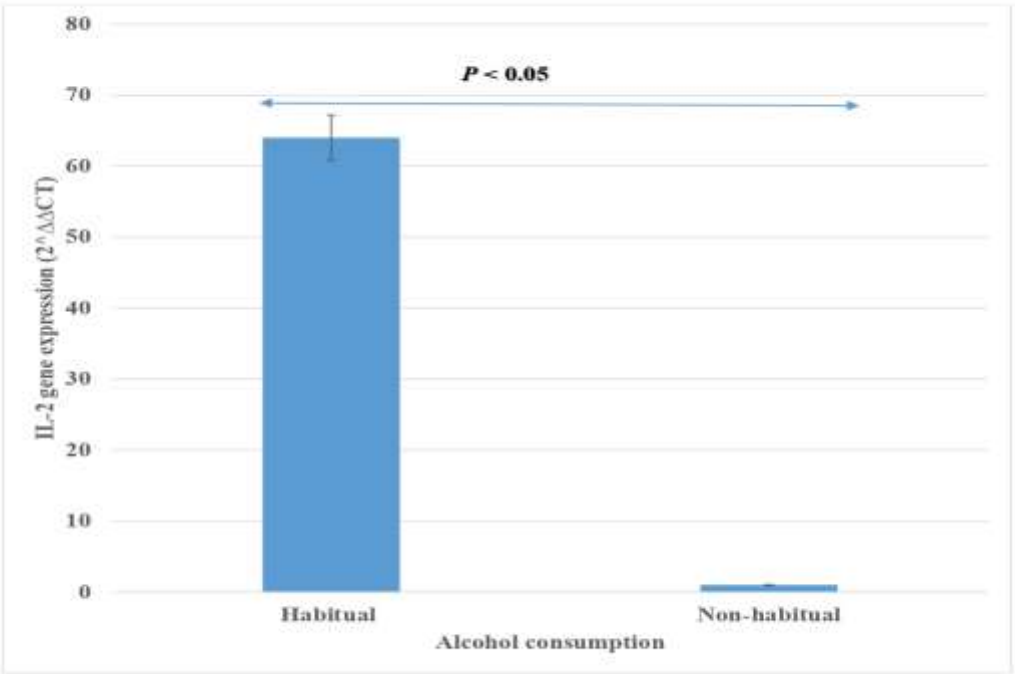

Figure 4 Differences in interleukin-2 gene expression between habitual and non-habitual alcohol consumers with NCDs

4. DISCUSSION

A high prevalence of current alcohol use (90.9%) was found among adults with NCDs in the present study, this was however lower than a prevalence of 100% found among bike riders in Edo State [22] and was lower than a prevalence of 29.3% found among pregnant women in Delta State [39]. This underscores the importance of studying alcohol consumption among subpopulations at health risk for alcohol consumption and calls for an urgent health promotion intervention with respect to alcohol abuse mitigation in Niger Delta, Nigeria.

Various patterns of alcohol abuse such as binge drinking, habitual alcohol consumption and alcohol dependence were found among adults with non-communicable diseases in the present study. A previous study carried out in the United States of America found that individuals that consume alcohol more than three times in a week had the highest prevalence of hypertension [40]. Given the limited number of studies on alcohol abuse among subpopulations of adults with non-communicable diseases in Nigeria, the present study has helped to fill a major gap in research. Further large scale study in Nigeria is recommended on alcohol abuse and individuals with non-communicable diseases in order to fully understand unique and novel methods of managing the disease.

In addition, the present study found that majority of habitual alcohol consumers with NCDs consumed distilled spirits. This brand of alcohol was also reported as the most consumed brand among inter-city commercial drivers in Edo State, Nigeria [22]. The present study also found that the availability of distilled spirits in 30 – 50 ml sachets at a cheap price increased affordability and consumption among the study population. Given the health risk associated with alcohol consumption [19, 30, 31], health promotion interventions to mitigate the habitual consumption of alcohol especially spirits packaged in low-cost sachets is highly recommended.

Also, the present study found that sex, age, drinking setting, and affordability were associated with alcohol abuse while education and occupation were not. This disagrees with the findings of a previous study carried out in Njoro-Kenya where no significant association between alcohol abuse and age was found [41], It also disagrees with another previous study carried out among a population of hospitalized patients in Ouagadougou (Burkina Faso) where education level was associated with alcohol consumption and age was not [42]. This shows that sociodemographic determinants of alcohol abuse are location specific. Thus, for future alcohol interventions, sociodemographic determinants of alcohol abuse in a given location should be taken into consideration.

The average standard drink (alcohol unit) consumed weekly by individuals with non-communicable diseases in the present study was very high. Although World Health Organization has stated that no amount of alcohol is safe for consumption [9], however, the National Health Service for England recommends the consumption of no more than 14 units (14 standard drinks) of alcohol per week for men and women and this amount has to be spread out over at least three days [19]. Although a previous study has evaluated the volume and concentration of alcoholic beverages offered for sale in Port Harcourt, Niger Delta, Nigeria [43], however research on the total standard drink (alcohol unit) consumed weekly by adults with non-communicable diseases in Niger Delta on a weekly basis has not been carried out. Thus the present study is novel and has helped to fill a major gap in research. Given the limited study population in the present study, further large scale study is highly recommended.

Reduced hematocrit level was found in the present study among habitual consumers of alcohol with non-communicable diseases. Low hematocrit has been previously reported among habitual consumers of alcohol in Japan [44]. Low hematocrit has also been reported among individuals with non-communicable diseases especially diabetes [45]. However the co-occurrence of these two risk factors and their synergistic effect on hematocrit has not been carried out. Thus health promotion interventions among adults with non-communicable diseases on the effect of alcohol on hematocrit is highly recommended.

In addition, the present study found a significantly elevated inflammation level among habitual consumers with NCDs compared with non-habitual consumers. In the present study, inflammation was measured in terms of IL-2 gene expression. Elevation of pro-inflammatory cytokines such as interleukin-2 and interferon gamma among individuals with communicable and non-communicable diseases have been previously reported [5, 6, 7, 35, 46]. However, the gene expression profile of IL-2 among habitual consumers of alcohol with non-communicable diseases is sparse in literature. Thus a major research gap has been filled by the present study. A previous study showed that elevated inflammation not controlled can results in death among persons with non-communicable diseases [47], however, further study is recommended to unravel the link between IL-2, habitual alcohol consumption and non-communicable diseases.

Although several plant herbs have been studied for their effectiveness in the treatment and management of non-communicable diseases [48, 49, 50], however, as observed among non-habitual consumers in the present study, avoiding habitual alcohol consumption may help in regulating the metabolic changes caused by habitual alcohol consumption among adults with non-communicable diseases.

5. CONCLUSION

The present study revealed a high prevalence of current alcohol consumption, habitual alcohol consumption, binge drinking and alcohol dependence among adults with non-communicable diseases in selected communities in Niger Delta. It also showed the occurrence of alterations in hematocrit and IL-2 gene expression levels among habitual alcohol consumers with non-communicable diseases. Furthermore, the present study revealed that age, sex, drinking settings and affordability were significant predictors of habitual alcohol consumption among NCDs individuals. It is hoped that findings from the present study will have implication for the management of non-communicable diseases among individuals with alcohol use disorder.

Conflict of interest

The authors declare no conflict of interest.

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