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Research Article

Electrolyte and oxidative stress profile of healthy adult population in Zaria, Nigeria, and their relationship with experimental pain response

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Keywords:
Electrolytes, oxidative stress, pressure pain, sex, age, ethnicity

ABSTRACT

Background: Electrolyte imbalance and oxidative stress (OS) are known to impair physiological functions, which can alter health and wellbeing. The reactive species produced due to OS are detoxified by endogenous antioxidants to maintain homeostasis. This study investigated the electrolyte and oxidative stress profile of a healthy adult population in Zaria, Nigeria and their relationship with experimental pain outcome. **Method:** Participants were apparently healthy adult volunteers between the ages of 20 to 65 years and drawn from the city of Zaria and its environs. Experimental pain was induced using pressure algometry. About 5 ml of blood was collected for determination of serum electrolytes, malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD). **Result:** The results showed that serum concentrations of sodium, potassium and chloride as well as oxidative stress profile did not vary with sex, age and ethnicity among the studied population. There was a significant negative correlation between pressure pain threshold and serum concentration of potassium ($r = 0.2330$, $p = 0.003$) and chloride ($r = 0.2126$, $p = 0.007$), while serum sodium correlated positively ($r = 0.3439$, $p = 0.000$). Serum MDA, SOD and GSH did not show statistically significant correlation with pressure pain threshold ($p > 0.05$). **Conclusion:** Serum electrolytes, but not oxidative stress markers, correlate significantly with experimental pressure pain threshold among healthy adult population in Zaria, Nigeria.

INTRODUCTION

Sodium, potassium, calcium and magnesium are important elements responsible for electrolyte balance, and their optimal concentration for proper biochemical and physiological activities of the cell is maintained by ion channels. Potassium, sodium, and calcium play integral roles in the electrophysiology of pain and myriad cellular functions (Kianifard and Chopra, 2018). The optimal concentration of elements responsible for electrolyte balance and proper biochemical and physiological activities of living cells is maintained by ion channels (Dutta et al., 2015), inactivation of which lead to altered homeostasis. These biologically important elements play a significant role in myriads of physiological functions (Soetan et al., 2010). Thus, altered levels of these elements may induce series of events such as slow movement, postural abnormality, impaired balance, extensive membrane damage and peripheral vascular resistance (Dutta et al., 2015). The well-being of living cells depends on salinity of extracellular fluids. The osmoregulatory system, by controlling water intake, normally keeps the plasma sodium concentration within its normal range (135 to 142 mmol per liter), failure of which exposes cells to hypotonic or hypertonic stress (Sterns, 2015).

Sodium, the most abundant cation in the body, plays a significant role in fluid balance, osmotic regulation, and maintenance of membrane potential. Changes in glomerular filtration rate (GFR) can affect normal body sodium concentration. Renal blood flow, prostaglandins,

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and natriuretic peptides also play a role in sodium regulation, with the Na+-K+-ATPase pump maintaining cellular membrane potential. Sodium intake, under normal physiologic condition, matches sodium losses. Abnormally high sodium concentration (hypernatremia) presents with increased thirst, fatigue, restlessness, and muscle irritability, and in severe conditions, cerebral cellular dehydration can occur, which can progress to hemorrhage, seizures, coma, and death (Blackmer, 2018). Hyponatremia, on the other hand, is associated with headache, nausea, myopathy, lethargy, and restlessness and seizures. Potassium, the primary intracellular cation, also play essential roles in cellular metabolism and maintains membrane potential as well as promotes neuromuscular and cardiac function (Blackmer, 2018).

Oxidative stress is the imbalance between the production of pro-oxidant reactive species and the ability of the living system to cope and prevent their adverse effects via antioxidant defence. It is the persistent imbalance between the production of Reactive oxygen species (ROS) and antioxidant defences, and it often results in irreversible cellular damage (Prasad et al., 2017). ROS are oxygen containing molecules including free radicals (hydroxyl radical, superoxide anion, hypochlorite ion), hydrogen peroxide, and nitric oxide. Due to the presence of unpaired electrons in the valence shell, free radicals are short-lived, unstable and highly reactive. ROS are generated by the mitochondria (through oxidative phosphorylation), non-mitochondrial membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and xanthine oxidase (XO) (Lepetsos and Papavassiliou, 2016). ROS are formed as byproduct of normal metabolism. These molecules play an important role in cell signalling pathways and likely act as key players in many disorders (e.g. metabolic syndromes) and homeostasis. They include oxygen radicals and reactive non-radicals (Banerjee et al., 2016). Nitric oxide (NO), a signaling molecule in the Central Nervous System (CNS), reacts with excess ROS to generate Reactive Nitrogen Species (RNS) like peroxynitrite, which is also a powerful oxidizing agent that can cause lipid peroxidation, thus contributing to free radical-mediated damage (Prasad et al., 2017). The ROS are detoxified by endogenous anti-oxidant system of enzymatic and non-enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), glutathione (GSH), NADPH ubiquinone oxidoreductase (NQO1), paraoxonases (PON), ascorbic acid (vitamin C), α-tocopherol (vitamin E) and carotinoids, which help to keep the system in a state of normal homeostasis. Increase in oxidative stress causes disturbance of homeostasis, leading to denaturation of intracellular molecules like nucleic acids, proteins, and lipids by ROS (Chang et al., 2013; Rubolini et al., 2012). Indeed, ROS can cause different degrees of oxidative damage to biological macromolecules and cells/ tissues. Although some ROS play a key role in cell signalling, oxidative stress is known to impair physiological functions, may accelerate ageing, and cause higher susceptibility to environmental stress or pathogens, thereby resulting in reduced fitness (Rubolini et al., 2012). Oxidative stress causes destruction of cell membrane and cartilage, breakage of DNA strand, rise in intracellular free Ca2+ and damage to membrane ion transporters, thus, leading to cell death and disease development (Dutta et al., 2015). Decrease in CAT and/or SOD and reduced levels of free radical scavengers such as glutathione, and vitamins C and E also contribute to oxidative stress (Baradaran et al., 2014).

Oxidative stress has been implicated in perception of pain. For example, NO is involved in perception and reduction of pain caused by osteoarthritis (OA) via normalization of blood flow pathway (which may help to decrease ischemic pain), the nerve transmission pathway (which decreases the irritation of the nerves in the synovium, bone, and soft tissues), the opioid receptor pathway (which might stimulate the body's normal pain reduction pathways), and the anti-inflammation pathway (Lepetsos and Papavassiliou, 2016). Both H2O2 and ONOO− are involved in inflammatory pain, mainly through COX2/PGE2 pathway.

The ROS generated during normal cellular metabolism can be scavenged by enzymatic antioxidants (SOD, GPx, CAT), or non-enzymatic antioxidants (mainly vitamin E and reduced form of Glutathione). However, oxidative stress results either due to increased ROS production or decreased antioxidant defense systems or both (Prasad et al., 2017). The brain contains high concentration of poly-unsaturated fatty acids that are highly susceptible to lipid peroxidation. Thus, assessment of MDA levels in biological materials can be used as an important indicator of lipid peroxidation in vitro and in vivo for various diseases

Free radicals attack polyunsaturated fatty acid of the membrane, leading to lipid peroxidation and excessive production of malondialdehyde (MDA), the most abundant product of lipid peroxidation. MDA reacts with membrane proteins and ion channels, affecting their normal function and causing electrolyte imbalance (Dutta et al., 2015). This study aimed to investigate the

hypothesis that serum electrolytes and oxidative stress does not correlate with experimental pain outcome among healthy adult population in Zaria, Nigeria.

MATERIALS AND METHODS

Subject recruitment

This research was carried out in nine randomly selected secondary schools under the Zaria Zonal Education Authority. Observational cross-sectional study was used for this research. One hundred and sixty one (161) apparently healthy volunteers of ≥ 20 years were recruited for the study. The subjects included mostly secondary schools staff population around Zaria and environs in Kaduna State, Northern Nigeria. Sample recruitment was based on convenience, agreeing with the study protocol, qualified and willing to participate. Selected individuals were taught and given basic understanding about Physiology of pain and were informed about the experimental procedures (verbally and in writing), as well as risks and contraindications of all procedures. Informed consent was obtained from the subjects, as well as ethical approval from the Ahmadu Bello University Teaching Hospital Health Research Ethics Committee (ABUTH/HREC/N22/2015) prior to the study. Subjects were excluded if they reported significant psychiatric co-morbidity, prior or present alcohol abuse, use daily analgesics or have 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. Patients with a diagnosis of dementia were also excluded.

Experimental pressure pain assessment

Pressure pain was assessed by applying pressure at the first dorsal interosseous muscle (Chesterton *et al.,* 2007), using pressure algometer, at the rate of 1Kg/sec. Subjects were told to press a button at the first perception of pain. The pressure at which pain was first reported was recorded as the pressure pain threshold (in Kg). Retest was not carried out as study has demonstrated good test–retest reliability for repeated measurements (Lacourt *et al.,* 2012). Also, pressure pain was recorded on the dominant hand only, as reports indicated no significant difference between dominant and non-dominant sides (Fischer, 1987; Park *et al.,* 2011). Hogeweg *et al.* (1992) and Rui *et al.* (2015) also reported no significant differences in pressure pain thresholds between the same points on either side of the body.

Determination of serum electrolytes:

Serum sodium was determined by colourimetry method of Maruna and Trinder (1958). A volume of 1000 µL of preparation reagent (sodium RI), 10 µL of standard and 10 µL of serum were vigorously mixed and incubated at room temperature for 5 minutes, followed by centrifugation at 1000 *g* to obtain a clear supernatant. Supernatant was transferred for standard and test, and the assay and absorbance was measured at 530 nm. Serum potassium was determined by colourimetric method of Tietz (1976). A volume of 1000 µL of reagent, 25 µL of standard and 25 µL of serum were mixed and incubated at room temperature for 5 minutes. The mixture was then centrifuged at 1000 *g* and the clear supernatant transferred for standard and test assay. Absorbance was measured at 530 nm against distilled water. Determination of serum cloride concentration was carried out by colourimetry method of Schonfeld and Lowellen (1964). Volume of 1000 µL of reagent, 10 µL of standard and 10 µL of serum were thoroughly mixed and allowed to incubate at room temperature for 1 minute. Absorbance of standard and test samples was measured at 530 nm against distilled water.

Determination of oxidative stress

Serum MDA was measured using the method of Buege and Aust, (1978), as described by Dsouza *et al.* (2012). 100 µL of serum was diluted to 500 µL with distilled water, and the samples were kept in boiling water bath for 15 min. 1 mL of Trichloroacetic acid (TCA)-2-thiobarbituric acid (TBA)–HCl reagent was added and the reaction mixture was cooled to room temperature, and centrifuged at 1000 *g* for 5 min. The supernatant was taken and the optical density of the pink colour formed was read in a spectrophotometer at 535 nm. Serum SOD activity was determined by its ability to inhibit auto-oxidation of epinephrine which was estimated by the increase in absorbance at 480 nm, as described by Sun and Zigma (1978). Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 minutes. Serum GSH concentration was estimated according to the method described by Sedlak and Lindsay (1968) and Awoyemi *et al.* (2014). To 0.5ml of serum, 2.5ml of 0.3M phosphate buffer was added, followed by 0.5 ml of Ellman's reagent. The mixture was incubated for 10 minutes and the absorbance was read at 412 nm using spectrophotometer.

Statistical Analysis

All analyses were carried out using SPSS version 23 software for windows (SPSS Inc, Chicago). Results are presented as mean ± SEM. Sex differences were analysed by Independent-Samples *t* test, while age and ethnic differences were analysed by analysis of variance

(ANOVA), followed by Tukey's *post hoc* tests to indicate significance (if any). Associations were determined using Pearson's correlation. Values of $p < 0.05$ were statistically significant.

RESULTS

Sociodemographic characteristics

Out of the 161 participants included in the study, 91 are males (56.5%), while 70 are females (43.5%). Age distribution showed that 36% are between 20 to 30 years, 31.1% between 31 to 40 years, 16.8% are between 41 to 50 years, while 14.9% are above 50 years. By ethnicity, 60.9% belong to Hausa ethnic group, 15.5% Yorubas, 8.1% Fulanis, and the other minority groups, classified as Others, constituted 13.7%

Pressure pain

This study was carried out on 161 apparently healthy volunteers, made of 70 (43.5%) females (table 1). Perception of pressure pain was found to be significantly lower in males than in females. Mean pressure pain threshold was significantly higher in males (6.90±0.14) compared to females (6.00±0.17) ($p = 0.00$; $T = 4.095$) (figure 1). Perception of pressure pain in the present study showed that subjects in the 41 – 50 age group had significantly lower pressure pain threshold (5.85±0.24) when compared to all the other groups (ANOVA; $p < 0.05$; $F = 2.221$). The result also showed that participants that fall in the youngest age group (20 to 30 years) had the highest pressure pain threshold (6.67±0.19), while those in the oldest age group (>50) had a threshold of 6.61(0.29), which is lower than the youngest age group. Participants in the 31 to 40 years group have a threshold of 6.63(0.21) (figure 2). Ethnicity was found to affect perception of pressure pain among healthy adult population in Zaria, Nigeria, in the present study, with Yorubas having statistically significant higher pressure pain threshold (7.32±0.30) compared to Hausas (6.28±0.12) ($p = 0.008$; $f = 4.143$). Participants belonging to Fulani ethnic group had a threshold of 6.17(0.43), showing that they have a higher pressure pain perception than the Hausas and Yorubas, though the differences were not statistically significant. Others ethnic group showed a mean threshold of 6.83(0.40), which is higher than Hausas and Fulanis, but lower than the Yorubas, though all the differences showed no statistical significance (figure 3).

Serum electrolytes

The result of sex differences in serum electrolytes showed that the male subjects had lower serum sodium concentration (143.65±52.95) than the females (148.83±48.71), though the difference was not statistically significant [p=0.527; T(155)=-0.634]. Difference in serum potassium concentration also showed no statistical sex significance [p=0.099; T(155)=-1.660], but male participants have lower concentration (5.09±2.08), than females (5.77±3.09). Analysis of serum chloride concentration shows that male participants had slightly lower serum chloride (93.42±11.89) than females (94.10±10.13), but the difference was not statistically significant [p=0.706; T(155)=-0.378] (table 3). The result of age differences in serum electrolyte concentration showed that serum sodium concentration was significantly higher among subjects in the younger age group of 20 – 30 years (165.06±50.11), than those in the 41 – 50 years group (119.54±44.61) (p=0.001) and those in the above 50 years group (129.76±11.73) (p=0.018). Age difference in serum potassium concentration shows no statistically significant variation among the studied subjects (p=0.076; f=2.342), though there was a decrease with increase in age, as participants in the youngest age group (20 – 30 years) had the highest concentration (6.03±2.91), and those in the oldest age group (above 50 years) had the lowest concentration (4.45±2.13). Serum chloride concentration analysis shows no statistically significant age variation among the studied participants (p=0.361; f=1.076), but the result shows that there is an age dependent increase, with participants in the youngest age group having the lowest concentration (92.25±10.29), and those in the oldest age group having the highest (96.04±13.32) (table 4)

Table 1: Socio-Demographic Characteristics of Study Volunteers, Showing Frequency and Percentage Distribution for Sex, Age and Ethnicity in an Adult Population of Zaria, Nigeria.

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: Age differences in mean anthropometric characteristics in a healthy adult Nigerian population in Zaria

Characteristic/ Age (yrs)	20 – 30 (Mean±SD)	31 – 40 (Mean±SD)	41 – 50 (Mean±SD)	Above 50 (Mean±SD)
Weight (Kg)	61.29±10.42[b]	71.02±14.06	73.16±10.19	76.68±15.39
Height (m)	1.65±0.09	1.67±0.08	1.66±0.09	1.64±0.08
BMI (Kg/m^2)	22.46±3.47[a]	25.44±4.64[b]	26.67±3.98[b,c]	28.40±5.38[c]
SBP (mmHg)	107.58±14.55[a]	114.79±9.84[a,b]	122.96±26.28[b,c]	133.54±15.07[c]
DBP (mmHg)	72.15±10.47[a]	76.26±7.61[a,b]	79.63±16.46[b]	88.12±11.87[c]

Mean difference of values with different superscript letters ([a,b,c]) is statistically significant ($p < 0.05$) across the table (ANOVA, df = 157). SBP (systolic blood pressure); DBP (diastolic blood pressure).

Table 3: Sex Differences in Serum Electrolytes and Oxidative Stress Markers Among Healthy Adult Population in Zaria, Nigeria

Parameters	Male	Female
[Na] (mmol/L)	142.15±5.75	148.83±5.95
[K] (mmol/L)	5.19±0.24	5.99±0.40
[Cl] (mmol/L)	93.42±1.25	94.10±1.23
MDA (nmol/ml)	23.68±1.53	23.15±1.31
GSH (µg/ml)	30.68±0.68	21.79±0.78
SOD (µg/ml)	13.51±1.01	14.07±0.98

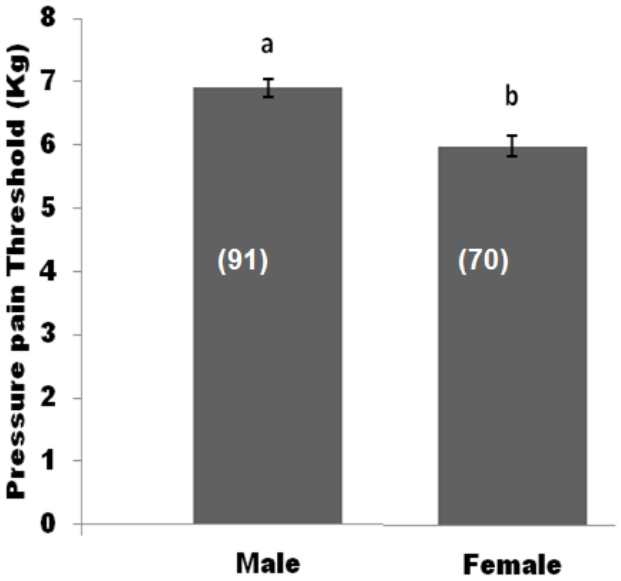

Fig. 1: Sex Differences in Experimental Pressure Pain Threshold in a Healthy Adult Population of Zaria, Nigeria. [a,b]mean difference is statistically significant between males and females (independent *t*-test; $p = 0.00$); (n).

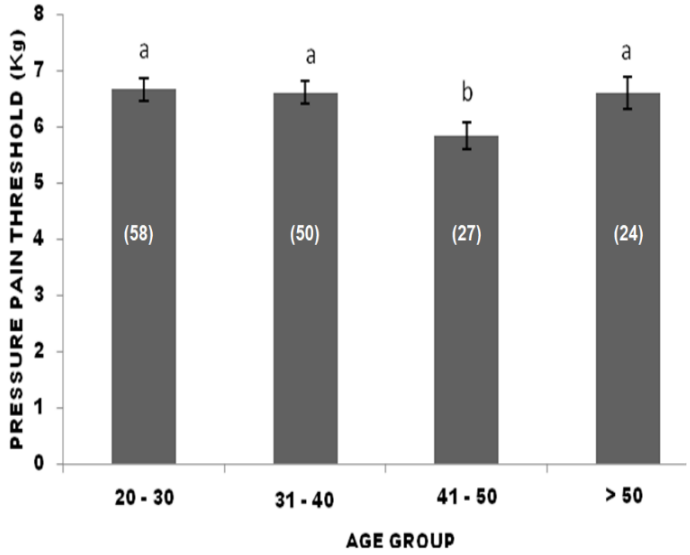

Fig. 2: Age Variation in Experimental Pressure Pain Threshold in a Healthy Adult Population in Zaria, Nigeria, Showing Age Differences in Pressure Pain Threshold. [a,b]Mean difference is statistically significant between groups (ANOVA; $p < 0.05$); (n).

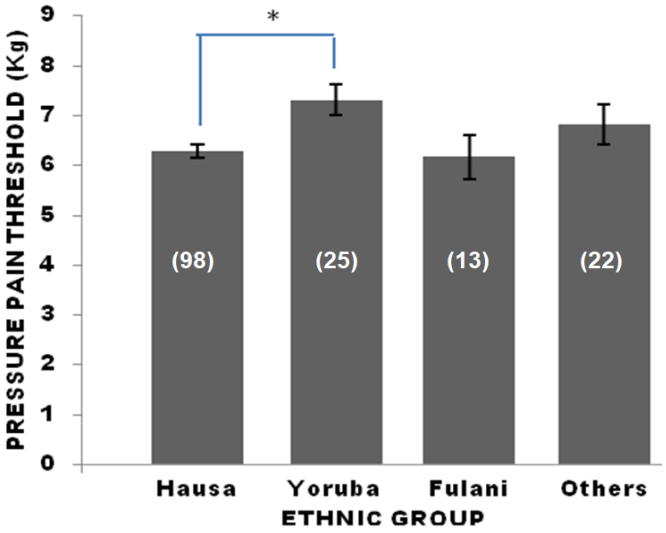

Fig. 3: Comparison of Pressure Pain Threshold between Ethnic Groups among a Healthy Adult Population in Zaria, Nigeria. *Mean difference between the groups is statistically significant (ANOVA; $p < 0.01$) ; (n).

Result of ethnic differences in serum concentration of sodium, potassium and chloride showed that subjects that belong to the Hausa tribe had statistically significant lower serum sodium (135.21±47.37) than the Yorubas (187.94±35.00) (p=0.000; f=11.054) and those in the others group (167.27±48.83) (p=0.023). The Yorubas had significantly higher serum sodium than the Fulanis (123.22±51.15) (p=0.001), and the difference between the Fulanis and Others was also statistically significant (p=0.045). Ethnic difference in serum potassium concentration showed no statistically significant variation among the studied subjects (p=0.063; f=2.486), though the Fulanis had the highest concentration (6.26±3.25), while those in the Others group had the least (4.20±2.03). Serum chloride concentration analysis showed no statistically significant ethnic differences among the studied population (p=0.193; f=3.148), but the result showed that subjects in the Fulani group had the highest (95.92±9.94), and the Yorubas had the lowest (89.40±13.14) (table 5)

Table 4: Age Differences in Serum Electrolytes and Oxidative Stress Markers Among Healthy Adult Population in Zaria, Nigeria

	Age (years)			
Parameters	20-30	31-40	41-50	> 50
[Na] (mmol/L)	165.06±6.69[a]	143.79±6.59[a,b]	119.54±8.75[b]	129.76±11.73[b]
[K] (mmol/L)	6.04±0.39	5.28±0.33	5.09±0.49	4.45±0.44
[Cl] (mmol/L)	92.25±1.37	93.08±1.71	96.04±1.79	96.04±2.78
MDA (nmol/ml)	24.47±2.19	22.33±1.02	22.48±1.30	23.59±1.39
GSH (µg/ml)	29.88±0.84	31.50±0.85	28.04±1.43	31.33±1.27
SOD (µg/ml)	15.68±1.54	12.76±0.52	11.24±0.69	12.83±0.63

Mean differences of values with different superscript letters [a,b,] are statistically significant along the column (ANOVA, $p < 0.05$)

Table 5: Ethnic Differences in Serum Electrolytes and Oxidative Stress Markers Among Healthy Adult Population in Zaria, Nigeria

	Ethnicity			
Parameters	Hausa	Yoruba	Fulani	Others
[Na] (mmol/L)	133.89±4.99[a]	187.94±7.00[b]	123.22±14.77[a]	167.27±10.65[b]
[K] (mmol/L)	5.63±0.27	4.96±0.41	6.26±0.94	4.20±0.44
[Cl] (mmol/L)	95.22±1.05	89.40±2.63	95.92±2.87	89.52±2.32
MDA (nmol/ml)	22.76±0.85	26.25±3.49	22.84±1.78	21.66±1.27
GSH (µg/ml)	30.58±0.77	30.12±1.07	28.74±2.13	30.44±1.05
SOD (µg/ml)	13.05±0.53	16.30±2.44	10.71±0.76	13.73±0.96

Mean differences of values with different superscript letters [a,b,] are statistically significant along the column (ANOVA, $p < 0.05$)

Oxidative stress

Sex differences in serum concentration of MDA, GSH and SOD in the present study showed that the male subjects had slightly higher serum MDA concentration (23.68±1.53) than the females (23.16±1.31), though the difference was not statistically significant [p=0.762; T(155)=0.259]. Difference in serum GSH concentration does not also statistically vary by sex [p=0.391; T(155)=0.865], but the males had higher GSH concentration (30.69±0.68) than the females (29.79±0.78). Analysis of serum SOD

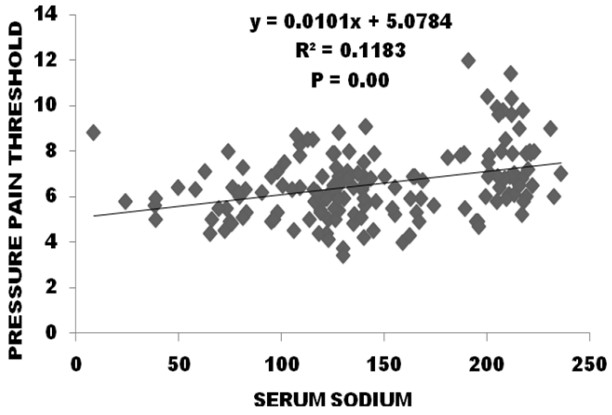

Figure 4: Scatter diagram showing relationship between serum sodium and experimental pressure pain threshold among healthy adult population in Zaria, Nigeria

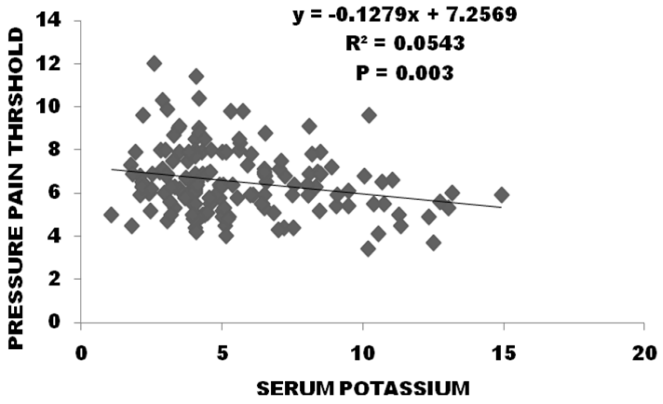

Fig. 5: Scatter diagram showing relationship between serum potassium and experimental pressure pain threshold among healthy adult population in Zaria, Nigeria

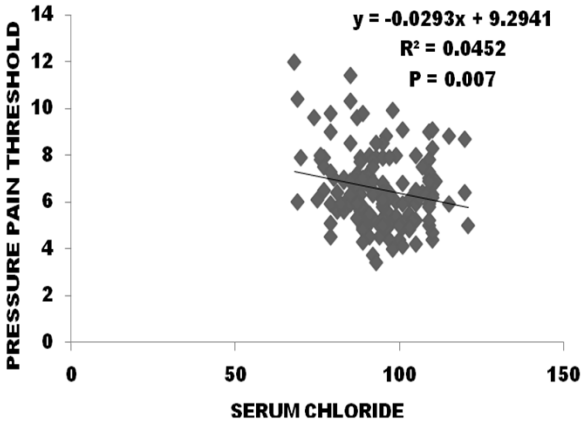

Fig. 6: Scatter diagram showing relationship between serum chloride and experimental pressure pain threshold among healthy adult population in Zaria, Nigeria

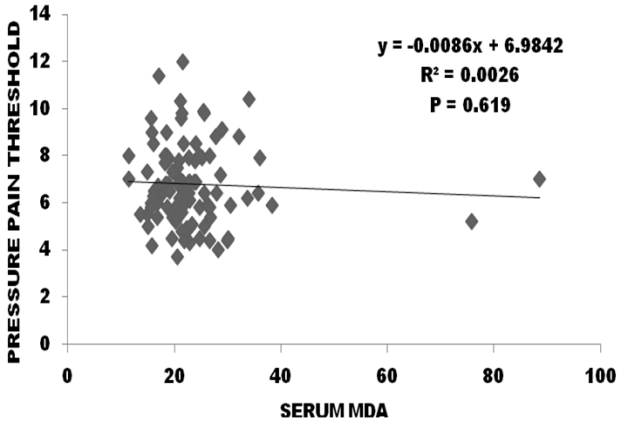

Fig. 7: Scatter diagram showing relationship between serum MDA and experimental pressure pain threshold among healthy adult population in Zaria, Nigeria

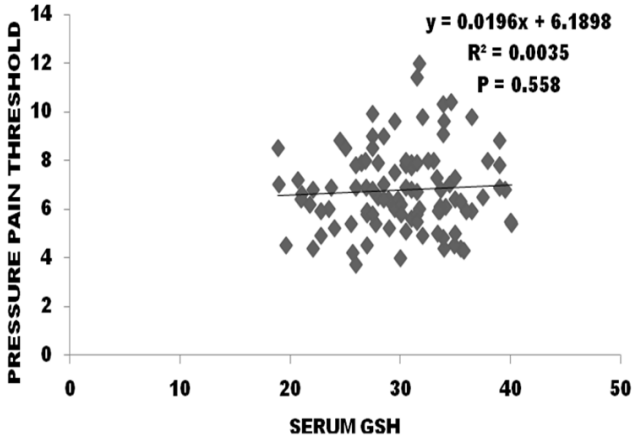

Fig. 8: Scatter diagram showing relationship between serum GSH and experimental pressure pain threshold among healthy adult population in Zaria, Nigeria `

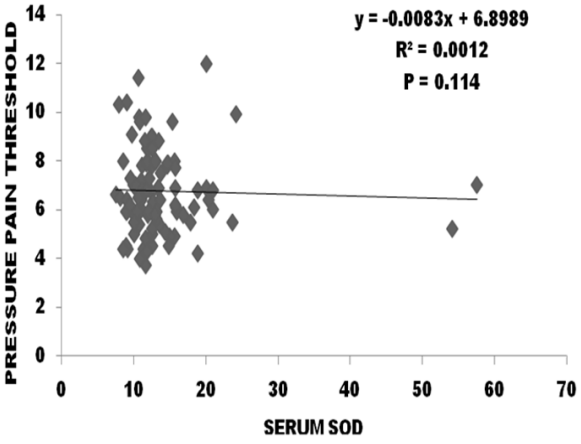

Fig. 9: Scatter diagram showing relationship between serum SOD and experimental pressure pain threshold among healthy adult population in Zaria, Nigeria

concentration showed that male participants had lower serum SOD (13.51±1.00) than the females (14.07±0.98), but the difference was also not statistically significant [p=0.740; T(155)=-0.402] (table 3).

The result of age differences in serum concentration of MDA, GSH and SOD showed that serum MDA concentration was highest among subjects in the youngest age group of 20 – 30 years (24.47±2.19), and lowest among the 31 – 40 years group (22.33±1.02), though the differences were not statistically significant (p=0.829; f=0.296). Age difference in serum GSH concentration showed no statistically significant variation among the studied population (p=0.159; f=1.764). Participants belonging to the 41 – 50 years group had the lowest serum GSH (28.04±1.43), while those in the 31 – 40 years group had the highest (31.50±0.85). Analysis of serum SOD concentration also showed no statistically significant age variation among the studied population (p=0.117; f=2.017), but there was a somewhat age dependent decrease, with participants in the youngest age group (20 – 30) having the highest concentration (15.68±1.54), and those in the 41 – 50 years group having the lowest concentration (11.24±0.69) (table 4).

Ethnic differences in serum concentration of MDA, GSH and SOD showed that subjects that belong to the Yoruba ethnic group had the highest serum MDA concentration (26.25±3.49), while those in the Others group had the least (21.66±1.27), but there were no statistically significant variations among all the ethnic groups (p=0.426; f=0.938). Ethnic difference in serum GSH concentration also showed no statistically-significant variation among the studied population (p=0.792; f=0.346), though the Fulanis had the lowest GSH concentration (28.74±2.13), while the Hausas had the highest (30.58±0.77). Serum SOD concentration analysis showed no statistically significant ethnic differences (p=0.136; f=1.894). The Fulani ethnic group had the least serum SOD concentration (10.71±0.76), while the Yorubas had the highest (16.30±2.44) (table 5).

There was a significant positive correlation between serum sodium and experimental pressure pain in the present study (p = 0.000) (figure 4), while serum potassium and chloride correlated negetively and significantly with experimental pressure pain (p = 0.003; p = 0.007) (figures 5 and 6). Serum MDA, GSH and SOD, on the other hand, showed no signifcant correlation with experimental pressure pain in this study.

DISCUSSION

Biologically important elements have significant role in maintenance of homeostasis and participate in various physiological activities such as neuromuscular irritability, nerve conduction, prevention of development of age-elated complications, among others. Alteration in the levels of these elements may induce series of events such as slow movement, postural abnormality, impaired balance, extensive membrane damage and peripheral vascular resistance (Dutta *et al.,* 2015). Relationship betwen electrolyte profile and clinical pain has not been well documented and measuring electrolytes in sickle cell aneamia patients presenting with painful vaso-occlusive crises has been shown to be un-necessary (Desai, 2014).

Although serum electrolytes concentrations did not statistically vary by sex in the present study, serum sodium concentration was found to decrease with age, as subjects in the younger age group of 21 – 30 years have significantly higher serum sodium than those in the 41 – 50 years group and those in the above 50 years group. Our result is in agreement with that of McLean *et al.* (2003), who found no sex difference in serum sodium and potassium concentrations. Singh *et al.* (2016) also reported no significant sex differences in serum sodium, potassium and chloride concentrations. Our result also agree with a previous work by Prabhunath *et al.* (2016) that reported a significantly lower serum sodium concentration in healthy elderly subjects than their younger counterparts, but they also reported a significantly higher serum potassium concentration, contrary to our finding. Ibrahim *et al.* (2012) reported no significant sex difference in serum sodium, but significantly higher serum potassium and chloride in female than male Turkeys, as well as higher serum sodium, potassium and chloride in adults. Decreased serum sodium may be as a result of hormonal imbalances, such as the syndrome of inappropriate ADH secretion, a common occurrence in elderly (Prabhunath *et al.,* 2016).

Potassium and sodium are known to play integral roles in the electrophysiology of cellular functions, including pain transmission (Kianifard and Chopra, 2018). Lidocaine, for example, is an amide-type local anaesthetic that exerts its pharmacological action through the block of sodium channels in neural tissues, thereby interrupting neuronal transmission (Eipe *et al.,* 2016), including blockage of pain transmission. The lack of significant effect of sex, age and ethnicity on serum potassium in the present study may explain the outcome of cold pressor pain responses, as potassium ion has been

implicated to play a role in abnormal pain signalling. Sex and age variations in experimental pressure pain threshold in the present study is in tandem with the observed differences in serum potassium concentration (higher pain threshold is associated with higher serum potassium, as seen in the results). The Kv7 (KCNQ or M channels) family is widely expressed in the nervous system, including nociceptors. Injection of M channel blocker in the hind paw of rats induced moderate pain, while injection of M channel enhancer produced an analgesic effect. Reduced Kv7 function was also involved in inflammatory pain (Kianifard and Chopra, 2018). Diclofenac which is popularly used to treat pain and inflammation was demonstrated to activate Kv7.2/Kv7.3 channels.

Though not significant, the female subjects were found to have higher sodium concentration than the males, which may be due to differences in sex hormone level, as plasma rennin activity has been reported to correlate positively with serum oestradiol level (Philips *et al.*, 1995). The lack of significant sex differences in serum electrolytes in this study may be due to inability to assess the menstrual status of the female subjects, as both serum sodium, potassium and chloride concentrations were reported to vary across the various stages of the menstrual cycle, with highest concentrations found during the ovulatory phase (Dadlani *et al.,* 1981). Pregnancy and lactation, which were also not assessed in this study, may also influence serum sodium concentration, as Faith *et al.* (2017) reported significantly higher serum sodium in lactating ewes when compared to pregnant ewes, though Obembe and Antai (2008) reported no significant difference in concentration of serum electrolytes between pregnant and non-pregnant women.

Studies indicate an age-related decrease in plasma sodium level by about 1 mEq/L per decade (Luckey and Parsa, 2003). Though subjects in the oldest age group have higher blood pressure (Table 2), they also have lower serum sodium. This may be associated with increase in plasma volume due to decrease in diluting capacity of the kidneys, accounted for as a result of increase in solute load delivery to the remaining functioning glomeruli. The Na-K-2Cl co-transporter, which plays dual roles in the kidney's capacity to concentrate and dilute the urine, has been shown to be down-regulated in aged rodents. Decrease in the reabsorption of NaCl increases solute delivery to the collecting tubule and decreases solute free water excretion (Schlanger *et al.,* 2010). Alterations in the level of aldosterone, antidiuretic hormone (ADH) and atrial natriuretic peptide (ANP) in the elderly are partly responsible for changes in fluid balance associated with ageing. Elderly patients are more likely to develop extracellular fluid volume depletion due to delay in the ability of the ageing kidneys to lower sodium excretion to minimal value (Luckey and Parsa, 2003).

The somewhat age-dependent decrease in serum sodium concentration in this study may be associated with an age related alteration in blood electrolyte balance. Kengne *et al.* (2008) reported hyponatraemia as a risk of fracture in ambulatory elderly. Older subject's kidneys have been reported to possess an inability to conserve urinary sodium. Decrease in serum aldosterone may account for an increase in excretion of sodium in the urine and an inability to decrease urinary sodium excretion in a hypovolemic state. Age induced decrease in glomerular filtration rate (GFR) is associated with a decline in the number of functioning glomeruli, and a corresponding reduction in number of active kidney tubules. This leads to a decrease in the ability to dilute urine and an increased prevalence of hyponatremia in the elderly (Schlanger *et al.,* 2010). The aged kidney is also associated with abnormal distal tubular activity, which leads to a decreased ability to reabsorb sodium. This can be a consequence of reduced GFR or decreased responsiveness of the tubules to ADH. There is also an age related decrease in renin activity with aldosterone secretion (Prabhunath *et al.,* 2016).

Age related changes in serum electrolyte concentrations may be due to age-related reduction in the serum concentrations of renin and aldosterone consequent to increased ANP activity, usually released in response to increased blood pressure and right atrial filling (El-Sharkawy *et al.,* 2014). The elderly are commonly known to be prescribed many medications that interfere with urinary excretion of electrolytes/potassium (Schlanger *et al.,* 2010).

The significant ethnic difference in serum sodium concentration observed in this study is likely to be attributed to dietary intake. Yousafzai *et al.* (2011) reported ethnic differences in serum sodium and chloride between Pathan, Baloch and Punjab patients, a difference they associated to diet. Chun *et al.* (2008) reported no significant difference in baseline sodium excretion between blacks and whites, as well as after furosemide injection, but there was statistically significant decrease in potassium excretion in blacks than in whites. A study by Kim *et al.* (2017) concluded that Hispanic haemodialysis patients have higher serum potassium levels than African-Americans and whites, while higher death risk is associated with higher potassium levels in African-Americans and whites, but not in Hispanics. This was postulated to be associated with socio-cultural factors such as high potassium tolerance due to very high potassium diet, or

racial/ethnic genetic differences in potassium adaptation. Rate of disposal of intravenous load and urinary excretion of potassium has been reported to be less in African-Americans when compared with Caucasian-American subjects, a difference associated with potassium uptake in skeletal muscle by Na/K ATPase (Suh *et al.,* 2004). African-Americans were reported to have lower serum potassium values than non–African Americans independent of demographic characteristics, comorbid conditions, and potassium-altering medications. African ancestry was associated with lower serum potassium levels, showing a genetic component to potassium homeostasis (Chen *et al.,* 2017). The African ancestry of the subjects in the present study could explain the lack of statistically significant age and ethnic differences in the measured serum electrolytes.

The present study showed no statistically significant sex variation in serum MDA, GSH and SOD levels. This agrees with previous findings that reported no significant sex difference in serum MDA level (Beg *et al.,* 2005; El-Badry 2006; Moreto *et al.,* 2014). Schmitt *et al.* (2016) also reported no significant sex difference in serum MDA in normal control rats. However, this study disagrees with the work of Tothova *et al.* (2012), who reported significantly lower MDA in males than females, while Bhutia *et al.* (2011) reported higher serum MDA levels in male than female diabetics. Lack of sex difference in serum MDA in the present study may be associated with menstrual status of the female subjects, as MDA level has been reported to be significantly higher during the luteal phase when compared to the follicular and ovulatory phases (Akande and Akinyinka, 2005).

Sex differences in oxidative stress markers and enzymatic antioxidant defence mechanisms are attributed to higher levels of sex hormones; oestradiol and other oestrogens in fertile females (Tothova *et al.,* 2012). Trevisan *et al.* (2001) reported lower glutathione level in men than in women, while Schmitt *et al.* (2016) reported no sex difference in erythrocyte GSH in control rats. GSH, a tripeptide representing the most abundant nonprotein thiol present in the cell, acts as an antioxidant defence system by its ability to scavenge reactive oxygen species (ROS) through the reversible oxidation to oxidized glutathione (GSSG) (Frijhoff *et al.,* 2015). Lack of significant difference in serum GSH in the present study may be associated with the fact that glutathione is minimally concentrated in serum as it is mainly stored within the cytosol. Diet, diurnal variation and health status affect GSH concentration, as glutathione synthesis depends on the availability of cysteines, the rate-limiting precursor, and diurnal variation in GSH and cysteine has been reported, and GSH levels and GSH/GSSG ratio has been related to pathological conditions (Marocco *et al.,* 2017).

Glutathione (GSH) is the most abundant thiol antioxidant present in the blood and tissues, together with its associated biosynthetic, redox and detoxification pathways represent the key defense system against oxidative stress and free radical damage in the cell (Thomas *et al.,* 2015). Though glutamate is significantly lower in males than females in the present study, GSH was found to be opposite, though not significant. The lack of significant difference in serum GSH may be similarity in diet among the studied population.

The present study agreed with the finding of Sobocanec *et al.* (2009) that reported no significant sex or age differences in brain SOD activity between male and female mice, but disagree with Pepe *et al.* (2009) that reported significantly higher resting SOD activities in men than women. SOD is an enzyme that is known to provide protection against free radical induced damage by converting superoxide radicals (O^{2-}) generated in peroxisomes and mitochondria to hydrogen peroxides. The hydrogen peroxide is then converted to water and molecular oxygen by catalase (CAT). Inhibition of SOD leads to increased oxidative stress as a result of the damageing activities of the superoxide free radicals, and also reduction in the activity of CAT (Awoyemi *et al.,* 2014). Sex differences in antioxidant enzymes may be attributed to factors such as age, sex hormones, cardiovascular health, genetics and lifestyle (Pepe *et al.,* 2009).

Age difference in serum markers of oxidative stress shows no statistically significant variation in the present study. Though ageing has been associated with increased oxidative stress supposedly due to increase free radical, the lack of significant age difference in the present study may be due to small number of aged participants, as only 24 subjects (14.9%) were above 50 years. The present study agrees with that of Bogdanska *et al.* (2003), but disagrees with the finding of Mecocci *et al.* (2000) that reported increase of both plasma and red blood cell SOD activity with age. Guemeun *et al.* (1991) reported no statistically significant variation of plasma and erythrocyte SOD activities related to sex, but plasma and erythrocyte SOD correlate negatively with age. Ho *et al.* (2005) reported a significant increase in SOD activities with age, but no significant sex variation.

The present study showed no significant effect of ethnicity on oxidative stress biomarkers. A study by Bhutia *et al.* (2011) showed that serum MDA levels of

type 2 diabetics in different ethnic populations of Sikkim showed no significant difference. Lipid peroxidation impairs nerve tissue structure and function by decreasing the activity of Na/K ATPase. Painful procedures are well recognized conditions leading to free radicals generation, and oxidative stress plays a role in pain mechanisms, especially in neuropathic and inflammatory pain. Antioxidants have also been used as anti-nociceptive agents. Reactive oxygen species play an important role in allodynia and hyperalgesia mainly through central sensitization, as well as in the pathogenesis of rheumatoid arthritis (Szallasi *et al.,* 2011).

The lack of significant difference in oxidative stress markers in this study may be due to the fact that healthy subjects with no apparent medical condition were used, and oxidative stress is a pathophysiologic imbalance between oxidants and antioxidants system (Chang *et al.*, 2014), which result from increased production of free radicals and/or decreased antioxidant defence mechanisms, and it plays a significant role in several pathological conditions and their complications, such as cardiovascular diseases, cancer, inflammation, and chronic stress. During pain processing, there is involvement of reactive species including superoxide, NO, and peroxynitrite, and increase in SOD activity is associated with decreased availability of superoxide (Kolberg *et al.*, 2015).

Lipid peroxidation has a wide range and depends upon several factors like diet and lifestyle of the individual (Beg *et al.,* 2005). Lipid peroxidation caused by ROS causes disarrangement and disruption of cell membranes, leading to necrotic cell death. It is a free radical–related process that is associated mostly with cellular damage as a result of oxidative stress (El-Badry, 2006). High sugar/energy intake, insulin resistance and waist circumference (central adiposity) have been reported to be associated with plasma MDA concentration (Moreto *et al.,* 2014). The activity of antioxidant enzymes may be enhanced or inhibited under chemical stress depending on the intensity and the duration of the stress applied, as well as, the susceptibility of the exposed species (Awoyemi *et al.*, 2014). SOD activity was reported to significantly increase after 10 sessions of high-voltage low amplitude spinal manipulation for 5 weeks in patients with chronic low back or neck pain (Kolberg *et al.*, 2015).

MDA level has been reported to be associated with altered serum electrolyte in osteoarthritic patients, indicating that lipid peroxidation initiates electrolyte imbalance (Dutta *et al.,* 2015). Free radical mediated lipid peroxidation causes electrolyte imbalance by injuring ion channels, including Na^+-K^+ ATPase, Na–Ka–2Cl co-transporter and K^+ channels, as well as production of protein radical in lipid membranes that effects normal ion transport (Dutta *et al.,* 2015). This is clearly demonstrated by low level of trace elements and electrolytes and high oxidative stress index in camel-calves with pasteurellosis (Shoieb *et al.,* 2016). Limitations of the present study include inability to determine the pregnancy and menstrual status of the female volunteers, as well as the diet of the studied population.

CONCLUSION

The present study was able to establish that sex differences does not determine the concentration of serum electrolytes among the studied population, but serum sodium decreases with age and is also affected by ethnicity, most likely due to difference in dietary intake. Serum MDA, SOD and GSH showed no variation by sex, age or ethnicity. Pressure pain threshold correlates positively with serum sodium concentration, but negatively with serum potassium and chloride concentrations.

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CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest regarding the publication of this paper.

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