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INTER-INDIVIDUAL VARIABILITY IN PAIN AMONG HEALTHY AFRICAN VOLUNTEERS: EXPERIMENTAL EVIDENCE

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ABSTRACT

BACKGROUND:
Pain, either clinical or experimental, is characterised by a high degree of inter-individual variability, associated with environmental, physiological and genetic factors. This study was designed to investigate sex and ethnic differences in pain perception and the roles of serum glutamate and serotonin concentrations on experimental pain responses among healthy Nigerian adult population.

METHODS/SUBJECTS:
One hundred and sixty-one (161) apparently healthy volunteers between the ages of 20 to 65 years were recruited for the study. Experimental pain was induced using ischaemic pain tests and pressure pain. Glutamate and serotonin were analysed in serum by ELISA.

RESULTS
The result showed that experimental pressure and ischaemic pain thresholds were significantly higher in males than in females Male subjects were found to have lower serum glutamate concentration than females. Serum glutamate concentration was found to correlate positively with pressure pain threshold, while serum serotonin concentration was found to correlate negatively with ischaemic pain tolerance.

CONCLUSION:
Perception of experimentally induced pressure and ischaemic pain among healthy adult Nigerian subjects showed variation by sex. There is a positive correlation between serum glutamate and experimental pressure pain threshold, and a negative correlation between serum serotonin and experimental ischaemic pain tolerance.

KEYWORD
Variability; ischaemic pain; pressure pain; glutamate; serotonin

INTRODUCTION

As a multi-dimensional sensory experience, pain is known to be intrinsically unpleasant[1], and its perception has been found to vary significantly even among individuals of the same population. What one categorises as low pain, another person may present same as moderate or even high pain. Other experimental reports demonstrate a high degree of inter-individual variability in pain, which is associated with multiple bio-psycho-social factors[2,5], including household income6. Differences in pain intensity have been found to exist even within the same individual over time[7]. Interpretation and expression of pain can be shaped by cultural values, beliefs and practices[8].

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Pain responsiveness can also be assessed by pain ratings (e.g. using visual analogue scale). Different studies use different modalities, such as temperature, electro-cutaneous stimuli and pressure, which do not elicit the same pain responses and outcome measures between them are not always related[9]. Pain treatment has also been shown to be associated with significant inter-individual variability, and as such, will require a personalized approach[10]. There is growing complexity on individual differences in response to opioids, as well as the differential responses that an individual may have to different opioids, which are influenced by genetic and molecular factors[11], as well as by phenotypic determinants such as psychosocial factors, gender and pain sensitivity[12]. There is growing need to personalise the prescription of pain medicine, so that patients receive treatments that are most suited to them.

Mechanically evoked pain, and in particular the pressure pain threshold (PPT), is a popular model for inducing acute experimental pain. Algometry is a useful technique in determining pressure pain measures, and has been used widely in evaluation of both clinical and experimental pain[13]. Pressure-induced tenderness of soft tissues causes sensitization of peripheral nociceptive receptors in the affected area, which is associated with painful conditions resulting from repetitive trauma, inflammation, excessive strain or psychosocial stress[14].

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The serotonin transporter (5-HTT) regulates the entire 5-HT system via modulation of extra-cellular fluid 5-HT concentration by a sodium-dependent reuptake of serotonin into the pre-synaptic neuron[16'17].Sex differences in 5-HT function may underlie the known sex difference (women > men) in the prevalence of depression and may also impact pharmacological treatments that target 5-HT neurotransmission[18], including pain severity.

METHODOLOGY

Data obtained from one hundred and sixty-one (161) apparently healthy volunteers aged 20 to 65 years was used for analysis in this study. Subjects were recruited based on convenience, agreeing with the study protocol, qualified and willing to participate. Selected individuals were taught and given basic understanding about pain physiology and were informed about the experimental procedure (verbally and in writing), as well as risks and contra-indications of all procedures. Informed consent was obtained from all the subjects, as well as well as ethical approval from the Ahmadu Bello University Teaching Hospital Health Research Ethics Committee (ABUTHHREC). Criteria for exclusion include signs of significant psychiatric disorder, prior or present alcohol abuse, daily use of analgesics or any neurological or inflammatory disease that could interfere with pain perception and pain report, such as diabetes, peripheral or central neuropathy, a chronic pain disorder, or current pain condition.

Pressure and ischaemic pain procedures were conducted in separate sessions by trained personnel who were blind to the experimental outcome. Pressure pain was assessed by applying pressure at the first dorsal interosseous muscle[13], using pressure algometer (66LB/30KG; Fabrication Enterprises. Inc. NW, USA). The pressure at which pain was first reported was recorded (in kg). Pressure pain was recorded on the dominant hand only, as reports indicated no significant difference between dominant and non-dominant sides[19,20] · Hogeweg et al.[21] and Rui et al.[22] also reported no significant differences in pressure pain thresholds between the same points on either side of the body.

Ischaemic pain was induced using the sub-maximal effort tourniquet procedure[23]. The left arm was exsanguinated by elevating it above heart level for 30 sec. The arm was then occluded using segmental blood pressure cuff inflated to 240 mmHg. Subjects were asked to perform 20 handgrip exercises of 2 seconds duration at 4 seconds intervals. They were asked to say ‚pain' when they first feel pain (threshold) and to continue until the pain

became intolerable (tolerance).

The procedure was terminated if the subject failed to respond after 15 min of initiation[24]. The time for pain threshold and pain tolerance were recorded in seconds. At the end of the experimental pain procedures, about 4 ml of venous blood was collected using syringe and placed in plain bottle, allowed to clot and centrifuged using bench centrifuge at 1000 × g for five minutes. Collected serum was used for glutamate and serotonin assays.

Serum glutamate concentration was determined according to the method described by Koochekpour et. al.[25], using human glutamate Enzyme Linked Immunosorbent Assay (ELISA) kit (GA-E5446HM, GenAsia Biotech Co. Ltd., Shanghai, China), following manufacturers' instructions. Briefly, about 25 µl of the prepared standards, controls and samples were pipetted into the Human Glutamate monoclonal antibody 96-well pre-coated plates. The plates were covered with adhesive foil and incubated for 2 hours at room temperature. The foil was removed and the plates were washed 3 times by adding 300 µl of Wash Buffer. One hundred microlitre of the enzyme conjugate was pipetted into the wells and incubated for 30 minutes at room temperature. The contents were discarded and the plates washed three times by adding 300 µl of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on paper towel. 100 µl of substrate was
pipetted into all wells and incubated for 30 minutes at room temperature, followed by 100 µl of stop solution. Absorbance of the solution in the wells was read using Rayto RT-2100C microplate reader at 450 nm. Standard curve was plotted with concentration of the standards on x-axis, and absorbance on the y-axis, from which corresponding glutamate concentrations of the samples were determined (in µg/ml).

Serum serotonin concentration was determined using human serotonin Enzyme-linked Immunosorbent Assay kit (GA-E1145HM, GenAsia Biotech Co. Ltd., Shanghai, China). Twenty microlitre of standard, control and samples were pipetted into the respective wells of the coated microtiter strips, followed by 100 µl anti ST antibodies labelled with biotin into all wells. The preparation was then incubated for 30 minutes at room temperature. The contents were discarded and the wells were washed thoroughly with each 250 µl Wash Buffer. Washing was repeated 3 times and wells were dried with paper towel. One hundred microlitre enzyme conjugate was pipetted into the wells and incubated for 15 minutes at room temperature and washing was repeated. One hundred microlitre of substrate was pipette into all wells and incubated for 20 minutes at room temperature, followed by addition of 100 µl stop solution into each well. Optical density was read in a Rayto RT-2100C microplate reader set at 450 nm wavelength. Standard curve was plotted with concentration of the standards on x-axis, and absorbance on the y-axis, from which corresponding concentrations were determined (in ng/ml).

Statistical analyses were carried out using SPSS version 23 software for windows (SPSS Inc, Chicago, IL, USA) and data obtained are presented here as mean ± SEM. Sex differences were analyzed using independent-samples t-test, while ethnic differences were analyzed using one way ANOVA. Associations between serum glutamate and serotonin concentrations with experimental pain outcomes were determined using linear regression. Values of $p \leq 0.05$ were considered to be statistically significant.

RESULTS

A total of two hundred and seventy nine (279) participants volunteered for the study. One hundred and eighteen (118) subjects were excluded using the exclusion criteria, thus, data obtained from one hundred and sixty one (161) participants were used for analysis. The subjects included 91 males (56.5%) and 70 females (43.5%). Two
subjects did not report their age, and information on ethnicity was also missing in three subjects. Among the studied population, 54 (33.5%) were single, 101 (62.7%) married, 4 (2.5%) divorced and 2 (1.2%) widowed. Two subjects (1.2%) reported having no formal education, one (1.2%) had only primary education, eight (5.0%) had secondary education, while the rest (148 or 91.9%) reported having tertiary education (Tables 1 and 2).

TABLE 1: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS, SHOWING FREQUENCY AND PERCENTAGE DISTRIBUTION FOR SEX, AGE AND TRIBE IN AN ADULT NIGERIAN POPULATION IN ZARIA

Characteristic	Description	Frequency	Percentage
Sex	Male	91	56.5
	Female	70	43.5
	Total	161	100
Age (years)	20 – 30	58	36.0
	31 – 40	50	31.1
	41 - 50	27	16.8
	>50	24	14.9
	Total	159	98.8
Ethnicity	Hausa	98	60.9
	Yoruba	25	15.5
	Fulani	13	8.1
	Others	22	13.7
	Total	158	98.1

TABLE 2: SOCIO-ECONOMIC CHARACTERISTICS, SHOWING FREQUENCY AND PERCENTAGE DISTRIBUTION FOR MARITAL STATUS AND EDUCATIONAL LEVEL IN AN ADULT NIGERIAN POPULATION IN ZARIA

Characteristic	Description	Frequency	Percentage
Marital Status	Single	54	33.5
	Married	101	62.7
	Divorced	4	2.5
	Widowed	2	1.2
	Total	**161**	**100**
Education	None	2	1.2
	Primary	1	0.6
	Secondary	8	5.0
	Tertiary	148	91.9
	Total	**161**	**100**

Ischaemic pain threshold was significantly higher in males (34.92±1.57) compared to females (26.89±1.39) (p = 0.000), but there was no statistically significant sex difference in ischaemic pain tolerance (p = 0.520), though it's slightly higher in males (22.85±1.38) than females (21.51±1.55).

Experimental pressure pain threshold was significantly higher in males (6.93±0.15) than females (5.99±0.18) (p = 0.000) (figure 1). Ischemic pain tolerance was significantly higher among Fulani (18.73±1.72) than all the other ethnic groups (p < 0.05), while pressure pain threshold was significantly higher among Yoruba (7.32±0.30) compared to Hausa (6.28±0.12) (p = 0.008). Male subjects had lower serum glutamate concentration (25.26±2.39) compared to the female subjects (35.85±3.56), and the difference was statistically significant (p = 0.019). Similarly, serum serotonin concentration was lower in males (120.22±8.30) than in females (123.27±8.67), but the difference was not statistically significant (p = 0.800) (Figure 3). There was a statistically significant positive correlation between serum glutamate concentration and experimental pressure pain threshold (p = 0.027; R^2 = 0.0549), and a significant negative correlation between serum serotonin concentration and ischaemic pain tolerance (p = 0.007; R^2 = 0.1107).

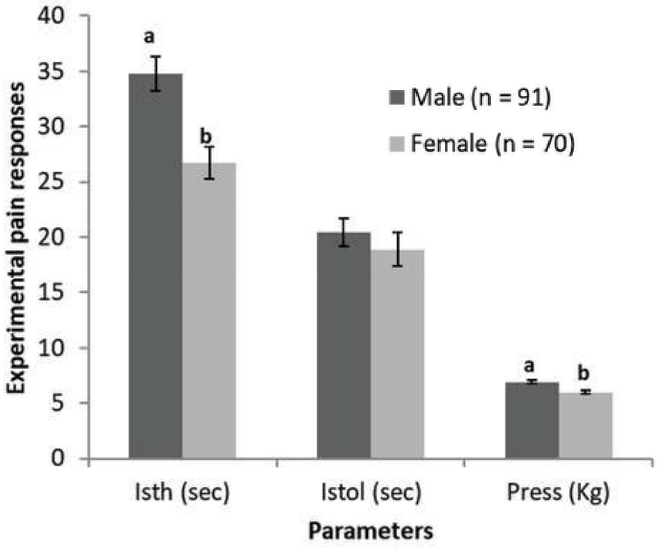


Figure 1: Sex differences in Experimental Pain Responses in a Healthy Nigerian Adult Population. a,bMean difference is statistically significant (p<0.05) for corresponding variable between male and female (t-test). Isth (ischaemic pain threshold); Istol (ischaemic pain tolerance); Press (pressure pain threshold).

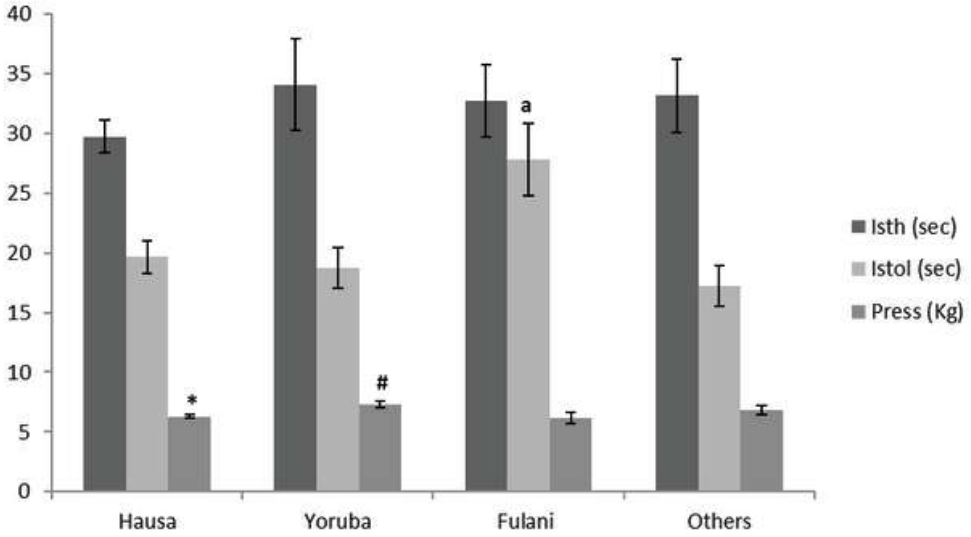


Figure 2: Ethnic differences in Experimental Pain Responses in a Healthy Nigerian Adult Population. Statistically significant difference compared to other groups (Istol; ANOVA). Mean difference is statistically significant (Isth; ANOVA).
Isth (ischaemic pain threshold); Istol (ischaemic pain tolerance); Press (pressure pain threshold).

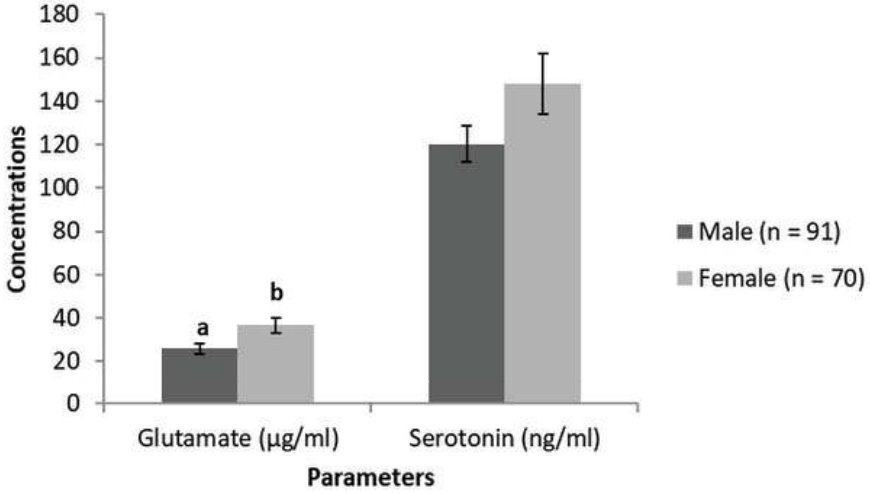
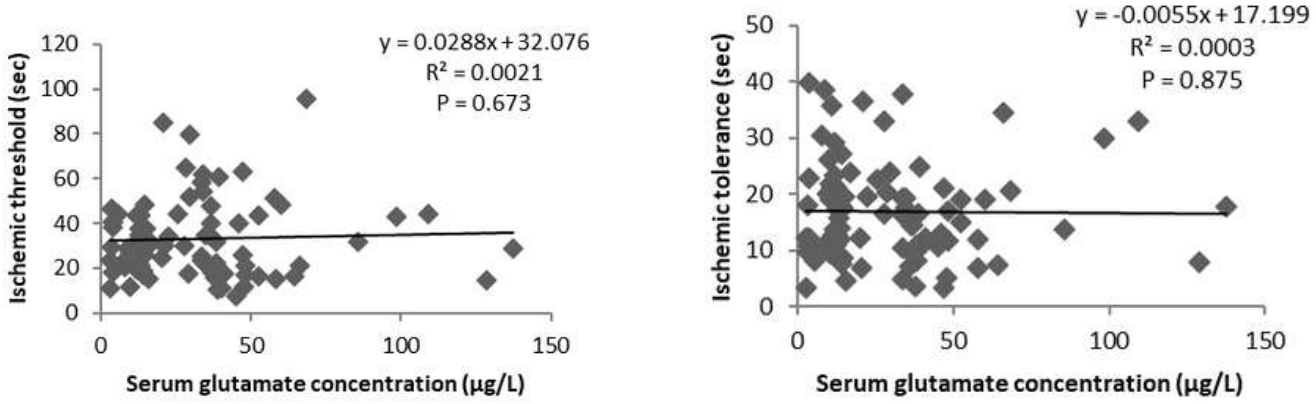


Figure 3: Sex Differences in Serum Glutamate and Serotonin Concentrations in a Healthy Nigerian Adult Population. a,bMean difference is statistically significant ($p < 0.05$) between males and females (t-test).



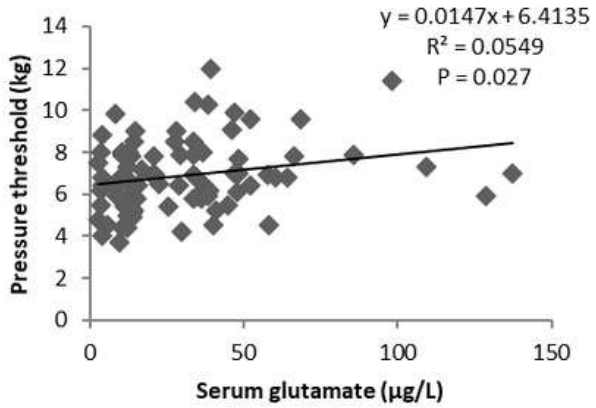
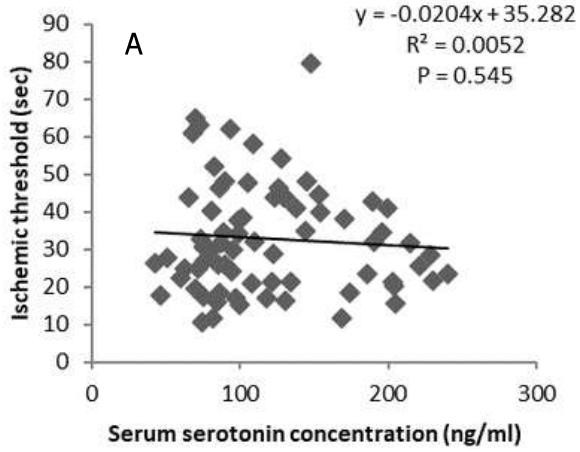
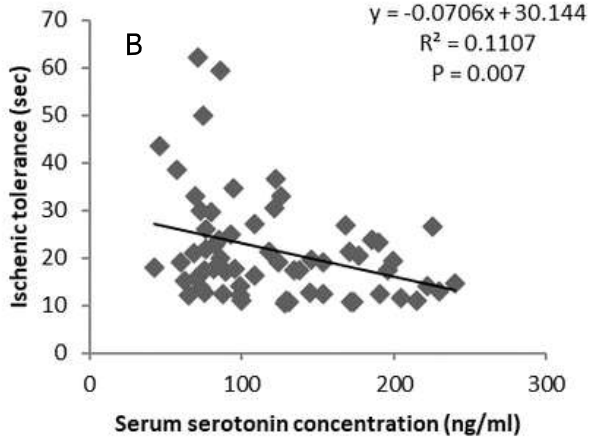


Figure 4: Relationships between serum glutamate concentration and ischaemic pain threshold (A), ischaemic pain tolerance (B) and pressure pain threshold (C) among healthy Nigerian adult population.





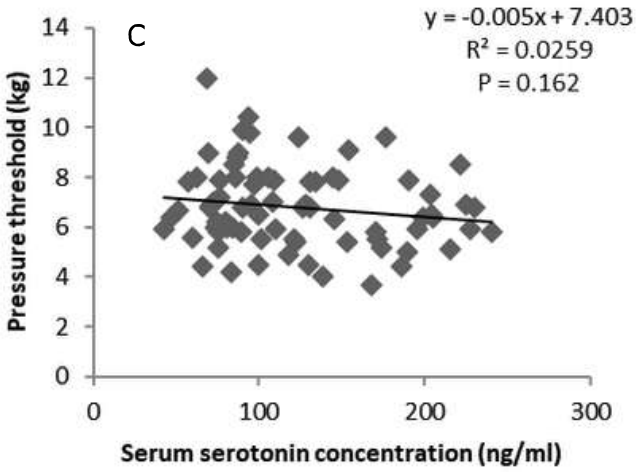


Figure 5: Relationships between serum serotonin concentration and ischaemic pain threshold (A), ischaemic pain tolerance (B) and pressure pain threshold (C) among healthy Nigerian adult population.

DISCUSSION

We have demonstrated presence of differences in experimental pain outcomes by sex, age, ethnicity and body mass index in previous studies[26'28]. In the present study, we investigated the relationship of serum glutamate and serotonin concentration with experimental pain outcomes in a healthy adult population. The present study demonstrated significant sex difference in experimental ischaemic and pressure pain thresholds, with males having higher threshold than females. A study by Ogedengbe et. al.[29] showed no significant sex difference in experimental cold pressor pain threshold. Significant difference has been demonstrated between males and females in relation to opioid requirement and OPMR1 signalling[30]. Glutamate, an amine excitatory neurotransmitter, is a major factor in pain neurotransmission. It is released by primary sensory neurons that transmit pain signals at the dorsal horn of the spinal cord. The lower serum glutamate concentration in males compared to females in the present study further demonstrates the role of glutamate in pain neurotransmission. Our result is not in line [A1] with the findings of Saito et. al. [31] and Zlotnik et. al.[32], whom reported significantly higher blood glutamate concentration in men than in women. Stover and Kempski[33], also reported a sustained increase in blood glutamate in male than in female patients following isoflurane anaesthesia.

The lower glutamate concentration recorded in this study may explain the observed higher experimental pain thresholds (lower pain sensitivity) in males than females in the present study, as the level of glutamate has been reported to be elevated in the spinal cord of rats during inflammation and following nerve injury in neuropathic pain[34], showing that increased glutamate level leads to decrease in pain threshold, thus increased pain sensitivity. Observed lower glutamate concentration in males than females may be due to activity of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT), which were found to be significantly higher in males than in females[35]. GOT and GPT are two enzymes that are able to metabolize blood glutamate, thus, facilitating the lowering of extracellular levels of brain glutamate.

Another factor that may explain the higher glutamate in females than males in our study is the non-pregnant status of the female participants, since pregnant women were excluded from the study. Pregnancy is associated with increased level of placental hormones, and blood glutamate correlate inversely with plasma oestrogen and progesterone[31]. Study in goldfish showed that testosterone and oestradiol cause a decrease in glutamate decarboxylase (GAD) mRNA expression in males, but increase in females[36].

Glutamate is regarded as an important pain mediator in peripheral tissue, at the DRG and in the brain[37], and low blood glutamate levels has been shown to strongly correlate with the brain's extracellular glutamate concentrations[32]. Glutamate, as the major excitatory neurotransmitter in the central nervous system, has been implicated in pain pathophysiology. Kainate glutamate receptors function as mediators and modulators of synaptic transmission and plasticity by regulating presynaptic neurotransmitter release, as well as postsynaptic potential. Evidence has shown activation and modulation of kainate receptors by nociceptive stimuli. Topiramate, a partial kainite receptor antagonist, is clinically effective as a migraine preventive and has been shown to reduce trigeminovascular and thalamic activation[38].

In the present study, mean serum serotonin concentration was found to be lower in males than in females, though the difference was not statistically significant, which agrees with some previous findings[39-41]. variation in serotonin concentration. Serotonin values have been reported to be low during the summer as compared to rest of the year in healthy volunteers[39].

CONCLUSION

Our study demonstrated the presence of sex differences in experimental pain outcomes among healthy Nigerian population. Serum glutamate concentration correlated positively with experimental pressure pain threshold, while serum serotonin concentration correlated negatively with ischemic pain tolerance. Lower serum glutamate concentration may explain the higher pain threshold in males compared to females.

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CONFLICT OF INTEREST

Authors declare no conflict of interests

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