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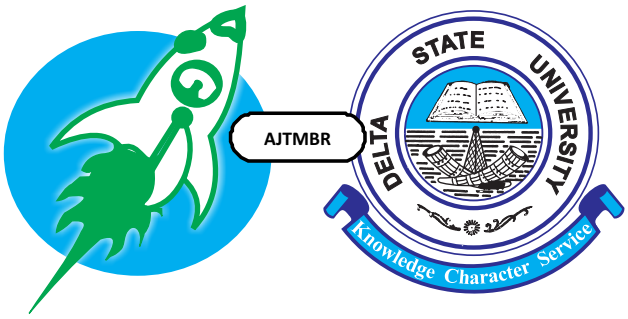

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The African Journal of Tropical Medicine and Biomedical Research is a multidisciplinary and international journal published by the College of Health Sciences, Delta State University of Abraka, Nigeria. It provides a forum for Authors working in Africa to share their research findings on all aspects of Tropical Medicine and Biomedical Sciences and to disseminate innovative, relevant and useful information on tropical medicine and biomedical sciences throughout the continent. The journal will publish original research articles, reviews, editorials, commentaries, short reports, case reports and letters to the editor. Articles are welcome in all branches of medicine and dentistry including basic sciences (Anatomy, Biochemistry, Physiology, Pharmacology, Psychology, Nursing etc) and clinical sciences (Internal Medicine, Surgery, Obstetrics and Gynaecology, Dental surgery, Child Health, Laboratory Sciences, Radiology, Community Medicine, etc). Articles are also welcome from social science researchers that document the intermediating and background social factors influencing health in countries of Africa. Priority will be given to publication of articles that describe the application of the principles of primary health care in the prevention and treatment of diseases.

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Table of Contents

Effect of Occupational exposure to Gasoline on Reproductive and Thyroid hormones among male Petrol station attendants in Kwara State

Adunmo Godwin O.; Seyi Taiwo; Ibrahim Muniru; Busari, A. O.

Abstract

Introduction: Petrol fume contain organic compounds that have been suggested to be endocrine disruptors. There is paucity of data on petrol fume-linked health challenges in Nigeria. Thus, we evaluated the effects of petrol fume exposure on blood levels of reproductive and thyroid hormones among petrol pump station attendants in Kwara state, Nigeria.

Material and Methods: Eighty (80) petrol station attendants and fifty (50) non-occupationally exposed subjects were recruited as controls. Anthropometric indices were taken and fasting blood samples were collected after signing the consent form. Free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), testosterone, follicle stimulating hormone and luteinizing hormone were evaluated using standard techniques.

Results: Average age and BMI of petrol fume-exposed subjects are 27.40±0.419 and 24.98±0.254 respectively and was not different from controls. Elevated FT3 and FT4 were evaluated with a decreased TSH level in the exposed group when compared with controls. In contrast, FSH, LH and testosterone were significantly reduced in exposed group when compared with control.

Conclusion: The result of this study shows that petrol fume toxicants adversely alter thyroid and gonadal functions thereby modulating circulating levels of reproductive and thyroid hormones in male petrol station attendants in Kwara state.

Keywords: Petrol fumes, thyroid hormones; reproductive hormones, infertility.

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Introduction

Global health concern has been raised on the account of continuous unintentional exposure of humans to toxic derivatives of petroleum in the recent years[1]. These exposures have been indicated to engender hormonal imbalance associated with several disorders[2,3]. Hydrocarbon components of petroleum products are of economic importance, but some are toxic and have been implicated in a number of diseases and environmental degradations[4,5]. In addition, the impacts of exposure are more felt in some occupations and work environments than others[6].

Previous report shows more than 10% of Nigeria's population is exposed to hydrocarbon products from petroleum[7]. It is therefore apparent that continuous unintentional inhalation of volatile organic compounds in petroleum products especially by petrol station attendants is

a major occupational health hazard across the country. Unfortunately, most of these attendants neither use protective devices to prevent inhalation of compounds in petroleum products; nor do they visit the hospital regularly for routine medical check-up while many have been on this job for an extended period of time[8].

Gasoline (petrol) is a popular derivative of petroleum used for automobiles, industrial engines and home generators in many developing countries including Nigeria. It is a complex mixture containing about 62% alkanes, 7% alkenes and 31% aromatics, alcohols, ethers and additives[9]. Aliphatic and aromatic hydrocarbons, such as benzene, toluene, ethylbenzene and xylene (BTEX) are released as vapors from gasoline during the processes of loading, offloading or fueling, and are considered among the most hazardous compounds for human health[10]. Gasoline fumes are widespread in the environment with the common sources of contact or exposure well known to be petrochemical industries (refineries, oil fields and filling stations), engineering workshops and homes. Inhalation of petrol vapors was shown to impair liver and renal function[11,12], alter hematopoiesis[13] in gasoline station attendants. Benzene and xylene have been more associated with defects due to oxidative stress, alterations in DNA repair and expression of oncogenes [14,15].

Infertility remains a major global health problem with multifactoral causes. Disorders of sexual function are underlined by endocrine/hormonal issues and have been correlated with exposures to toxic environmental chemicals. Importantly, spermatogenesis is known to be more sensitive to environmental toxicants[16]. Studies documented that occupational exposure to gasoline decrease blood levels of reproductive hormones with attendant impairments in the gonadal function[2,17]. The effects of occupational exposure to petrol on thyroid function are not fully clear. Previous studies observed fluctuations in thyroid hormone levels upon exposures to petroleum-derived fuels[18,19]. Thyroid hormones imbalance has been previously linked with testicular dysfunction and infertility in males[20]. In this study, we investigated the effects of occupational exposure to gasoline on serum levels of reproductive and thyroid hormones among male petrol station attendants in Kwara state.

Study population

A total of eighty (80) adult male subjects with not less than four(4) years petroleum exposures were recruited from Ilorin and Offa; Kwara State, Nigeria. Fifty (50) non-exposed adult males were also recruited from the same metropolitan area to serve as study control.

Exclusion criteria

Female subjects among petroleum station's attendants were excluded from the study. Study participants were exclusively adult males that satisfy the inclusion criteria. Only adult male subjects who have worked for 5 years and above are considered.

Data collection

Information on demographic factors, anthropometric details and life style factors of each study participants were derived through an accurately structured questionnaire. Other information about personal data such as smoking habits, alcoholism and other addictions were enquired for and noted.

Height: This parameter was measured against a flat vertical surface with the subject standing without raising the heel from the ground. The feet were brought together without shoes aligned with a ruled bar against a vertical surface. The reading

was obtained and recorded to the nearest metre (m).

Weight: The weighing device is placed on a parallel surface. It was used to take measurement of each study participants. The subjects were made to stand on the scale with shoe off and the reading was obtained and recorded to the nearest kilogram (kg).
Body Mass Index (BMI)
This was derived from the expression; BMI= Weight (kg)/Height (m).

Statistical analysis
Statistical analysis of the data was done using SPSS version 17 software (SPSS inc, Chicago, IL, USA). The comparison of the mean of the two groups was done using student's *t*- test. Analysis of variance (ANOVA) two-tailed was used when the means of more than three groups were to be compared. Honestly significant difference turkey test was used in conjunction with ANOVA to find means that were significantly different from each other.

Collection of blood samples
The blood sample was obtained from the study participants during the day light without any special requirement for collection. No fasting episode is required throughout the sample collection.

The venepuncture was performed on the cubital fossa with tourniquet applied on the upper arm for easy access to the vein. 5.0ml of blood sample was collected following aseptic procedure from each participant and dispensed into Lithium heparin bottles. The blood obtained was thereafter spun at 3,000xg for 10minutes. The plasma was separated and refrigerated at -20oC. Analysis of biochemical parameters was done in the laboratory.

Biochemical Assays

Determination of Serum FSH, LH, Testosterone, TSH, T3 and T4
The determination of FSH, LH, Testosterone, TSH, T3 and T4 in the serum was conducted using enzyme-linked immunosorbent assay (ELISA) principles. Hormone concentrations were measured spectrophotometrically at 450 nm.

Results
Demographic characteristics
Average age and BMI of petrol fume-exposed subjects are 27.40±0.419 and 24.98±0.254 respectively (compared with 25.96±0.328 and 24.15±0.192 in non-exposed controls)

Effect of petrol exposure on reproductive hormones
Serum Follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone level were all significantly reduced in exposed subject when compared with the control.

Effect of petrol exposure on the thyroid hormones
Means of FT3 and FT4 were significantly elevated in subject exposed petrol when compared with the control. However, thyroid stimulating hormone (TSH) was significantly reduced in exposed subject when compared with the control.

Discussion
Endocrine glands regulate physiological processes through release of hormone substances, while any impairment in synthesis, transport and/or bioavailability of the hormones could lead to hormonal imbalance and systemic disorders[21,22]. Petrol fume contain organic compounds such as BTEX and heavy metals that act as endocrine disruptors rendering cyto- and hemo-toxic effects[8]. This study provides data on

the effect of occupational exposure to gasoline fume on serum levels of reproductive and thyroid hormones in male petrol station attendants in Kwara state. Average age and BMI of petrol fume-exposed subjects are 27.40±0.419 and 24.98±0.254 respectively (compared with 25.96±0.328 and 24.15±0.192 in non-exposed controls) (Table 4.1). This shows that healthy weight, young male subjects were recruited and there is no significant difference in BMI of petrol station attendants compared with controls.

Our results further revealed that occupational exposure to petrol fume caused plausible decreases in serum levels of follicle stimulating hormone and testosterone with slight reduction in luteinizing hormone level when compared with control (Table 4.2). This suggest that inhalation of organic constituents in petrol fume may have suppressing effect on the hypothalamic-anterior pituitary-testicular axis, by altering hypothalamic production of gonadotropin releasing hormone, which in turn could alter synthesis or release of FSH, LH and testosterone. Previous study in line with our observation reported significant attenuations in males reproductive hormones (FSH, LSH and testosterone) in petrol station attendants in Edo state, Nigeria[6]. Male infertility is a common public health challenge in Nigeria and exposures to environmental toxicants may mediate oxidative stress in Leydig cells causing impaired steroidogenesis and hypotestosteronemia[23]. Exposure to petrol fumes in rats has been implicated at the level of the gonads to induce testicular dysfunction and reduce sperm parameters with oxidative stress and decreased antioxidant enzymes as key pathogenic mechanisms[17].

Thyroid pathophysiology is a growing area of interest in evaluating toxicological effects due to the involvement of thyroid-secreting hormones in key processes including metabolism, growth and development. The thyroid gland produces thyroxine (T4), which is a relatively an inactive prohormone, while the highly active hormone is triiodothyronine (T3). There are reports that toxic chemicals can disrupt thyroid hormone balance directly or indirectly through various mechanisms. From our data, there was elevation in the levels of both T3 and T4 with a reduced TSH level in petrol fume-exposed subjects when compared with control (Table 4.3). Meludu *et al* shows that higher serum T3 (active thyroid hormone) but not T4 levels was accompanied with a decrease in TSH level in gasoline exposed workers in Anambra, South-East Nigeria[19]. Conversely, a study investigated the thyroid effect of diesel petroleum intoxication experimentally and found significant attenuations in serum thyroid hormones levels with increased TSH level[18]. Evidences show that thyroid hormones regulate male reproductive function from developmental stage up till spermatogenesis, erectile and ejaculatory processes. Interestingly, both hypothyroidism and hyperthyroidism have been implicated in the impairment of testicular functions and worsened semen quality[24,25,26]. The scope of this study did not investigate whether the reductions in male reproductive hormones in petrol station attendants are due to fluctuating thyroid hormone concentration. Nevertheless, exposure to endocrine disruptors mediates their deleterious systemic effects through common pathways of oxidative stress and lipid peroxidation[27,28] that must have caused both reproductive and thyroid hormones imbalance in this study.

In conclusion, findings from this study validates that inhalation of gasoline fumes cause disruptions in reproductive and thyroid hormones systems, providing data on

occupational exposure of male petrol station attendants in Kwara state, Nigeria. Prolonged exposure in these subjects would cause alterations in gonadal functions and exacerbate hormonal imbalance leading to infertility. It is therefore recommended that petrol station attendants especially males, should adhere to the use of protective masks to prevent inhalation of gasoline fume and a medical checkup routine should be adopted by workers at gasoline stations.

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Production of L-lysine under submerged fermentation by *Corynebacterium glutamicum* using different agricultural plants leaves

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ABSTRACT

Introduction: This study investigated the production of L-lysine using *Corynebacterium glutamicum* and various leaf extracts (cassava, palm tree, maize, cowpea, cocoyam, and plantain). The study also explored the activities of amylases and proteases, as well as the levels of total soluble proteins, reducing sugars, glucose, flavonoid and phenolic contents, and pH changes.

Materials and Methods: Different treatments (extract, boiled extract, extract + *C. glutamicum*, boiled extract + *C. glutamicum*) were examined for their effects on L-lysine concentration. Additionally, the activities of amylases and proteases, as well as levels of total soluble proteins, reducing sugars, glucose, flavonoid and phenolic contents, and pH changes, were analyzed.

Results: Maize leaf extract + *C. glutamicum* exhibited the highest L-lysine concentration (1.771a±0.1 mg/g), while boiled cassava leaf extract showed the lowest concentration (0.023b±0.1 mg/g). Palm tree leaf extract had significantly higher reducing sugar levels compared to other extracts. Boiled plantain leaf extract fermented by *C. glutamicum* had the highest total soluble protein level (9.5±0.2 mg/g), while cassava leaf extract had the lowest (2.1±1.2 mg/g).

Conclusion: Submerged fermentation of leaf extracts using *C. glutamicum* can be utilized for L-lysine production. The study highlights the influence of different leaf extracts and treatments on L-lysine production, as well as on amylase and protease activities, total soluble protein levels, reducing sugars, glucose, flavonoid and phenolic contents, and pH values. These findings provide valuable insights into the potential application of this approach for lysine production.

Key words: Agricultural leaves, submerged fermentation, *Corynebacterium glutamicum,* extract, boiling

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Introduction

Agricultural products are currently being used as low-cost carbohydrate source for the production of high value-added products such as amino acids[1-3].Amino acids are very important macromolecules required for both human and animal to function properly. L-Lysine is one of the nine essential amino acids required for human and animal nutrition. Studies have shown that the demand for L-lysine has been steadily increasing[4], with several hundred thousand tonnes produced annually[1,5]. Being an essential amino acid, its addition to foods and feeds improves protein quality resulting to better growth and tissue synthesis[5]. Different methods utilized in the production of lysine include chemical synthesis, fermentation, enzymatic method, and protein hydrolyzate extraction[6]. Of

these methods, fermentation remains the most practical and economical approach for the production of lysine as it requires low-cost carbon sources, low pressure and even moderate temperatures[7].

Corynebacterium glutamicum is majorly used for the production of amino acids such as L-glutamate and L-lysine industrially[8]. *C. glutamicum* is capable of utilizing different carbohydrates, organic acids and alcohols as sources of carbon and energy required for growth and production of amino acids[9-10]. Various organic and inorganic salts and compounds such as urea, ammonium salts and yeast extract can be utilized as nitrogen sources[11].

The use of agricultural plants or products as substrates for fermentative production of amino acids might provide cheap alternative for the manufacturing of lysine. Large-scale production of lysine using leaf extracts has not been reported in literature. The present study investigates the application of leaf extracts of tropical agricultural plants (cassava leaves, palm tree leaves, maize leaves, cowpea leaves, plantain leaves and cocoyam leaves) as alternative substrates for the production of L-lysine by *C. glutamicum*.

MATERIALS AND METHODS

Collection of materials

Leaves of cassava, palm tree, maize, cowpea, plantain, and cocoyam were harvested, identified, and authenticated by Plant Taxonomy and Molecular Systematics, Department of Botany, Delta State University, Abraka (Voucher numbers DELSUH 047, 132, 117, 068, 064, respectively). They were blended with water (1:10) using a commercial grinding machine and stored at room temperature.

Microorganism and preparation of inoculum for submerged fermentation

Corynebacterium glutamicum was obtained from Tonukari Biotechnology Laboratory in Delta State, Nigeria, and stored on glycerol at 4°C. For inoculation, a loop full of microorganisms was transferred to an inoculum media containing glucose, yeast extract, tryptone, NaCl, agar, and distilled water at pH 7. Homogenization was performed using 10 g of the pulverized sample. A 2 ml culture was used to inoculate a 100 ml Erlenmeyer flask with 20 ml fermentation broth. After a 72-hour incubation period at 30°C and 160 rpm on a rotary shaker, growth and lysine accumulation were assessed. Control flasks were not inoculated. Each extract underwent four different treatments: extract, boiled extract, extract + *C. glutamicum*, and boiled extract + *C. glutamicum*, with boiling lasting for 30 minutes. The reported values represent the average of at least two congruent samples.

Estimation of L-lysine and other biochemical parameters

L-lysine concentration was estimated using the acid ninhydrin method of Chinard[12]. Total soluble proteins were determined following the procedure by Gornall *et al.*[13] with bovine serum albumin as the standard. Reducing sugars were estimated using the 3,5-dinitrosalicylic acid (DNS) colorimetric technique[14]. Glucose content was determined using the Randox glucose kit as per the manufacturer's instructions. α-amylase activities were determined based on the procedure of Nouadri *et al.*[15], while protease (caseinolytic) activity was assayed using the method of Kunitz[16]. Total flavonoid and phenolic contents were determined using colorimetry methods described by Jia *et al.*[17] and Singleton and Rossi[18], respectively. pH measurements were taken using a Mettler Toledo pH meter.

Statistical analysis

All data were subjected to statistical analysis. Values were reported as mean ± standard deviation and the experimental results were analyzed using analysis of variance (ANOVA). The results were considered significant at p-values of less than 0.05 (95% confidence level; $p<0.05$).

Results

The results of the study on the determination of L-lysine concentration in submerged fermented agricultural by-products using *C. glutamicum* are summarized in Figure 1. Maize leaf extract + *C. glutamicum* showed the highest lysine production (1.771±0.1 mg/g), while boiled cassava leaves exhibited the lowest concentration (0.023±0.1 mg/g).

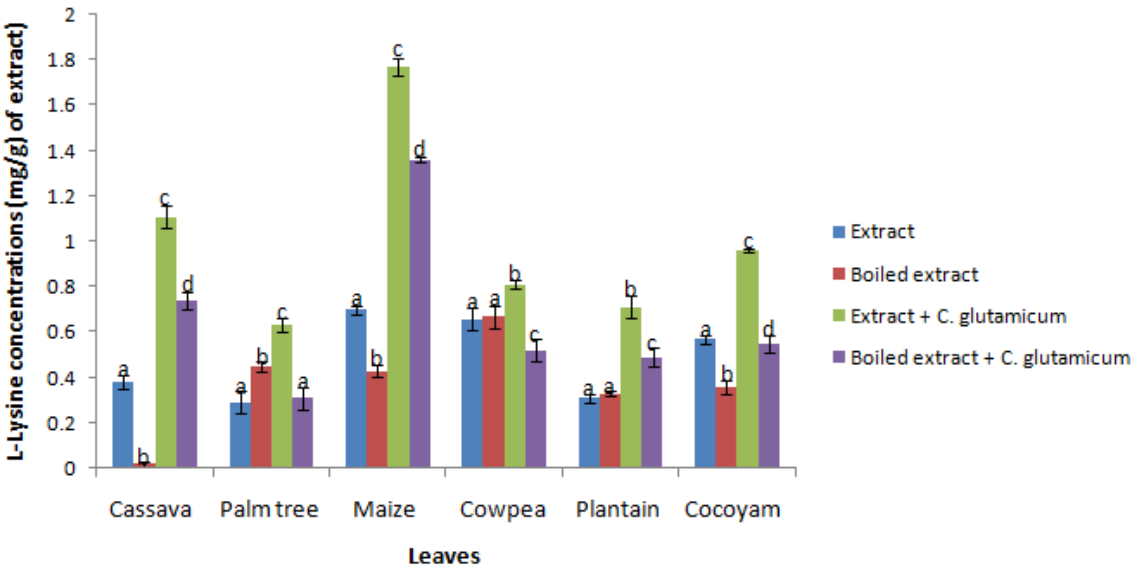

Figure 1. L-Lysine concentrations in leaves extracts fermented by *C. glutamicum*. Bars with different superscript differs significantly at ($p<0.05$).

Figures 2, 3, and 4 present the levels of total soluble proteins, reducing sugars, and glucose, respectively. Palm tree leaves showed a significant increase in reducing sugar content, while plantain leaves exhibited a significant decrease in all test groups compared to the extract. Palm tree leaves boiled before introducing C. glutamicum had the highest level of total soluble proteins (9.5±0.2 mg/g), whereas cassava leaf extract had the lowest (2.1±1.2 mg/g). Glucose levels significantly increased in all test groups compared to the control.

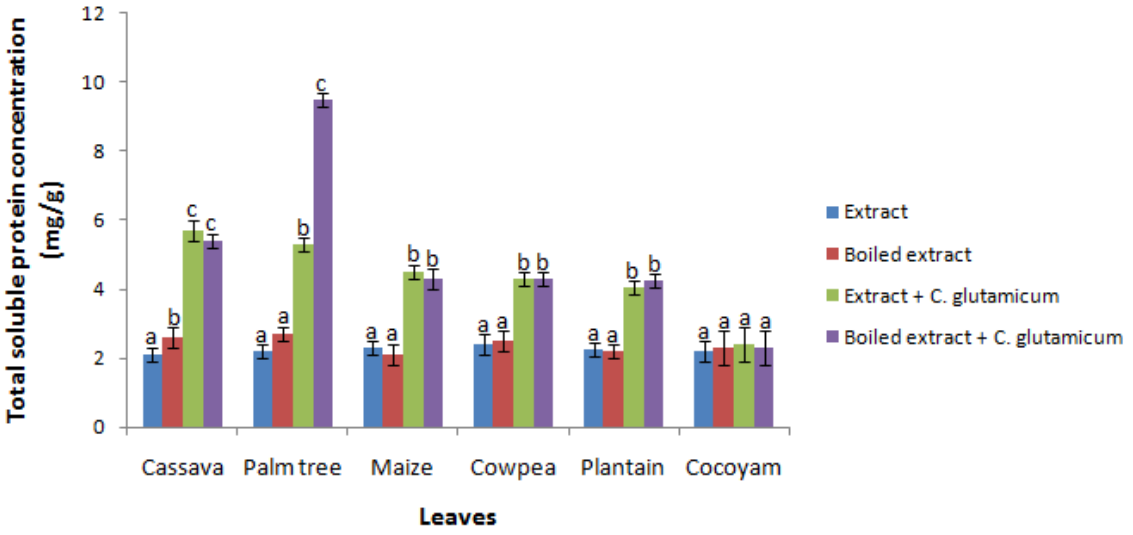

Figure 2. Total soluble protein concentrations in leaves extracts fermented by *C. glutamicum*. Bars with different superscript differs significantly at ($p<0.05$).

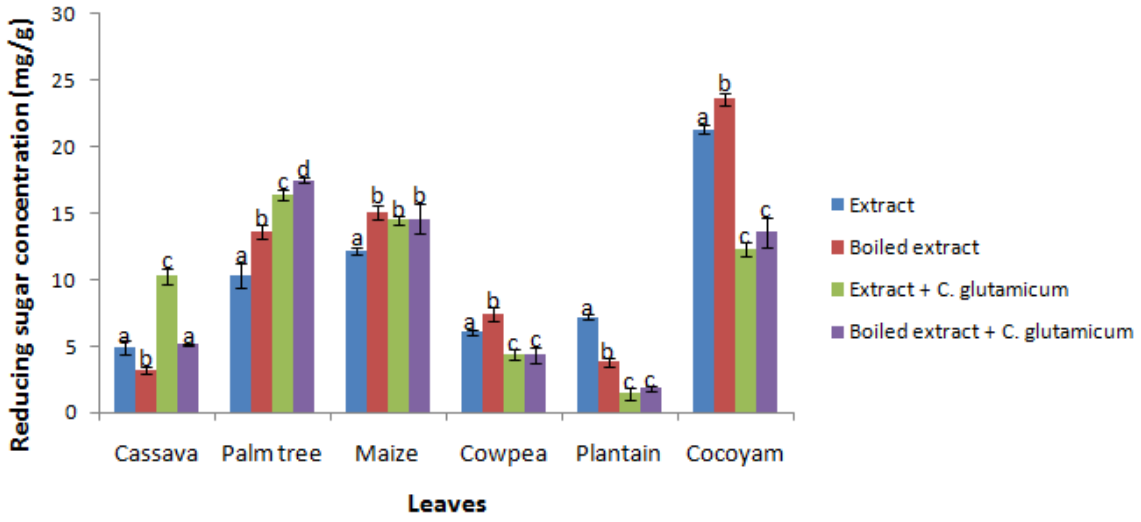

Figure 3. Reducing sugar concentrations in leaves extracts fermented by *C. glutamicum*. Bars with different superscript differs significantly at ($p<0.05$).

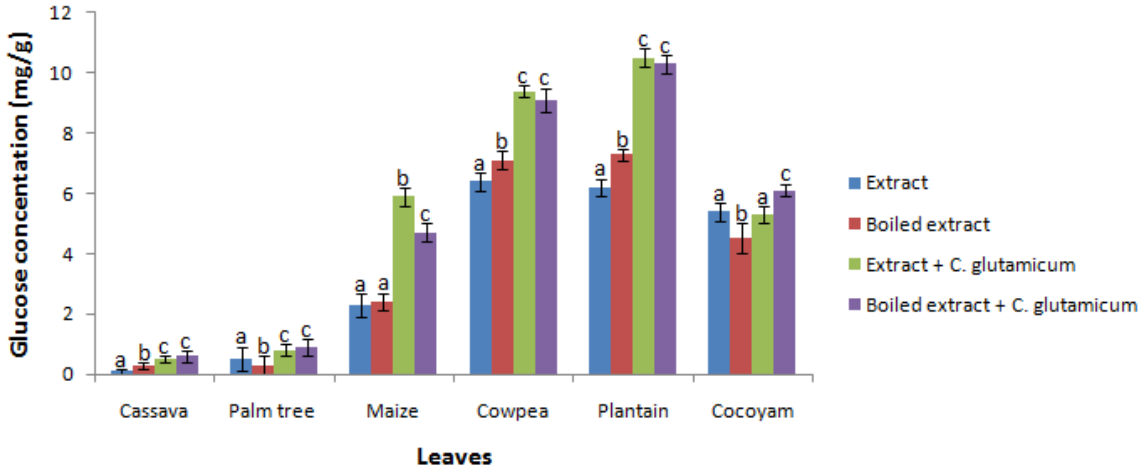

Figure 4. Glucose concentrations (mg/g) in leaves extracts fermented by *C. glutamicum*. Bars with different superscript differs significantly at ($p<0.05$).

Figures 5 and 6 illustrate the effect of submerged fermentation using C. glutamicum on amylase and protease activities, respectively. Cassava leaves boiled in the presence of C. glutamicum showed the highest amylase activity, while the combination of extract and C. glutamicum exhibited the highest protease activity. Beans extract and boiled plantain leaves had the lowest amylase and protease activities, respectively.

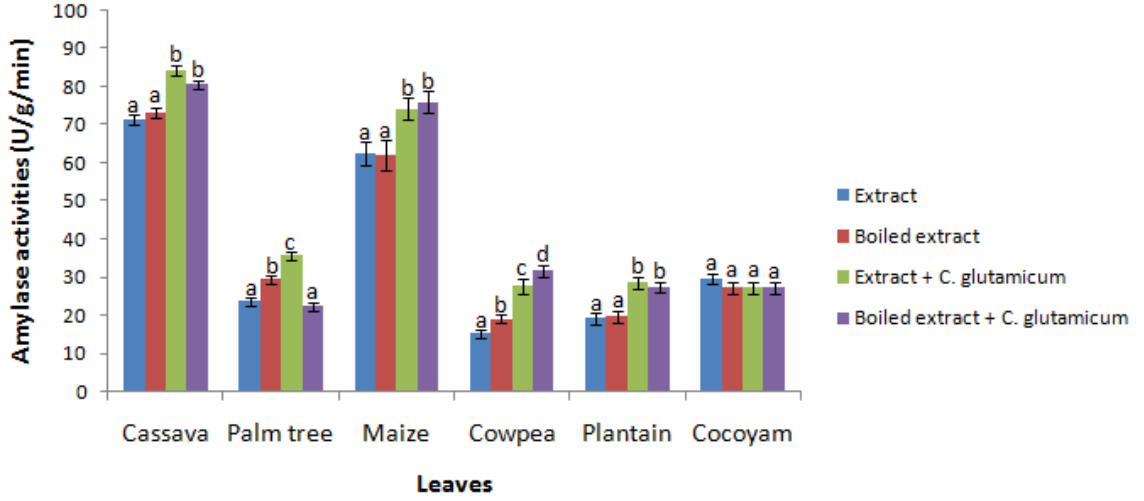

Figure 5. Amylase activities (U/g/min) in leaves extracts fermented by *C. glutamicum*. Bars with different superscript differs significantly at ($p<0.05$).

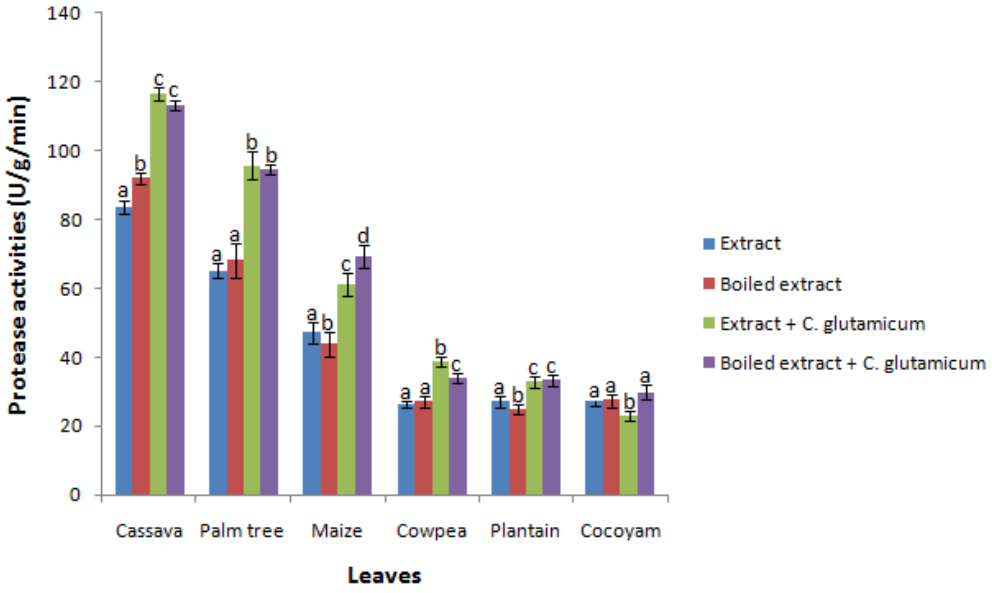

Figure 6. Protease activities (U/g/min) in leaves extracts fermented by *C. glutamicum*. Bars with different superscript differs significantly at ($p<0.05$).

The effects of the experimental treatment on total flavonoid and phenolic contents are shown in Figures 7 and 8, respectively. All test groups for the agricultural products displayed a significant increase in total flavonoid content.

The total phenolic content in palm tree leaves varied among the treatment groups (extract, boiled extract, extract + C. glutamicum, boiled extract + C. glutamicum) with values of 17.3±2.1, 19.5±2.1, 34.3±1.9, and 37.5±1.5 µg/ml, respectively (Figure 8).

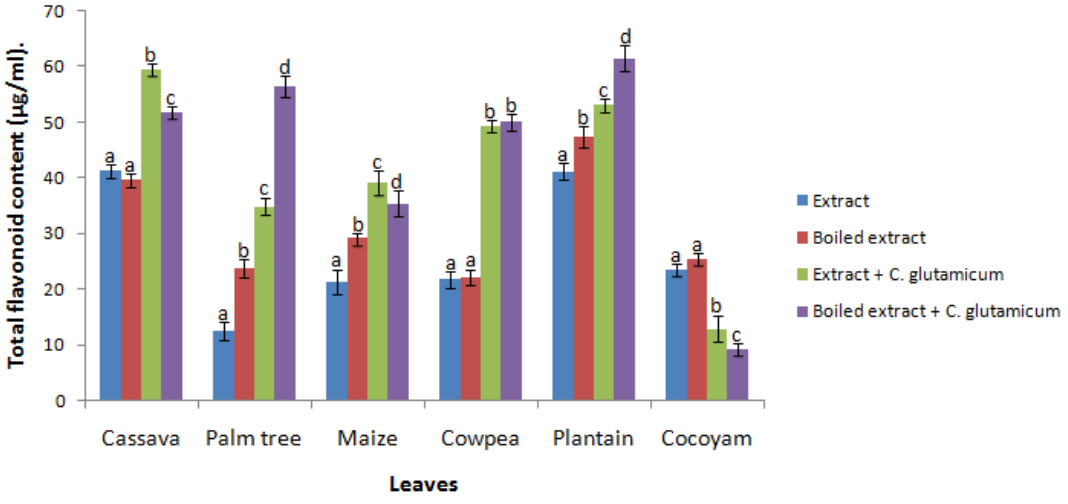

Figure 7. Total flavonoid content (TFC) (µg/ml) in leaves extracts fermented by *C. glutamicum*. Bars with different superscript differs significantly at ($p<0.05$).

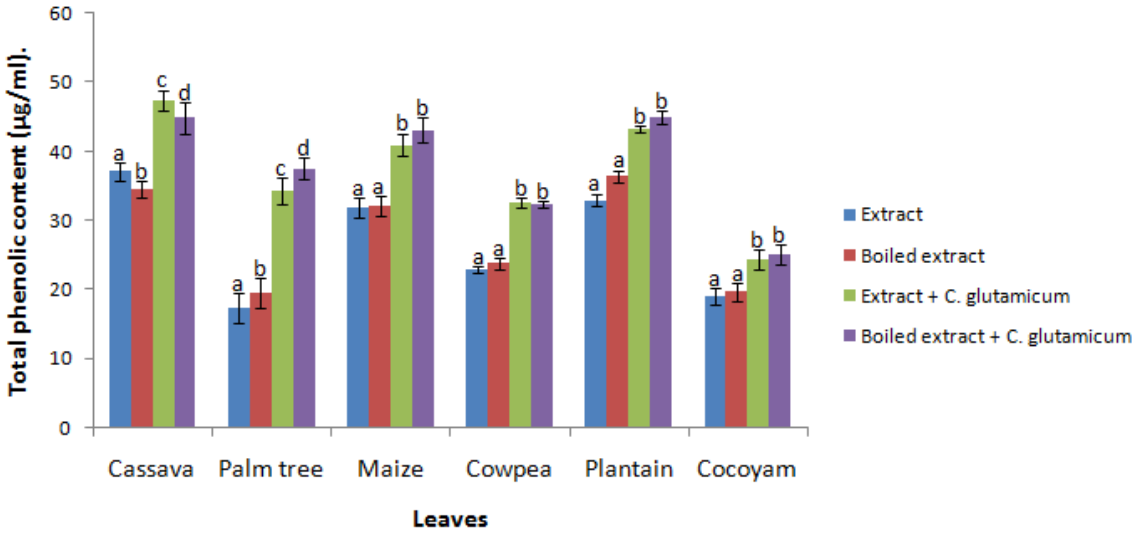

Figure 8. Total phenolic content (TFC) (µg/ml) in leaves extracts fermented by *C. glutamicum*. Bars with different superscript differs significantly at ($p<0.05$).

Figure 9 demonstrates that the treatment had no significant effect on pH, except for maize, plantain, and cocoyam leaf extracts + C. glutamicum, and maize leaf boiled extract + C. glutamicum.

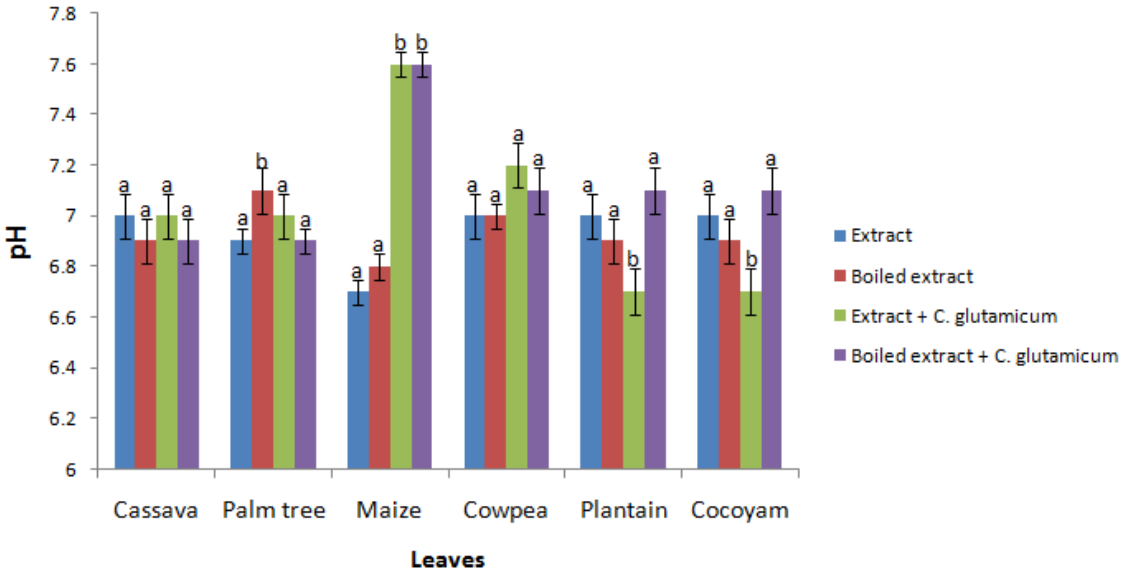

Figure 9. pH values in leaves extracts fermented by *C. glutamicum*. The leaf extracts were made by grinding 1g of leaf in 10 ml water. Bars with different superscript differs significantly at ($p<0.05$).

Discussion

The study revealed variations in lysine concentration among different treatments. Maize extract combined with *C. glutamicum* exhibited the highest lysine concentration, consistent with previous reports by Teniola [19] and Cui *et al.* [20], highlighting the potential of fermentation to enhance lysine levels. Ezemba *et al.* [21] also observed a significant increase in lysine yield through submerged fermentation. *Corynebacterium*, *Arthrobacter*, *Bacillus*, and *Brevi bacterium* are among the yielding strains used for fermentation, as reported by Ekwealor and Obeta [7]. Boiling agricultural products, with or without *C. glutamicum*, significantly reduced lysine concentration, as shown in the current study. Amaechi and Oluagha [22] and Hurrell and Finot [23] emphasized the susceptibility of lysine to damage during processing, and boiling has been shown to reduce its levels. Lysine, an essential amino acid, inhibits viral growth, aids in calcium absorption, reduces serum triglyceride levels, and contributes to the production of hormones, antibodies, and enzymes [24-25].

The study revealed variations in the levels of total soluble protein among different agricultural leaves and treatments. The highest level of total soluble protein was observed in palm tree leaves boiled in the presence of *C. glutamicum*, consistent with the findings of Zhu *et al.* [26], who reported increased proteinase activity during fermentation. Similar increases in protein levels have been reported by Ezekiel *et al.* [27] for cassava peels fermented with Trichoderma viride and by Egbune *et al.* [28] for fermented Pearl millet using *Rhizopus oligosporus*. Amaechi and Oluagha [22] highlighted the protein-increasing effect of boiling, which may contribute to the observed results. Specific *C. glutamicum* strains can secrete hydrolytic enzymes and utilize lipids and starch to produce proteins, as reported by Ezedom *et al.* [29], Vijayaraghavan *et al.* [30], and Ray *et al.* [31].

In this study, the levels of reducing sugar varied among the different agricultural by-products and treatments. Cocoyam leaf, beans, and maize exhibited the highest levels of reducing sugar after boiling. Wei *et al.* [32] demonstrated that cooking can increase total sugar content, particularly reducing sugars, with variations depending on cultivars and cooking methods. The study also observed a significant reduction in reducing sugar levels in plantain leaves, palm tree leaves, and cassava leaves compared to the control. This reduction could be attributed to the loss of components in hot moist conditions and other factors such as cooking temperature, time, and species differences, as reported by Lai *et al.* [33] and Bian and Liu [34]. Lu *et al.* [35] attributed the decrease in reducing sugars in sweet potatoes to the loss of amylase activity.

The levels of glucose were significantly increased in all agricultural leaves treated with extract + *C. glutamicum* and boiled extract + *C. glutamicum*. This increase can be attributed to the fermentative ability of *C. glutamicum*. Nkhata *et al.* [36] and Osman [37] demonstrated that fermentation activates starch-hydrolyzing enzymes like α-amylase and maltase, leading to the degradation of starch into malto-dextrins and simple sugars. El-Hag *et al.* [38] also reported an increase in glucose levels during the early stages of fermentation. Anigboro *et al.* [39] observed an increase in glucose levels during the solid-state fermentation of maize (Zea mays) offal. Conversely, Wei *et al.* [32] reported a decrease in glucose levels in agricultural by-products subjected to boiling.

Amylases play a crucial role in various biotechnological applications across industries such as food, fermentation, detergent, pharmaceutical, brewing, textile, and paper [40-41]. In the present study, a significant increase in amylase activities was observed in the groups treated with extract + *C. glutamicum* and boiled extract + *C.*

glutamicum, particularly in cassava leaves and maize leaves. Similar increases in amylase activities due to fermentation have been reported by Dou *et al.* [42] and Divakaran *et al.* [43]. The heat stability of amylase, retaining about 80% enzyme activity at high temperatures, makes it valuable for industrial applications [44]. The enzyme exhibits activity under acidic to neutral conditions (pH 6-7), enabling its use in various food industry processes such as dough preparation, juice and fruit processing, baking, and brewing [1,45].

Submerged fermentation of agricultural by-products using *C. glutamicum* resulted in the highest protease activities in cassava leaves, palm tree leaves, and maize compared to the control group. Punniyakotti *et al.* [46] demonstrated enhanced protease production using agricultural waste materials in submerged fermentation with *Bacillus subtilis* B22. Mathias *et al.* [47] also reported increased proteolytic activity values in residual brewer's waste utilizing Lactobacillus delbrueckii. Proteases find wide biotechnological applications in the food, detergent, textile, pharmaceutical, peptide synthesis, leather, and paper industries [48-49]. They play a crucial role in hydrolyzing protein molecules, breaking down bonds to produce peptides or amino acid units.

The total flavonoid content of the agricultural by-products was determined and showed significant increases in the fermented groups (extract + *C. glutamicum* and boiled extract + *C. glutamicum*) compared to the unfermented by-products, except for cocoyam leaf. Similar findings were reported by Adetuyi and Ibrahim [50], Yao *et al.* [51], Moktan *et al.* [52], and Ademiluyi and Oboh [53]. The increase in flavonoid content could be attributed to the increase in acidity during fermentation, which liberates bound flavonoid components and enhances their bioavailability. Microbial enzymes generated during fermentation have been reported to play a significant role in breaking down the plant matrix and extracting flavonoids [54-56]. However, β-glucosidase of microbial origin can hydrolyze flavonoids, resulting in either an increase or decrease in flavonoid levels, as observed in cocoyam leaves [59]. Flavonoids are polyphenolic compounds known for their antioxidant and free radical scavenging abilities [60].

The fermented agricultural by-products exhibited significantly higher total phenolic contents compared to the non-fermented samples, consistent with previous studies [61-62]. Phenolic compounds in their natural form are often bound to sugars, reducing their bioavailability. However, during fermentation, microbial proteolytic enzymes hydrolyze these complexes, releasing soluble free phenols [63].

The pH values of the fermented and non-fermented agricultural leaves showed non-significant changes compared to the control, except for maize, which exhibited a significant increase in pH. Generally, during fermentation, the pH value tends to decrease initially due to the production of organic acids by microorganisms. However, as fermentation progresses and nutrients are depleted, microorganisms start utilizing the organic acids as a nutrient source, leading to a reduction in their levels and subsequent increase in pH [64,65].

In conclusion, the present study demonstrates the advantage of submerged fermentation using *C. glutamicum* in increasing lysine yield compared to non-fermented methods. The study also highlights the effects of various treatments on the nutritional value, pH, and activities of amylase and proteases in agricultural leaves. The observed variations can be further optimized for specific lysine production by employing suitable methods

and conditions. Given the increasing demand for L-lysine in the food, pharmaceutical, and animal feed industries, these methods offer potential for improving production technology, reducing costs, and utilizing unconventional resources.

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Use of Cognitive Enhancers among students of Nigerian Tertiary Institutions

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ABSTRACT

Introduction: Nonmedical use of pharmaceutical cognitive enhancers PCE in Universities has been documented in several studies. However, there is paucity of research on this subject among Nigerian University students. This survey hopes to investigate the awareness, use and effect of PCE among undergraduate medical students of a Nigerian University.

Materials and method: The study utilized self-administered questionnaire on the awareness and usage of PCE with respondents composed of 327 medical students at Delta State University, Nigeria.

Results: The respondents have a male to female ratio of 1:0.85 with 99.1% being single. Those living on campus, off campus, family home and dual accommodation, accounted for 65.4%, 30.6%, 2.8% and 0.3% of the participants. In total, 81.7% of the participants are aware of PCE usage for academic purpose. Overall, 40.1% of the respondents reported using one or more of the PCE during the period considered. Caffeinated drinks and beverages, energy drinks, cigarette, marijuana, and Ginseng were used by 82.5%, 50.4%, 3.1%, 1.5% and 1.5% of the users respectively. The motivation for using both caffeinate beverages and energy drinks are for increased study time, concentration and alertness. The more frequent periods of use are during examination period and personal study time. Most of these PCE are self-purchased and choice of usage are mainly influenced by friends. Both set of users reported insomnia, headache, palpitation, dizziness and fatigue as undesirable experience. Among the respondents, 45% are interested in using PCE if there are no undesirable effects; 30% rejected such, while the rest are indifferent.

Conclusion: Our survey showed significant association between the age, male gender and academic level and the usage of PCE. Our respondents used mainly caffeinated beverages and energy drink. Their choice of PCE is strongly influenced by peer pressure and the motivations are mainly to boost studying time, concentration and alertness.

Keywords: Pharmacological cognitive enhancers, Medical students, Caffeine, Nigeria,

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INTRODUCTION

Pharmacological cognitive enhancement is defined as "amplification or extension of core capacity of the mind by improving the internal and external information processing systems by using drugs or substances".[1] Many of such substances were primarily used to treat neuropsychiatric conditions that are often associated by cognitive deficit.[2] These pharmacological cognitive enhancers (PCE) are also taken by healthy individuals to boost energy, and to improve mental alertness, concentration and memory, despite the drawback of its use such as development of dependence and psychiatric disorders.[2,3,4]

These PCE are broadly classified into prescription and non-prescription drugs or substances. Prescription PCE include methylphenidate, modafinil, piracetam and amphetamine salt mixture; and many other drugs which have not been approved for human use.[5,6] The non-prescription PCE include caffeine and caffeine containing beverages, vinpocetine, cobalamine (vitamin B12), guarana and pyridoxine (vitamine B6).[5]

Their mode of action have not been fully elucidated. However, most of them, including modafinil, amphetamine salt and methylphenidate are central nervous system stimulants.[7]

There is a rising interest on use of PCE in university setting among researchers. In United Kingdom (UK), among 1614 student respondents, 33% of them have used off-label prescription PCE.[8] In another study, involving 877 students from Irish and UK Universities, lifetime incidence of use of amphetamine, methylphenidate and modafinil was 2%, 5.9% and 6.2% respectively.[9] Report from United States (US), showed that misuse of PCE among university students was up to 17%.[10]

Scientific data regarding the use of PCE among Nigerian University students is scanty.

This study was performed in Delta State University and is hoped to unveil the pattern of PCE usage among medical students, including relationship with age, gender, academic level and place of residence. We hope that results from this study will guide policy makers as well as add to already existing literature.

MATERIALS AND METHOD

The sample comprises of the medical students of Delta State University (DELSU) during the study period. The participants were asked to complete a self-administered questionnaire about awareness and usage of PCE in their academic environment. The study was conducted from 4th to 16th June 2023 in line with the ethical standard of the institution. Filling of the questionnaire was voluntary, and the participants were assured of anonymity and confidentiality of information provided. Before participating in the study, the framework of the study was explained to the participants. Informed consent was obtained from all participants and the right to withdraw from participation at any time during the survey was reserved.

Demographic information was limited to age, sex, academic year, marital status and nature of residence of the respondents. A 10-item questionnaire was used for this survey to assess: 1) respondents' awareness of the use of PCE; 2) the usage of PCE among participants; 3) reasons for usage of PCE; 4) positive effects of PCE on participants; 5) time of usage of PCE; 6) duration of usage of PCE; 7) source of acquisition of PCE; 8) source of information on the PCE; 9) Undesirable effects during usage of PCE; and 10) their readiness to use PCE if they are assured of minimal side effects and safety.

All the data were analyzed using SPSS for windows, version 24.0 (SPSS Inc, Chicago, II, USA) and results were summarized using tables and figures. Finding was regarded as statistically significant using $p<0.05$.

The study was approved by the Research and Ethics Committee in the Faculty of Basic Clinical Sciences, Delta State University, with approval number FBCS/REC/23/01. The authors also secured permission and approval for data collection from the provost, College of Health Sciences, Delta State University.

RESULTS

In total, 327 completed questionnaires were retrieved, which is equivalent to 90% of the expected participants in the study, with a male to female ratio of 1: 0.85. Of all the respondents, ninety-nine-point one percent of them are married. Those living on campus, off campus, family home and dual accommodation, accounted for 65.4%, 30.6%, 2.8% and 0.3% of the respondents. The detail of the socio-demographic profile is shown in Table I.

The awareness of PCE by respondents is shown in Figure I. In total, 81.7% of the respondents are aware of the use of PCE in the university setting. Amongst them, 68.2%, 45.3%, 20.8%, 13.1%, and 5.5% are aware of use of caffeinated beverages, energy drinks, tramadol, marijuana, and kola nut as PCE. There is a significant association between age and academic level and awareness of PCE (as is depicted in Table II).

The usage of PCE is shown in Figure II. Overall, 40.1% of the respondents reported using one or more of the PCE during the period considered. Caffeinated beverages, energy drinks, cigarette, marijuana, and ginseng were used by 82.5%, 50.4%, 3.1%, 1.5% and 1.5% 2 of the respondents that admitted using PCE respectively. There is also significant association between age, gender and academic level and usage of PCE as is depicted in Table III. Details of impact of use of energy drink and caffeinated beverage is shown in Table IV.

The motivation for using these drugs, in order of frequency are increased study time, concentration and alertness. The more frequent periods of use are during examination period and personal study time. Most of the PCE are self-purchased and the choice of PCE are mainly influenced by friends. Both sets of users reported insomnia, headache, palpitation, dizziness and fatigue as undesirable side effects.

Among the respondents, 45% agreed that they will use PCE if they are guaranteed of its safety, 30% disagreed to its usage while the rest are indifferent.

Table I: Demographic characteristics of respondents (N=327)

Demographic Parameters	Frequency	Percentage (%)
Age (Years)		
16 – 20	154	47.1
21 – 25	142	43.4
26 – 30	28	8.6
>30	3	0.9
Gender		
Male	177	54.1
Female	150	45.9
Academic Level		
100	57	17.4
200	77	23.5
300	54	16.5
400	39	11.9
500	64	19.6
600	36	11.0
Marital Status		
Single	324	99.1
Married	3	0.9
Residence		
On Campus	214	65.4
Off Campus	100	30.6
Individual home	9	2.8
Family home	3	0.9
Dual accommodation	1	0.3

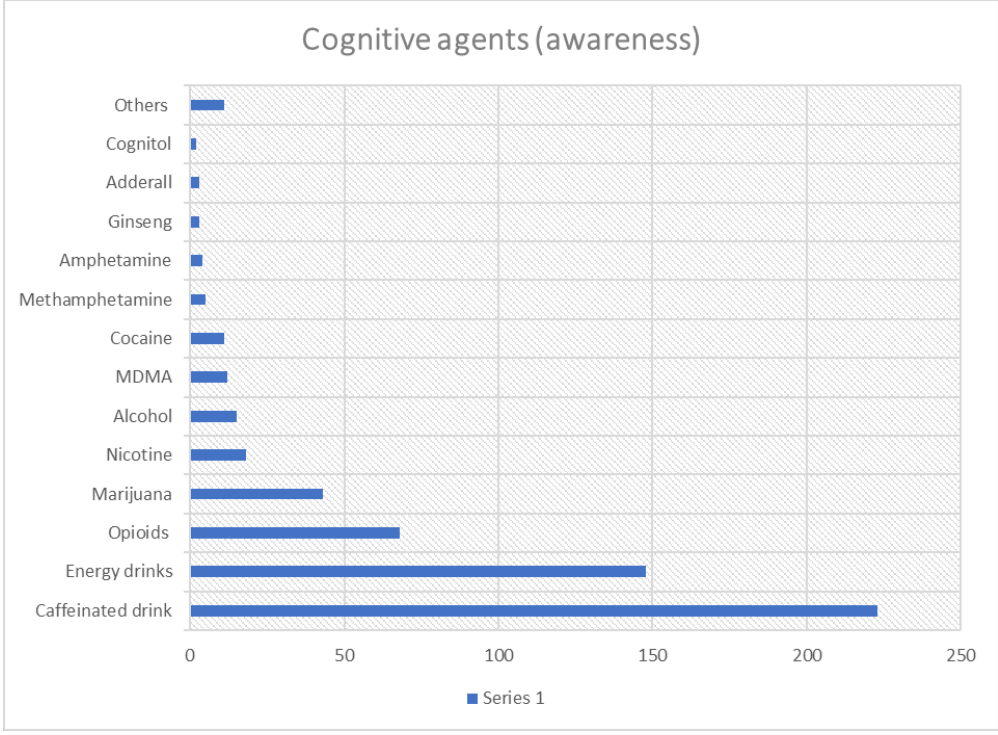

Figure 1: Awareness of the use of PCEs among medical students

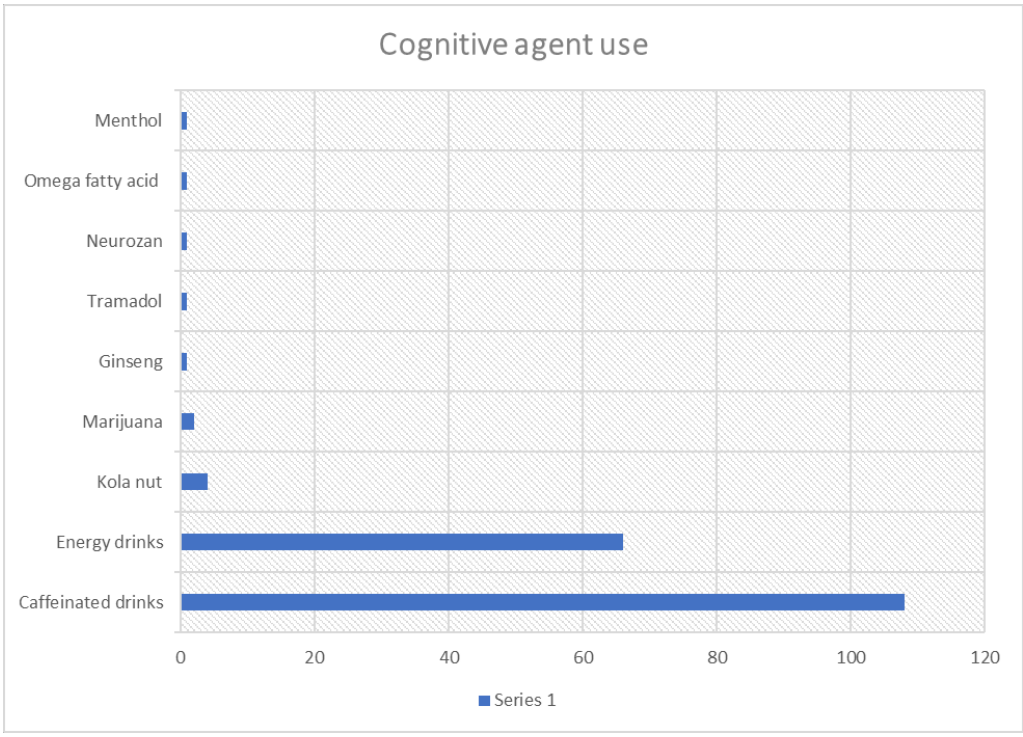

Figure II: The use of PCEs among medical students

Table II. The distribution of students' awareness of PCEs by their demographic features

	Awareness of PCEs			X^2	P value
	Yes	No	Total		
Age (Year)					
16 – 20	116 (43.4%)	38 (63.3%)	154 (47.1%)	0.727	0.033
21 – 25	125 (46.8%)	17 (28.3%)	142 (43.4%)		
26 – 30	24 (9.0%)	4 (6.7%)	28 (8.6%)		
>30	2 (0.7%)	1 (1.7%)	3 (0.9%)		
Sex					
Male	143 (53.6%)	34 (56.7%)	177 (54.1%)	0.191	0.662
Female	124 (46.4%)	26 (43.3%)	150 (45.9%)		
Academic Level					
100	36 (13.5%)	21 (35.0%)	57 (17.4%)	30.938	0.000
200	57 (21.3%)	20 (16.7%)	77 (23.5%)		
300	44 (16.5%)	10 (16.7%)	54 (16.5%)		
400	38 (14.2%)	1 (1.7%)	39 (11.9%)		
500	61 (22.8%)	3 (5.0%)	64 (19.6%)		
600	31 (11.6%)	5 (8.3%)	36 (11.0%)		
Marital Status					
Single	265 (99.3%)	59 (98.3%)	324 (99.1%)	0.454	0.501
Married	2 (0.7%)	1 (1.7%)	3 (0.9%)		
Residence					
On Campus	183 (68.5%)	31 (51.7%)	214 (65.4%)	6.803	0.147
Off Campus	74 (27.7%)	26 (43.3%)	100 (30.6%)		
Individual home	7 (2.6%)	2 (3.3%)	6 (2.8%)		
Family home	2 (0.7%)	1 (1.7%)	3 (0.9%)		
Dual accommodation	1 (0.8%)	0 (0.0%)	1 (0.3%)		

Table III. The distribution of students' use of PCE by their demographic features

	Use of PCEs				
	Yes	**No**	**Total**	**X^2**	**P value**
Age (Year)					
16 – 20	44 (33.6%)	110 (56.1%)	154 (47.1%)	16.969	0.001
21 – 25	70 (53.45%)	72 (36.7%)	142 (43.4%)		
26 – 30	16 (12.2%)	12 (6.1%)	28 (8.6%)		
>30	1 (0.8%)	2 (1.0%)	3 (0.9%)		
Sex					
Male	83 (63.4%)	94 (48.0%)	177 (54.1%)	7.499	0.006
Female	48 (36.6%)	102 (52.0%)	150 (45.9%)		
Academic Level					
100	16 (12.2%)	41 (20.9%)	57 (17.4%)	44.887	0.000
200	20 (15.3%)	57 (29.1%)	77 (23.5%)		
300	11 (8.4%)	43 (21.9%)	54 (16.5%)		
400	24 (18.3%)	15 (7.7%)	39 (11.9%)		
500	42 (32.1%)	22 (11.2%)	64 (19.6%)		
600	18 (13.7%)	18 (9.2%)	36 (11.0%)		
Marital Status					
Single	130 (99.2%)	194 (99.0%)	324 (99.1%)	0.057	0.811
Married	1 (0.8%)	2 (1.0%)	3 (0.9%)		
Residence					
On Campus	90 (68.7%)	124 (63.3%)	214 (65.4%)	3.046	0.550
Off Campus	35 (26.7%)	65 (33.2%)	100 (30.6%)		
Individual home	4 (3.1%)	5 (2.6%)	9 (2.8%)		
Family home	1 (0.8%)	2 (1.0%)	3 (0.8%)		
Dual accommodation	1 (0.8%)	0 (0.0%)	1 (0.3%)		

Table IV. Impact of specific PCE

Descriptions	Energy drink	Caffeine
Reasons for use		
Concentration	13 (20.6%)	24 (19.5%)
Increased study time	49 (77.8%)	74 (60.2%)
Improve academic performance	9 (14.3%)	14 (11.4%)
Relaxation	1 (1.6%)	1 (0.8%)
Effect		
Positive	34 (54.0%)	49 (39.8%)
Negative	8 (12.7%)	16 (13.0%)
Indifferent	21 (33.3%)	58 (47.2%)
Time of use		
Daily	1 (1.6%)	7 (5.7%)
Study period	27 (42.9%)	44 (35.8%)
Exams	34 (54.0%)	48 (39.0%)
Duration of use (months)		
1	9 (14.3%)	14 (11.4%)
1 – 6	9 (14.3%)	11 (8.9%)
6 – 12	5 (7.9%)	5 (4.1%)
13 – 24	11 (17.5%)	11 (8.9%)
>24	14 (22.2%)	27 (22.0%)
Source of acquisition		
Friends	6 (9.5%)	10 (8.1%)
Self-purchased	27 (43.9%)	38 (30.9%)
Prescribed	4 (6.3%)	4 (3.3%)
Others	11 (17.5%)	8 (6.5%)
Cost		
Very expensive		1 (0.8%)
Expensive	1 (1.6%)	4 (3.3%)
Fair	22 (34.9%)	27 (22.0%)
Cheap	30 (84.1%)	53 (43.1%)
How do you learn about them		
Social media	4 (6.3%)	10 (8.1%)
Internet	4 (6.3%)	8 (6.5%)
Friends	37 (58.7%)	43 (35.0%)
Others	5 (7.9%)	16 (13.0%)
Side effects		
Insomnia	8 (12.7%)	16 (13.0%)
Headache	7 (11.1%)	11 (8.9%)
Palpitation	5 (7.9%)	6 (4.9%)
Dizziness	3 (4.8%)	5 (4.1%)
Fatigue	3 (4.8%)	3 (2.4%)
Chest pain	1 (1.6%)	1 (0.8%)
Sweating	1 (1.6%)	
Abdominal pain	1 (1.6%)	1 (0.8%)
Willingness to buy in the future		
Yes	32 (50.8%)	45 (36.6%)
No	9 (14.3%)	19 (15.4%)
Indifferent	22 (34.9%)	59 (48.0%)

DISCUSSION

The usage of PCE in University environment in Nigeria has not been examined scientifically. This survey therefore provides first-hand epidemiological data on this subject in this part of the globe.[8] The choice of medical students among other University students is in consideration of the relatively higher workload and competitiveness of the course. These medical students may therefore be motivated to use any available means, including use of PCE to ensure better grades.

Our survey showed that usage of prescription PCE for non-medical reasons in our academic setting is relatively uncommon. This is contrary to reports from western countries, where the lifetime prevalence of use of modafinil, methylphenidate and amphetamine among university students is within the range of 2% to 43%.[5] This epidemiological difference may be attributed to fear of undesirable effects and unavailability of such prescription drugs. This is however a welcome development, considering the risk of psychiatric problems that may arise from use of these drugs.[2,4]

The most used non-prescription PCE in our study is caffeinated beverages and energy drinks. This concurs with global view as caffeine remains the most consumed stimulant across the globe which are mainly in form of coffee or energy drink.[11] This is likely because they are freely accessible, quite affordable, does not require doctor's prescription before it can be purchased. They have also been known for ages for its use for memory and concentration enhancement. This raises concern of associated health hazards, which includes addiction, anxiety, mood disorder, panic attack, sleeping disorder and cardiovascular problems.[12]

We observed a significant correlation between male gender and the use of these PCE.

This is consistent with earlier reports.[13-17] This may be attributed to gender difference in perception of risk, differences in attitude towards substance and drug use and the social stigma associated with drug and substance use among females.

Our survey showed that increased duration of studying, concentration and improved alertness were the most frequently cited reasons for taking PCE. Other reports also concurred with our observation.[18-22] Unexpectedly, report from Lengvenyte et al., showed no relationship between grade point average (GPA) and use of PCE among university students.[23] Some studies have even shown that some students that engaged in PCE even had a lower GPA relative to those that abstained from them.[24-26] There is therefore need to discourage this trend and explore non-pharmacological approaches using nutrition, physical exercise, sleep, meditation, mnemonic strategies, computer training, and brain stimulation.[27]

Our study showed that major influence or source of information is from friends, showing that most decisions on use of PCE are strongly influenced by peer-pressure. This peer pressure relationship has earlier been demonstrated by Carroll and collegues.[28] Our study showed that most of the PCE used by respondents are self-purchased. This is at variance with report from western countries where most PCE are purchased through online sales.[29-31] It may however be explained by the choice of PCE in the study environment which are mainly caffeinated products that are readily available and affordable.

The survey also observed that PCE usage among senior medical students is more than usage among junior medical school. This view is supported by earlier report and may be related to addictive

effect as well as relatively higher workload with higher academic levels.[32]

Going forward from here, there is need to build a system that reduces peer pressure among students and explore non-pharmacological methods of cognitive enhancement.

There is also need to run campaign in universities that will build students' confidence, so that they can believe more in themselves without depending on PCE.

In conclusion, our survey showed significant association between the age, male gender and academic level and use of PCE. Our respondents used mainly caffeinated beverages and energy drink. Their choice is strongly influenced by peer pressure and the motivations are mainly to increase reading time, concentration, and alertness.

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Prevalence of gestational diabetes mellitus, fetal and maternal outcomes of parturients with risk factors versus parturients without risk factors for gestational diabetes mellitus: A preliminary analysis of the comparative study of blood sugar levels at a tertiary hospital in southern Nigeria

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Abstract

Background: Typically asymptomatic, gestational diabetes mellitus (GDM) has been associated with myriads of maternal and fetal complications and has been shown to predict morbidity in both mother and the newborn much later in life. The incidence of these complications in GDM has been strongly associated with a maternal glycemic level. As a generalization, the degree of maternal hyperglycemia dictates the fetal outcome.

Methods: This study was a prospective cohort analytical observational study of blood glucose levels amongst two cohorts of women who attended antenatal care at the obstetric unit of Delta State University Teaching Hospital, Oghara.

Results: The prevalence of GDM was 31.3% and 9.4%, respectively, for cases and controls. The difference in prevalence and glycemic control was statistically significant. The subjects were recruited based on a positive history of - previously haven had macrosomic babies, maternal weight greater than 90kg, unexplained intrauterine fetal death/stillbirth, fasting glycosuria, and the presence of a family history of GDM in first-degree relatives. Interventional deliveries and maternal and fetal complications were statistically significantly higher in cases than in controls.

Discussion: The prevalence of GDM in cases was significantly higher than in the controls; this seems to have given some credence to the fact that the risk factors based on which the patients were recruited may indeed be predictive of the risk of developing GDM in the pregnant parturients in Delta State Nigeria.

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Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance of variable severity with onset or first recognition during pregnancy[1-5]. Undoubtedly, some women with gestational diabetes have previously unrecognized overt diabetes. Incidence of GDM depends on the population studied and the diagnostic criteria employed[6] but is said to affect about 3-10% of pregnancies generally and accounts for 90% of diabetes mellitus in pregnancy [4,7]. GDM is pregnancy-induced, and it

occurs when the β-cell reserve cannot counter-balance the insulin resistance caused by placental hormones [6,8].

Even though various diagnostic criteria which predict adverse pregnancy outcomes exist[9,10], diagnosis of GDM is based on World Health Organization (WHO) criteria for Glucose tolerance based on a 75 g -oral glucose tolerance test (OGTT):

> Normal glucose tolerance: *Fasting plasma glucose <7.0 mmol/l; 2hrs after 75g oral glucose load <7.8 mmol/l*
> Impaired glucose tolerance: *Fasting plasma glucose <7.0 mmol/l; 2hrs after 75g oral glucose load >7.8 - <11.1 mmol/l*
> Diabetes: *Fasting plasma glucose >7.0 mmol/l; 2 hrs after 75g oral glucose load >11.1 mmol/l*

Although many studies have lent credence to the benefit of screening, there is no consensus on the timing of the screening, but it is usually done at 24-28 weeks[11].

Though typically asymptomatic, GDM has been associated with myriads of maternal and fetal complications and has been shown to predict morbidity in both mother and the newborn much later in life. The incidence of these complications in GDM has been strongly associated with a maternal glycemic level, and as a generalization, it is the degree of maternal hyperglycemia that dictates fetal outcome[6,8]. Fetal complications include congenital abnormalities, stillbirth, macrosomia, increased risk of birth trauma, neonatal hyperbilirubinemia, neonatal hypoglycemia, and long-term complications like childhood obesity and type II DM in the offspring[4,5,8,8,12]. Maternal complications include increased risk of preeclampsia, obstructed labor as a result of macrosomia, increased risk of operative deliveries; shoulder dystocia, genital track injuries, and development of type II DM later in life with more than half of women with gestational diabetes ultimately developing overt diabetes in the ensuing 20 years[1,5,13]

Overall, reductions in perinatal complications among women actively treated for GDM have been demonstrated in several studies. These include an Australian randomized clinical trial that demonstrated better perinatal outcomes in the intervention (with diet or insulin) group[14]; and an analysis of the effects of carbohydrate-restricted diet in patients with diet-controlled Gestational Diabetes in California, USA which demonstrated an improved glycemic control, less need for insulin therapy, decrease in the incidence of large for gestational age infant, and a decreased in cesarean deliveries for cephalo-pelvic disproportion (CPD) and macrosomia[15].

Therefore, the ability to recognize parturients at risk, promptly diagnose the disorder through screening, and institute appropriate management will avert many of these complications.

Studies have linked GDM with various risk factors which predict the occurrence of the disease[16], though about 40-60% of cases have no risk factors. These established Risk factors for GDM are Body mass index >30 kg/m2, Age>25 years old, GDM in a previous pregnancy, Family history of diabetes, Previous delivery of a large baby, and Previous stillbirth. Whereas previous studies[17] had sought to identify the presence of risk factors in parturients already independently recruited into such studies evaluating GDM, we, however, believe that using identified risk factors as a basis *ab initio* for recruiting our cases and the absence of any such risk factors for controls, may help to authenticate in our population the validity of these factors being predictive of developing GDM. This is against the background of our low

resources settings, where resources may be unable to support routine screening for all our pregnant population. We believe that the outcomes of this study could serve as a basis to design relevant interventions, including public health-related advocacy activities that would help optimize the health outcomes of pregnant women and their babies, thereby helping to achieve the maternal and perinatal global targets of the sustainable development goals.

It is against this backdrop that this study was conceptualized to determine the incidence of GDM in the women seen at the antenatal clinic of Delta State University Teaching Hospital if significant differences existed in the glycemic levels and in the maternal and fetal outcomes among parturients who have antenatal risk factors for GDM and parturients who have no antenatal risk factors for GDM.

Methods

This study was a prospective cohort analytical observational study of blood glucose levels amongst two cohorts of women who attended antenatal care at the obstetric unit of Delta State University Teaching Hospital, Oghara. The first cohort (cases) were women with proven risk factors for GDM, while the second cohort of women (control) were those without risk factors for GDM. Any parturient with one or more of these established risk factors for the development of Diabetes mellitus: *Previous history of macrosomic (>4kg) babies, Maternal weight >90kg; Previous unexplained intrauterine fetal death/stillbirth; Previous congenital malformation; Fasting glycosuria on two occasions; and Family history of GDM in any 1st-degree relative was recruited as a case upon given informed consent.* For every recruited patient with risk factors for GDM, the next presenting patient to the antenatal clinic matched for Age, height, and gestational Age of the pregnancy without any of the established predisposing risk factors for GDM were recruited as controls. Both groups of parturients underwent glucose screening during pregnancy between 24 to 28 weeks and were followed up to establish the maternal and fetal outcomes when they presented in labor.

There was a liaison with the DELSUTH laboratory unit that ensured quality control in the analysis of blood samples for glucose levels.

The authors bore the cost of screening the parturients with risk factors for GDM (cases) and the cost of screening parturients without antenatal risk factors for GDM (controls).

Ethical clearance was sought and obtained from DELSUTH Ethical Committee. Informed consent was obtained from all the parturients recruited into the study. Those who declined consent were excluded from the study.

The sample size was calculated using the formula: $n = (Z^2 \times PQ)/ d2$, and the expected figure was obtained using a degree of accuracy of 5% with a confidence interval of 95%. The power of analysis was based on a previous study with a prevalence of gestational diabetes of 6.8%.[18,19] Where n = desired minimum size; Z = score for a confidence interval of 95%, which is 1.96; P = proportion of women with gestational diabetes mellitus from the previous study is 6.8%; Q = complementary proportion equivalent to one (1) minus P, Q = 1- 0.068=0.932; and d = degree of accuracy desired which is 5% = 0.05. Therefore, n = 97.4. We assumed an attrition rate of 10%, giving a computed minimum sample size of 108 pregnant women. Thus, 108 parturients with risk factors for GDM and 108 parturients without risk factors for GDM (a total of two hundred and sixteen parturients) would be selected for this study from among parturients presenting for antenatal care at the DELSUTH, Oghara. Thus

far, we have recruited 32 cases and 32 controls. Selected patients were informed and counseled about the study, and only those who gave written consent were enrolled.

A datasheet designed for this study was employed to collect information about each parturient. The variables that were retrieved and entered into the data forms are *the Sociodemographic profile: (Names, Hospital No, Age (yrs), Parity, Level of Education (either None, Primary, Secondary, or Tertiary); Husband's occupation; Vital signs (Weight, Height, BMI, BP); Booking status: (Booked or Un-booked); Risk factors for the development of Diabetes mellitus, Previous history of macrosomic (>4kg) babies, Maternal weight >90kg; Previous unexplained intrauterine fetal death/stillbirth; Previous congenital malformation; Fasting glycosuria on two occasions; and Family history of GDM in any 1st degree relative*.

Between 24 weeks and 28 weeks gestation, glucose screening was conducted for each enrolled parturient. Following an 8-14 hours overnight fast, 5ml of venous blood was collected from each parturient's forearms into a Fluoride oxalate bottle after the patient was requested to drink a 75g of glucose in 100 ml of water over 5-10 mins, and 5 ml of venous blood sample was again collected from the forearm after 2 hrs. Samples were immediately sent to the laboratory for analysis. Results of the Blood sugar were obtained and entered into each parturient's data sheets. The categories of sugar pattern for this study on OGTT were:

Normal <7.0 mmol/l
Impaired glucose tolerance >7.0 <11.1 mmol/l
Diabetic >11.1 mmol/l

The above is based on World Health Organization (WHO) criteria for Glucose tolerance based on a 75 g -oral glucose tolerance test (OGTT):

Normal glucose tolerance: *Fasting plasma glucose <7.0 mmol/l; 2hrs after 75g oral glucose load <7.8 mmol/l*
Impaired glucose tolerance: *Fasting plasma glucose <7.0 mmol/l; 2hrs after 75g oral glucose load >7.8 - <11.1 mmol/l*
Diabetes: *Fasting plasma glucose >7.0 mmol/l; 2 hrs after 75g oral glucose load >11.1 mmol/l*

The parturients with results consistent with impaired glucose tolerance and with frankly diabetic values were grouped in line with the standard definition of GDM. All parturients diagnosed with GDM were commenced on immediate treatment and were co-managed with the endocrine Physician.

All Parturients were followed up from when they presented in labor. After delivery, information on maternal and neonatal outcomes was obtained from the mother's case note and the baby's record and entered into the datasheet. This information was *Mode of delivery (SVD, Forceps, Vacuum, or CS), a complication of delivery, EBL, Live birth or Stillbirth (FSB or MSB), Gestational Age @ delivery, birth weight, APGAR score in 5 minutes, Fetal complications*.

Data captured on the data sheets from all the 64 participants so far was collated, coded, and entered into the computer using Statistical Package for Social Sciences (SPSS PC+), and data was then analyzed with univariate and bivariate statistics using the same SPSS PC+. Differences in rates of outcomes between the two cohorts of parturients were compared using the Chi-square test with Yates correction, as appropriate and relevant deductions were made. The level of significance was set at a P value <0.05.

Results

Overall, 64 booked parturients have been

evaluated, with 32 each as cases and controls, respectively. The Average Age of the parturients (cases and controls) was 33.00 (3.586-3.928), average height was 1.7 (0901-0973), and average gestational at delivery was 38.25 (0.463-0.886). These average parameters were the same in cases and controls as they were matched. The tables and associated explanatory text below present the other findings and outcomes.

Analysis of the sociodemographic variables (table 1) shows that the majority (87.5%) of the respondents had secondary (50%) and tertiary (37.5%) levels of education. Fifty percent of the cases were married to skilled personnel as their husbands, 50% of the controls, on the other hand, were married to professionals as their husbands, and 37.5% were skilled workers. The differences observed were not statistically significant.

The prevalence of GDM between 24 weeks and 28 weeks (table 2) was 31.3% and 9.4%, respectively, for cases and controls. The difference in prevalence and glycemic control was statistically significant.

The analysis of the history of identified risk factors for GDM based on which the cases were recruited (table 3) revealed that fifty percent (16/32) of the cases had a positive history of previous macrosomic babies. The other risk factors that served as a basis for recruiting the study participants were maternal weight greater than 90kg, unexplained intrauterine fetal death/stillbirth, fasting glycosuria, and the presence of a family history of GDM in first-degree relative being the basis of recruitment. The controls were recruited from amongst the parturients without these risk factors.

Table 1: Sociodemographic Characteristics

Parameter	Cases n (%)	Controls n (%)	p-value
Level of education			
None	0	0	
Primary	4 (12.5)	4 (12.5)	NS
Secondary	16 (50)	16 (50)	
Tertiary	12 (37.5)	12 (37.5)	
Husband's occupation,			
Unskilled	8 (25)	4 (12.5)	
Skilled	16 (50)	12 (37.5)	0.1017
Professional	8 (25)	16 (50)	
Average Parity	3.13 (1.126)	2.75 (1.035)	1.000
Average maternal Weight	77.88 (9.687)	68.00 (5.014)	0.023
Total	**32**	**32**	

Table 2: Pattern of blood sugar and the prevalence of GDM

Parameter	Cases n (%)	Controls n (%)	p-value
OGTT @ **24-28 weeks**			
<7.0 mmol/l	19 (59.4)	28 (87.5)	
>7.0 <11.1 mmol/l	3 (9.3)	1 (3.1)	0.0389
>11.1 mmol/	10 (31.3)	3 ((9.4)	

Fifty percent of the cases (parturients with risk factors for GDM) had spontaneous vaginal delivery (SVD). In contrast, 12.5% (4/32) had forceps delivery, and 37.5% (12/32) had cesarean sections - 4 were elective cases on account of identified fetal macrosomia at term, 25% (8/32) were emergency cases with 15.6% (5/32) of the cases due to fetal distress and another 9.4% (3/32) due to fetopelvic disproportion in labor (table 4). Twenty-nine controls (90.6%) had SVD, and only two controls had cesarean sections, which were done as emergencies on account of fetal distress before full cervical dilatation. Eight (12.5%) of the parturients with risk factors for GDM suffered complications, and of these, 5 (15.6%) had genital tract lacerations, and 6 (18.8) had postpartum hemorrhage (PPH). Only one of the controls had complications of PPH. The differences in the complication rates between the cases and controls were statistically significant. Twelve (37.5%) cases had blood loss less than 500mls, and twenty (62.5%) had blood loss greater than 500mls. Only one of the controls had blood loss greater than 500mls. The differences between cases and controls are statistically significant.

All babies were live birth in cases and controls (table 5). However, over half (56.2%) of the babies of parturients with risk factors for GDM had complications, while only 1 (3.1%) of babies in the control arm had complications. Over half (55.6%) of the babies that had complications had birth trauma, 38.9% (7/18) suffered hypoglycemia within the first hour of birth requiring correction by the neonatologists, and one baby had a femoral fracture following a difficult vaginal delivery. The baby in the control arm that had complications suffered birth trauma. The differences between cases and controls were statistically significant (p-values <0.05).

Discussion

This report presents a preliminary analysis of the ongoing study amongst two cohorts of parturients recruited as cases and controls based on a positive or negative history of one or more of the traditionally identified risk factors for developing GDM. Of the six leading established risk factors[16] that served as the basis for recruiting parturients as cases and the absence of which they were classified as controls, family history in first-degree relative (68.7%), previous history of macrosomic babies (50%), fasting glycosuria on two occasions (56.2%), maternal weight >90kg (37.5%) and previous unexplained intrauterine fetal death/stillbirth (25%) were the risk factors volunteered by the parturients in the order of frequency as enunciated. This finding is in keeping with the results of previous studies[16,19-23], in which these factors were identified to increase the risk of developing GDM. In this study, positive history of previous congenital malformation was not reported; however, earlier reports[16,20-23] had indicated it to be associated with an increased incidence of GDM. The Average Age of the parturient in this study was 33.00 ($\pm$3.586-3.928), and existing data[21,22,24,25] suggest an increasing incidence of GDM after the Age of 25 years, with an incidence as high as 11.3% in parturients in the 30-39 age bracket.[21,25]

The overall incidence of gestational diabetes in this preliminary data was 26.7%, the incidence in the cases was 40.6%, and in the controls, it was 12.5%. A huge systematic review and meta-analysis of the prevalence and determinants of gestational diabetes mellitus in Nigeria in 2021 by Azeez et al. revealed that the prevalence of GDM in Nigeria was 0.5-38%.[17] The cohort of women with risk factors for GDM (cases) in our ongoing series had a significantly higher incidence than the controls (p-value =0.0389). This pattern gives credence to the fact that these established risk factors may be predictive of GDM in our

Table 3: Quantification of the History of Risks factors used for recruiting the cases

Parameter	Cases n (%)
Previous history of macrosomic (>4kg)	
Yes	16 (50)
No	16 (50)
Maternal weight >90kg	
Yes	12 (37.5)
No	20 (62.5)
Previous unexplained intrauterine fetal death/stillbirth	
Yes	8 (25)
No	24 (75)
Previous congenital malformation	
Yes	0
No	32 (100)
Fasting glycosuria on two occasions	
Yes	18 (56.2)
No	14 (43.8)
Family history in 1st degree relative	
Yes	22 (68.7)
No	10 (31.3)
Total	32

population, particularly against the backdrop that the incidence reported in the controls is consistent with the national average prevalence rate of 10-15% for the general antenatal population recruited without recourse to the presence of identified risk factors for GDM.[17,26] This report further reveals that a significant proportion of parturients with GDM have no known established risk factors - 12.5% of controls in this study. Evidence[16,20,22,27,28] from previously available data is in tandem with this observation, as about 40-60% of parturients from the earlier reports have no risk factors. This underscores the very critical question Moses et al.[29] raised in their seminal publication, whose title is the question: *Gestational Diabetes: Do all women need to be tested?* We hope to attempt to provide a response to this question at the end of this study when putting together the final report.

Interventional deliveries and adverse maternal outcomes were significantly more prevalent in the cases than controls. This compares favorably with the findings of previous studies.[19] The significantly higher incidence of cesarean sections and operative vaginal deliveries (forceps) associated with the cases compared with controls is consistent with previous reports.[19] Similarly, the cesarean sections' indications compare favorably with earlier reports.[19] Similarly, postpartum hemorrhage was significantly higher in the cases than in the controls.

One striking feature regarding complications was that the rates of maternal and neonatal complications were higher in the cases arm compared to the control arm despite the prompt commencement of all diagnosed with GDM on immediate treatment. This is at variance with the recent (May 2023) report of Simmons et al. in the New England Journal of Medicine[30], in which they showed that there was a modest reduction in some of the composite complication rates and no material differences in some other complications rates in the neonates in the group that was commenced on immediate treatment as compared with those that had no immediate treatment. Additionally, in the ACHOIS study,[31] the composite endpoint (neonatal death, perinatal injury, hyperbilirubinemia, neonatal hypoglycemia, and hyperinsulinemia) was significantly reduced with antihyperglycemic intervention, and there was also a lower weight gain (by 1.7 kg on average) and a lower incidence of LGA. This is instructive, and its implication for this ongoing study is that the treatment regime and compliance, particularly amongst parturients on treatment, needs to be closely monitored and appraised regularly as the survey progresses.

To further buttress the need to monitor and evaluate our treatment and management of parturients diagnosed with GDM for compliance, the fact that the neonates of the parturients that had risk factors for GDM had a significantly higher average birth weight (macrosomia) compared with the neonates of the controls (p-value = 0.000). Fetal macrosomia results from maternal hyperglycemia, which translates to the fetus having higher blood glucose levels and subsequent hyper-insulinemia that increases fetal body weight.[17,32] Fetal macrosomia is largely reflected in a higher incidence of complications in the newborns of diabetic mothers, as aptly seen with the fetal complications in this study, with 56.2% of neonates of cases having one form of complication or the other. In contrast, only 3.1% of neonates suffered complications in the controls. This is also the trend and pattern seen in reports of previous studies.[32]

Both cases and controls showed similar average gestational Age at delivery. This agrees with other authors' findings, which showed that the average gestational age at delivery was similar in both parturients with GDM and no GDM.[30]

Thus far in this study, the estimation of the prevalence rates in cases and controls was significantly higher in cases compared to the controls, and this seems to have given some credence to the fact that the risk factors based on which the cases were recruited may indeed be predictive of the risk of developing GDM in the pregnant parturients in Delta State Nigeria. It does appear that some complications still occurred even though parturients diagnosed with GDM were commenced on medications. This calls for close monitoring and evaluation of our treatment regimen and compliance with prescribed medications and other ancillary treatment modalities to achieve the pattern described in earlier reports.[14,15] We will follow up with a more detailed and comprehensive report after the ongoing study.

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There are, however, no conflicts of interest.

Table 4: Maternal outcomes

Parameter	Cases n (%)	Controls n (%)	p-value
Mode o delivery			
SVD	16 (50)	29 (90.6)	0.0010
Forceps	4 (12.5)	0 (0)	
Vacuum	0	1 (3.1)	
CS	12 (37.5)	2 (6.3)	0.0065
If vaginal delivery, any complication?			
Yes	8 (12.5)	1 (3.1)	0.0309
No	24 (75. 5)	31 (96.9)	
If yes, please specify			
Genital laceration	5 (15.6)	0	
PPH	**6 (18.8)	1 (3.1)	0.0452
If CS, indication (please specify)			
Fetal distress	5 (15.6)	2 (6.25)	0.8442
Fetal macrosomia	4 (12.5)	0	<0.001
Fetopelvic disproportion	3 (9.4)	0	
EBL			
<500ml	12 (37.5)	31 (96.9)	
≥500ml	20 (62.5)	1 (3.1)	0.00001.

**Some cases that had genital tract lacerations also had PPH

Table 5: Fetal outcomes

Parameter	Cases n (%)	Controls n (%)	p-value
Live birth			
Yes	32 (100)	32 (100)	NS
No	0	0	
Stillbirth			
Yes	0	0	
No	32 (100)	32 (100)	NS
Average Gestational age	38.25 (0.886)	38.25 (0.463)	1.000
Average birth weight	4.02 (0.231)	3.44 (0.226)	0.000
APGAR score in 5 mins	9.75 (0.707)	10.00 (0.000)	0.351
Neonatal complications*			
Yes	18 (56.2)	1 (3.1)	
No	14 (43.8)	31 (96.9)	0.00001.
If yes, specify			
Trauma**	10 (55.6)	1 (3.1)	0.0317
Hypoglycemia	7 (38.9)	0	
Fracture	1 (5.5)	0	

*some neonates suffered more than one complication ** Bruises, minor lacerations

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Comparative assessment of renal volume and doppler velocimetric indices among subjects with sickle cell disease and controls in Benin, Nigeria.

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Abstract

Background: Sickle Cell Disease (SCD), a hereditary blood disorder caused by an abnormality in haemoglobin can be complicated by impairment of renal function. Renal Doppler ultrasound has been found to be an effective method of evaluating reno-vascular events prior to abnormal laboratory renal function tests.

Aim and Objectives: This study aimed to evaluate and compare the renal volume (RV), intra-renal resistive and pulsatility indices (RI, PI) among sickle cell patients and controls in UBTH, Benin City using ultrasonography.

Materials and Method: This was a cross-sectional comparative study of renal volume, intra-renal resistive and pulsatility indices among 50 sickle cell disease patients attending sickle cell clinic of the University of Benin Teaching Hospital and equal number of "Age and Sex" matched controls. The study was conducted using a 2-8MHz curvilinear transducer of a SONOACE X6 (Medison Inc., Korea 2010) Doppler ultrasound machine.

Data analysis: Collated data was analysed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp, Armonk. N.Y., USA)

Results: The mean age for the HbSS subjects was 22.4±3.7years while that of the control group was 23.3 ±4.5 years. The left RV was higher than the right RV in HbSS and HbAA: 169.3±40.2cm^3 versus 162.2±40.3cm^3 and 153.9±30.9cm^3 versus 134.7±26.4cm^3 respectively. The mean RV, RI and PI was significantly higher in HbSS than controls (RV: 165.8±39.8cm^3 versus 122.9±13.4cm^3; p = 0.0001, RI: 0.74±0.02 versus 0.61±0.04; p = 0.0001, PI: 1.43±0.06 cm^3 versus 0.90±0.05; p = 0.0001).

Conclusion: Renal volume, RI and PI were statistically significantly higher in HbSS patients than controls.

Keyword: Sickle cell disease, renal volume, renal Doppler indices, ultrasonography

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INTRODUCTION

Sickle cell disease (SCD) is a genetic disorder in which there is an alteration in the normal globin chain within the red blood cell (RBC), which causes rigid sickling of the cell, leading to vascular occlusion, and ischemia in multiple organs[1].

Mutation in HbB (haemoglobin subunit beta) gene which encodes for the beta-globin of haemoglobin molecule results in SCD. The normal human haemoglobin molecule is composed of two alpha and two beta globin chains[2]. A single mutation leads to replacement of Glutamic acid with Valine in position 6 of the beta-globin chain resulting in mutant form of Hb known as sickle Hb (HbS), double mutation involving both globin chains results in HbSS also known as sickle cell anemia (SCA)[2,3].

Nearly 90% of the world's SCD population live in three countries: Nigeria, India and the Democratic Republic of Congo where the disease affects up to 2% of the population and the carrier prevalence rate (sickle cell trait) is as high as 10 to 30%[4-7]. In Nigeria, carrier prevalence is about 20 to 30%[8,9]. Nigeria alone has been estimated to have at least 150,000 newborn with sickle cell annually[4,10]. The prevalence of SCD in newborn is 3% in Benin City[11]. A retrospective study in Benin City, Nigeria revealed SCD prevalence of 2.39% and a carrier rate of about 23%[12].

DeoxyHbS polymerizes when its concentration reaches a critical threshold leading to RBC sickling. Sickled RBC is prone to haemolysis and causes vascular stasis[13]. Stasis induces vascular occlusion, which either leads to infarction or resolves and causes ischemia-reperfusion[13]. Recurrent episodes of ischemia-reperfusion in the microcirculatory bed amplify organ injury because they induce inflammation and endothelial dysfunction, both regionally and systemically[13]. Endothelial dysfunction promotes the adhesion of RBCs and WBCs to the endothelium. Adhesion impedes the transit of RBCs through the microcirculation, thereby promoting RBC sickling, vascular stasis and vascular occlusion[13]. Additionally, sickle RBCs exhibit abnormally high adhesion to the endothelium, owing to acquired membrane changes and to retained adhesion receptors on the reticulocytes[14,15].

Renal vasculopathy in SCD include cortical hyper-perfusion, medullary hypo-perfusion and increased stress-induced vaso-constrictive response which manifests by increased pulsatility index (PI) and resistive index (RI) values[16].

Studies in Nigeria have reported a range of intra-renal resistive index (RI) of 0.56 to 0.68 in healthy subjects[17,18]. An RI value of 0.70 is considered as the upper limit of normal, when increased; it is regarded as a non-invasive marker of renal histological damage in various pathological conditions and an early sign of renal impairement[19].

The study was carried out to sonographically evaluate and compare renal volume, intra-renal RI and PI among sickle cell disease and non-sickle cell disease patients in UBTH, Benin City.

MATERIALS AND METHODS

The study was a prospective cross-sectional study comparing renal volume and Doppler indices amongst 50 sickle cell disease patients and 50 non-sickle cell disease volunteers as control in UBTH, Benin City carried out between February 2020 and August 2020.

Approval for this study was obtained from the Ethics and Research Committee of the University of Benin Teaching Hospital. Subjects were examined after informed consent has been granted following a thorough explanation of the study objectives and method of examination.

Inclusion criteria for subjects were diagnosed HbSS adults attending the sickle cell clinic of the

University of Benin Teaching Hospital and subjects in steady state (no history of painful crisis, inter-current illness in the past 4weeks, no history of blood transfusion in the previous 4 months, no treatment with medications such as antibiotics in the past 3 weeks[20]). Inclusion criteria for control included adults of both sexes with no co-morbidity and laboratory confirmation of HbAA.

The subjects were encouraged to fast overnight (12 hours) on the day of the examination. Assurance of confidentiality was given. A detailed history was obtained from both groups of patients and entered into a questionnaire assessing socio-demographic and medical history.

The temperature of the subjects was obtained using an infrared thermometer and recorded. The respiratory rate was recorded by counting the number of breaths per minute with the aid of a stopwatch. The radial pulse was counted for a minute and recorded with the aid of a stopwatch. The blood pressure of the subjects and volunteers was recorded using the mercury sphygmomanometer with the cuff tied and inflated around the mid-arm with the subject in sitting position. The height and weight were measured using a clinic stadiometer and a calibrated scale (Avery Co. Ltd, England 1981). The body mass index BMI was calculated using the formula shown below[21].

$$BMI\ (kg/m^2) = Weight/Height^2$$

The blood sample was obtained from the thumb pulp of all subjects following a needle prick and their fasting blood sugar was determined using calibrated test strips of an ACCU-CHEK glucometer (Roche, Mannheim, Germany). PCV, urine micro-albuminuria was obtained from sickle cell patient case notes if done within the last 4 weeks or samples were obtained for the test. Samples from HbAA volunteers were sent for urine micro-albuminuria and PCV. Those with abnormal test parameters were excluded from the study.

Grey Scale and Doppler Ultrasonography

Subjects were made to lie on the couch in supine position and the abdomen was exposed from the xiphisternum to the symphysis pubis. Acoustic gel was applied on the abdomen. All scans were done using a 3.5- 5.0MHz curvilinear array transducer of a SONOACE X6 ultrasound machine and utilizing colour Doppler.

To obtain renal length and thickness the transducer was placed on the longitudinal axis of the kidney. The length (L) was obtained by determination of the longest distance between the superior and inferior poles of the kidney using electronic calipers and the thickness (T) was taken as the widest distance between the anterior and posterior walls of the kidney (figures 1). Renal width (W) was taken as the maximum transverse distance on a transverse scan at the level of the hilum (fig 2). These measurements (L, T, and W) were taken three times for both sides and the average was taken to reduce intra-observer variation. The ellipsoid formula in built within the machine was used to determine the renal volume (L x T x W x 0.523) by obtaining the length, thickness, and width of each kidney.

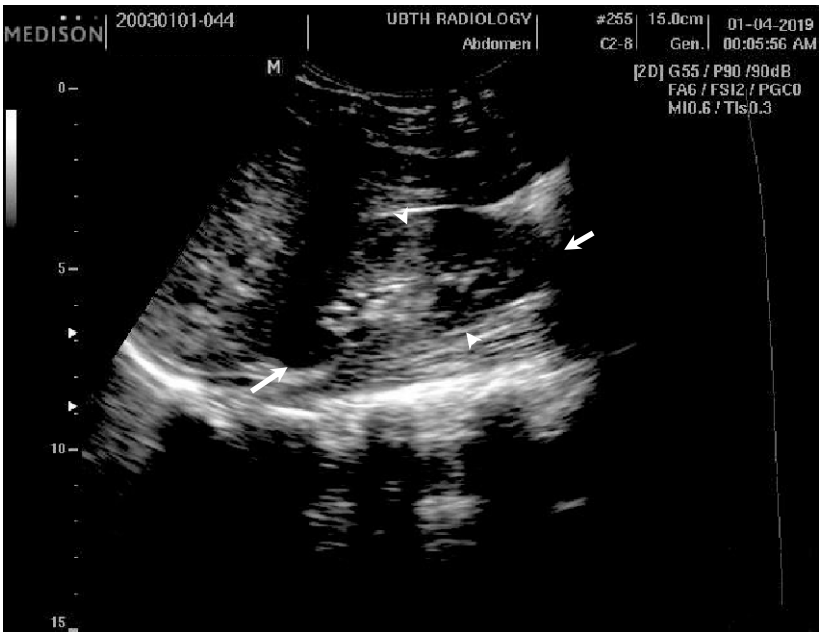

Figure 1:A trans-abdominal B-mode sonogram showing a longitudinal section of the right kidney and the points of measurement of renal length (long arrows) and thickness (arrow heads)

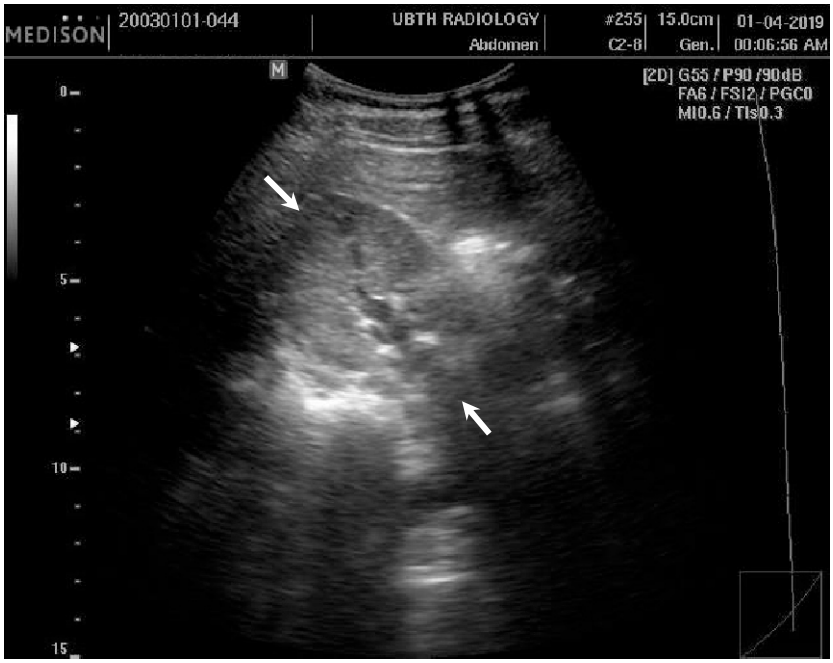

Figure 2**:** A trans-abdominal B-mode sonogram showing a transverse section of the right kidney at the level of the hilum and points of measurement of the renal width (arrows).

The resistive index (RI) is the ratio of the difference in the peak systolic velocity (PSV) and end diastolic velocity (EDV) to the peak systolic velocity within a vessel while the pulsatility index (PI) is the ratio of the difference in peak systolic velocity and end diastolic velocity to the mean velocity.

The patients were positioned in the right lateral decubitus to visualize the left renal artery and the left lateral decubitus to visualize the right renal artery. Using a 3.5MHz curvilinear array transducer of a SONOACE X6 ultrasound machine and utilizing colour Doppler with a wide colour box, the segmental vessels were located in the renal hilum. They were traced as they branch out to become the inter-lobar arteries which lie between the renal pyramids. The arcuate arteries were traced as they branch off from the inter-lobar arteries at the base of the renal pyramids.

The colour box size was minimized prior to sampling with a focus on the vessels of interest. Pulse wave Doppler with an incidence angle at 0 degrees, a Doppler gate of 1mm and a minimum pulse repetition frequency that does not produce aliasing was used to acquire the waveform. The patients were asked to hold their breath and spectral tracing for the inter-lobar arteries was obtained with a stable signal and adequate waveform. The following Doppler parameters were obtained for the inter-lobar arteries on each side: PSV, EDV, RI and PI. Three values were obtained for inter-lobar arteries from the upper, middle, lower poles and the mean values of RI and PI were recorded (fig 3). At the completion of the evaluation, the acoustic gel was gently cleaned off the abdomen using a hand towel and the subject was asked to come down from the examination couch.

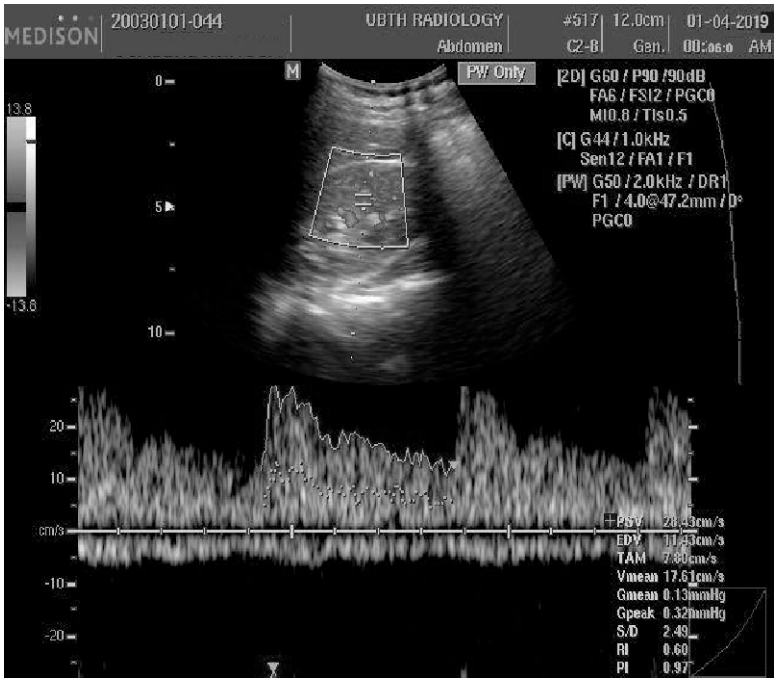

Figure 3: Spectral Doppler tracing of the right middle pole inter-lobar artery.

Data Analysis

The data obtained was entered into excel spread sheet and analysis was done using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp, Armonk. N.Y., USA). Data comparison (statistical test of significance) was done with a t-test and ANOVA (analysis of variance) for continuous variables and a Chi-square test for categorical data. Frequency and contingency tables, charts and graphs as appropriate were used to present the results. A 95% confidence interval was used and statistical significance was considered only for $p \leq 0.05$.

RESULTS

Socio-Demographic Characteristics of Participants

A total of 100 subjects consisting of 50 subjects with HbSS and an equal number of age and sex matched HbAA controls participated in this study.

Table 1 shows the socio-demographic parameters (age, gender, educational status and occupation) of the study participants. The age range for both study groups was 18-35 years. The mean age for the HbSS subjects was 22.4±3.7years while the mean age for the control group was 23.3 ±4.5 years. The difference in the mean age between both groups was not statistically significant (p=0.258).

The HbSS group and the HbAA group were aged matched, the difference in age distribution between the HbSS group and the HbAA groups was not statistically significant ($p = 0.476$). This is shown in Table 1.

The distribution according to gender was equally matched between the groups; 24(48.0%) males and 26(52.0%) females for the HbSS group and 30(60.0%) males and 20(40.0%) females in the HbAA group. The difference in the sex distribution between both groups was not statistically significant (p=0.229) as shown in Table 1.

The secondary level of education was highest among the SCD patients 32 (64.0%) while most of the control had a tertiary level of education 29 (58.0%).

Majority of the study participants were students [34 (68.0%) and 32 (36.0%) for HbSS and HbAA groups respectively]. The difference in occupation between the study groups was not statistically significant (p= 0.117). These findings are shown in Table 1.

Table 1:Socio-demographic Characteristics of Study Subjects.

Parameters	SCD (N = 50) Frequency (%)	Control (N = 50) Frequency (%)	Chi-square (c^2)	*p*-value
Age group				
18 – 23	31 (62.0)	25 (50.0)	1.468	0.476
24 – 29	15 (30.0)	20 (40.0)		
30 – 35	4 (8.0)	5 (10.0)		
Mean age (years)	22.4±3.7	23.3±4.5	–1.138†	0.258
Gender				
Male	24 (48.0)	30 (60.0)	1.449	0.229
Female	26 (52.0)	20 (40.0)		
Level of Education				
Primary	0 (0.0)	2 (4.0)	7.471§	**0.015***
Secondary	32 (64.0)	19 (38.0)		
Tertiary	18 (36.0)	29 (58.0)		
Occupation				
Civil servant	4 (8.0)	11 (22.0)	5.418	0.117
Self employed	10 (20.0)	7 (14.0)		
Student	34 (68.0)	32 (64.0)		
Unemployed	2 (4.0)	0 (0.0)		

§ Fisher's Exact Test ‡Yates Continuity Correction † Independent t-test

***Statistically significant**

Anthropometric, Clinical and Biochemical Parameters of Study Subjects.

The HbAA group had statistically significantly higher mean height, weight and BMI than HbSS group(1.66±0.06 m^2 against 1.63±0.03 m^2; p=0.001), (72.1 ± 9.3kg against 61.9 ± 6.8kg; *p*=0.0001) and (26.0±2.0kg/m^2 against 23.3±1.9kg/m^2;*p*=0.0001).

Temperature, Pulse rate, respiratory rate and fasting blood sugar were statistically significantly higher in HbSS compared to HbAA: Temperature (36.5±0.8 °C against 36.2±0.4 °C; *p* = 0.028), Pulse rate (80.6±8.6 bmp against 73.8±7.6 bmp; *p* = 0.0001), respiratory rate (18.3±2.6 cpm against 16.8±2.2 cpm; *p* = 0.002) and fasting blood sugar (81.0±7.5mg/dl versus 75.5 ± 9.2mg/dl; *p* = 0.001). Haemoglobin was statistically significantly higher in HbAA than HbSS (15.9±1.2 g/dl against 7.2±0.3 g/dl; *p* = 0.001). These are shown in table 2.

Table 3: Comparison of Renal volume, Resistive and Pulsatility indices of the right with the left kidney in HbSS and Control Subjects

Variables		HbSS (N = 50) Mean ± SD	Control (N = 50) Mean ± SD
Right kidney mean volume		162.2±40.3	117.5±18.3
Left kidney mean volume		169.3±40.2	128.3±16.8
	t-test	–0.885	–3.079
	p value	0.379	**0.003***
Right kidney mean RI		0.7±0.0	0.6±0.0
Left kidney mean RI		0.7±0.0	0.6±0.0
	t-test	–1.363	1.232
	p value	0.176	0.221
Right kidney mean PI		1.4±0.1	0.9±0.1
Left kidney mean PI		1.4±0.1	0.9±0.1
	t-test	–0.135	–0.320
	p value	0.893	0.750

RI: Resistive index, PI: Pulsatility index
***Statistically significant**

Comparison of Renal Volume, Resistive and Pulsatility Indices in HbSS and Control Subjects

The mean RV, RI and PI was significantly higher in HbSS than controls (RV: 165.77±39.85cm^3 versus 122.94±13.39cm^3; p = 0.0001, RI: 0.74±0.02 versus 0.60±0.03; p = 0.0001, PI: 1.43±0.06 versus 0.90±0.05; p = 0.0001) as shown in Table 4.

Table 2: Comparison of Anthropometric, Clinical and Biochemical Parameters of Study Subjects.

Parameters	HbSS (N = 50) Mean ± SD	HbAA (N = 50) Mean ± SD	t-test	df	*p*-value
Height (m)	1.63±0.03	1.66±0.06	–3.599	**98**	**0.001***
Weight (kg)	61.9±6.8	72.1±9.3	–6.282	**98**	**0.0001***
Body Mass Index (kg/m^2)	23.3±1.9	26.0±2.0	–3.599	**98**	**0.0001***
Temperature (°C)	36.5±0.8	36.2±0.4	2.231	98	**0.028***
Pulse rate (bpm)	80.6±8.6	73.8±7.6	–4.148	98	**0.0001***
Respiratory rate (cpm)	18.3±2.6	16.8±2.2	–3.188	98	**0.002***
Haemoglobin concentration (g/dl)	7.2±0.3	15.9±1.2	–49.136	98	**0.0001***
Fasting blood sugar (mg/dl)	81.0±7.5	75.5±9.2	46.321	98	**0.001***

***Statistically significant**

Renal Size in HbSS Group

In the HbSS group, a total of 26 (52.0%) patients had normal kidney size, 21 (42.0%) patients had enlarged kidneys and 3 (6.0%) patients had shrunken kidneys.

Comparison of Renal Volume, Resistive and Pulsatility Indices of the Right Kidney with the Left Kidney in HbSS and Control Subjects

The left mean RV was higher than the right mean RV in both HbSS and HbAA: 169.3±40.2cm^3 versus 162.2±40.3cm^3 and 128.3±16.8cm^3 versus 117.5±18.3cm^3 respectively. These differences were not statistically significant in the HbSS group (p=0.379) but was statistically significant in the HbAA group (p=0.003). There was no difference between the right and left mean intra-renal RI and PI in both HbSS and HbAA (p>0.05) as shown in Table 3.

Table 4: Comparison of Renal Volume, Resistive and Pulsatility Indices in HbSS and Control Subjects

Variables	HbSS (N = 50) Mean ± SD	Control (N = 50) Mean ± SD	t-test	*p*-value
Right renal volume	162.21±40.37	117.54±18.28	4.040	**0.0001***
Left renal volume	169.33±40.15	128.34±16.76	2.157	**0.0001***
Average (right and left) renal volume	165.77±39.85	122.94±13.39	7.205	**0.0001***
Right kidney mean RI	0.74±0.02	0.61±0.04	21.939	**0.0001***
Left kidney mean RI	0.75±0.02	0.60±0.04	24.749	**0.0001***
Average (right and left) mean RI	0.74±0.02	0.60±0.03	25.729	**0.0001***
Right kidney mean PI	1.43±0.06	0.90±0.06	45.205	**0.0001***
Left kidney mean PI	1.43±0.07	0.90±0.05	45.177	**0.0001***
Average (right and left) mean PI	1.43±0.06	0.90±0.05	47.538	**0.0001***

RI: Resistive index, PI: Pulsatility index,
***statistically significant**

Correlation of Resistive and Pulsatility Indices with Age, BMI and Hb Among HbSS Subjects

RI showed a statistically insignificant positive correlation with age in the HbSS group (r=0.268; p=0.060), a positive but statistically insignificant correlation with BMI (r=0.215; p=0.133) and a statistically insignificant negative correlation with Hb (r= -0.042; p=0.770) as shown in Table 5. PI showed a statistically significant positive correlation with age in the HbSS group (p=0.022), a positive but statistically insignificant correlation with BMI (p=0.244) and a statistically insignificant negative correlation with Hb (p=0.159) as shown in Table 5.

Table 5: Correlation of Resistive and Pulsatility Indices with Age, BMI and Hb among HbSS Subjects.

Parameters	PI		RI	
	Pearson's correlation coefficient	***p*-value**	**Pearson's correlation coefficient**	***p*-value**
Age	0.322	**0.022***	0.268	0.060
Body mass index	0.168	0.244	0.215	0.133
Haemoglobin concentration	–0.202	0.159	–0.042	0.770

***Statistically significant**

CORRELATION OF RENAL VOLUME, RESISTIVE AND PULSATILITY INDICES WITH AGE AND BMI AMONG HbAA SUBJECTS

RV showed a statistically insignificant positive correlation with age among the HbAA group (r = 0.190; p = 0.186) while it was statistically significant and positively correlated with BMI (r = 0.280; p = 0.049) as shown in Table 6.

RI showed a statistically significant positive correlation with age among the HbAA group (r = 0.456; p=0.001) while it was statistically insignificant and positively correlated with BMI (r = 0.020; p=0.888) as shown in Table 6.

PI was negatively correlated with age and BMI among the HbAA group but it was not statistically significant (p=0.456 and p=0.208) as shown in table 6.

Table 6: Correlation of Renal Volume with Age and BMI among HbAA Subjects.

Parameters	RI		PI		RV	
	Pearson's correlation coefficient	***p*-value**	**Pearson's correlation coefficient**	***p*-value**	**Pearson's correlation coefficient**	***p*-value**
Age	0.456	**0.001***	–0.106	0.465	0.190	0.186
Body mass index	0.020	0.888	–0.181	0.208	0.280	**0.049***

***Statistically significant**

DISCUSSION

This study sets out to compare renal volume and Doppler indices among sickle cell disease patients with age and sex-matched volunteers as controls. In this study, 50 SCD patients and 50 HbAA volunteers were recruited with an age range of 18 to 35 years and a mean age of 22.4±3.7years and 23.3±4.5years respectively. Aikimbaev *et al.,*[22] worked on spectral pulsed Doppler sonography in sickle cell disease among 40 patients in Turkey and Ibinaiye *et al.,*[23] worked on the incidence of abdominal ultrasound abnormalities in patients with sickle cell anaemia in Zaria, Nigeria among 74 patients. In both studies, the mean ages were 24.2±7.6years and 23.2±5.3years respectively which are similar to the present study because both studies were on adult SCD patients.

There were more females in this study which is similar to the findings in other studies done by Shogbesan *et al.,*[24] and Hamim[25]. This has been attributed to higher health-seeking behaviour among females[26,27].

The age group with the highest number of SCD patients and HbAA volunteers in this study was 18-23years. However, a study done by Geofery *et al.,*[28] on sonographic evaluation of abdominal organs in SCD among 252 subjects in Nigeria showed the highest number of patients and control in the age group 10-16years. This difference in the age group with the highest number of patients and controls may be due to a much higher sample size of 252 used in their study.

In this study HbAA group had significantly higher mean height, weight and BMI than HbSS group. During childhood and adolescence, SCD is associated with growth retardation, delayed sexual maturation and being underweight. Growth delay during puberty in adolescence with SCD is independently associated with decreased Hb concentration and increased total energy expenditure[29].

HbSS group had statistically significant higher mean pulse rate of 80.6±8.6. This finding is similar to that of Aikimbaev *et al.,*[22] who reported a mean pulse rate of 86.5.8±12.3 for HbSS group. This finding may be related to the compensatory mechanisms in SCD patients in response to anemia which is part of the complications of sickle cell disease.

Haemoglobin was significantly higher in HbAA group than HbSS group. Repeated crisis in SCD patients predispose them to lower Hb concentration.

The left mean RV was higher than the right mean RV in HbSS group and HbAA group in this study. These differences were not statistically significant in the HbSS group. Shilan *et al.,*[30] in Iraq and Udoaka *et al.,*[31] in Nigeria found significantly high mean left renal volume in comparison to the right mean renal volume in their study of healthy adult volunteers. This was believed to be due to the location of the liver which may not allow comparable vertical growth of the right kidney to that which is attained by the left kidney[30].

The mean RV was higher in the HbSS group than the HbAA group. This finding was statistically significant ($p = 0.0001$). Previous studies reported renal enlargement in SCD patients[23-25, 28, 32-35]. Glomerular hypertrophy, vascular dilatation, increased renal blood volume, engorgement of vessels and interstitial oedema have been suggested as likely contributors to renal enlargement in SCD patients[36,37].

In the HbSS group, 52.0% had normal kidney size, 42.0% had enlarged kidneys and 6.0% had shrunken kidneys. Previous studies done by Mapp

et al.,[38] in the United States of America found renal enlargement in 50%, Ibinaiye *et al.,*[23] in Nigeria found renal enlargement in 2.7% and renal size reduction in 27.1%, Nosiba *et al.,*[35] in Sudan found renal enlargement in 22.3%, Balci *et al.,*[33] in Turkey found renal enlargement in 30.1%, and Bhushita *et al.,*[39] in India found renal enlargement in 19% and shrunken kidney in 37%. The differences in the findings may be due to variation in ethnicity or race, methodology and differences in population size. Other factors suggested include; focal scarring, interstitial fibrosis and analgesic abuse as likely contributors to reduced renal size[40]

There was a positive correlation of RV with age among the HbAA group but it was not statistically significant. However, RV was positively correlated with BMI among the HbAA group and this was statistically significant. Studies done by Shilan *et al.,*[30] in Iraq and Udoaka *et al.,*[31] in Nigeria on healthy volunteers showed a statistically significant positive correlation between RV with age and BMI.

The mean RI and PI were significantly higher in HbSS than in HbAA in our study. These findings are in agreement with previous studies[22,24,41]. The difference in the values of RI was believed to be due to the difference in the haplotype of the HbSS gene in the study population[42]. The Arab-Indian haplotype with high foetal haemoglobin and low severity may account for the lower RI value found by Kishor *et al.,*[41] in India while the Benin haplotype found in Nigeria with intermediate foetal haemoglobin and intermediate severity may account for the higher RI found in this study[42]. The increase in RI and PI among SCD patients has been ascribed to increased renal vascular tone resulting from various vascular occlusive mechanisms (vascular intima hyperplasia, thrombosis, altered vascular reactivity and frank vasospasm) occurring in sickle affected kidneys[16]. High RI among individuals with HbSS compared to those with HbAA has been described as an early predictor of reno-vascular changes in SCD which can guide clinicians in monitoring other laboratory values to expedite early and appropriate treatment[41].

This present study found a positive correlation of RI with age among the HbSS group but it was not statistically significant. A previous study in Sudan[43] showed a significant positive correlation of RI with age in SCD patients. Renal RI is a complex interaction between renal interstitial pressure, peripheral vascular compliance and systemic hemo-dynamics, all of which are deranged with increasing age in SCD patients[19].

In this study there was a positive correlation of RI with BMI among HbSS and HbAA group respectively but it was not statistically significant. Mohamed *et al.,*[43] in their renal Doppler study of SCD patients and control reported a positive correlation of RI with BMI in SCD patients and control. An increase in BMI is associated with an increase in renal vascular resistance and glomerular filtration fraction[44].

RI was negatively correlated with Hb concentration among HbSS group but it was not statistically significant. Aikimbaev *et al.,*[22] in their study reported RI to be negatively correlated with Hb concentration. Haematological abnormalities are associated with alteration in renal vascular resistance; hypoxaemia is associated with an increase in renal vascular tone in SCD patients[45].

A thorough literature search showed that apart from one study in Southwest Nigeria,[24] this is the second documented study on renal Doppler velocimetry in in Sickle cell patients in Nigeria.

In conclusion, renal volume, RI and PI were

found to be significantly higher in the HbSS group than the HbAA group.

RECOMMENDATIONS

We recommend that renal Doppler scan should be an integral part of routine follow up examination of sickle cell disease patients and that further studies using large scale, community-based cohort of sickle cell disease patients in different centres in Nigeria should also be done to validate the accuracy of the findings in this study.

LIMITATION OF STUDY

- Ultrasound is operator dependent and thus may result in intra-observer error. This was minimized by taking the average of three measurements.
- Respiratory movement of the abdomen made it difficult taking Doppler spectral tracing but this was minimized by asking the patient to hold his/her breathe at the time of Doppler insonation of the intra-renal arteries.·

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