INFLUENCE OF SEX, AGE AND ETHNICITY ON PERCEPTION AND RESPONSE TO EXPERIMENTALLY INDUCED PAIN AMONG AN ADULT NIGERIAN POPULATION IN ZARIA

BY

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHYLOSOPHY IN HUMAN PHYSIOLOGY

DEPARTMENT OF HUMAN PHYSIOLOGY,
FACULTY OF BASIC MEDICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

MARCH, 2019

Declaration

I declare that the work in this thesis entitled **"INFLUENCE OF SEX, AGE AND ETHNICITY ON PERCEPTION AND RESPONSE TO EXPERIMENTALLY INDUCED PAIN AMONG AN ADULT NIGERIAN POPULATION IN ZARIA"** has been performed by me in the Department of Human Physiology, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

_____ _____

ABDULLAHI HUSSEIN UMAR Date

Certification

This thesis entitled **"INFLUENCE OF SEX, AGE AND ETHNICITY ON PERCEPTION AND RESPONSE TO EXPERIMENTALLY INDUCED PAIN AMONG AN ADULT NIGERIAN POPULATION IN ZARIA"** by Abdullahi Hussein UMAR meets the regulations governing the award of the degree of Doctor of Philosophy of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Dedication

This work is dedicated to my mother, Hajiya Laraba Hussaini Umar, and to my late father, Alh. Hussein Umar Kaigama.

Acknowledgement

All thanks, praise, glory and honour is to Allah Almighty, the giver of life, health and talent. I thank Him for sustaining me and giving me the ability, wisdom, strength and courage to conduct and see through to the end of my Ph.D. research. ALHAMDU-LILLAH.

My sincere gratitude goes to my mother for her spiritual, moral and financial support throughout the course of my study. May Allah reward, guide, protect, and give her a fruitful long life and prosperity, ameen. I am highly grateful to my supervisors and mentors, Prof. Aliyu Mohammed, Prof. J.O. Ayo and Prof. N. M. Danjuma for their overwhelming support. They taught me scientific research and gave me substantial advise. May Allah continue to guide and support them in all their endeavours and give them the strength, ability and courage to continue the good work of mentoring us for the greater tasks ahead.

I particularly acknowledge the great assistance of Mal. Bala Muhammad of Human Physiology Department for his assistance in the experimental proceedings during the field work, particularly his role in the collection of blood samples from the subjects. May Allah guide, protect and reward him abundantly. My gratitude also goes to Mr. Adetiba Bamidele of Keymog Medical Diagnostic center, Kaduna. I also acknowledge the assistance of my colleagues and friends; Dr. Isa ahmed-Sherif, Dr. Mustapha Shehu, Dr. Suleiman Ibrahim, Dr. Abdulmalik Muhammad, Umar Adam, Dr. Lawan Amadu, Mal. Yusuf Yusha'u, Dr. Jimoh Abdulazeez and Mrs. Maryam Isa Imam for their tremendous assistance during my field and laboratory work, as well as their untiring guidance and support during my statistical analysis and throughout the period

of my study. I pray that the Almighty will also guide and support them in all their endeavours.

I also thank my Head of Department, Dr. M.B. Akor-Dewu, departmenntal PG coordinator, Dr. F.A. Dawud and all my lecturers, including Dr. M.I.A. Saleh, Dr. R.A. Magaji, Dr. A. Abdulwahab, Dr. I. G. Bako and Dr. J.A. Tende for their good pieces of advise and support. May Allah reward you all. I also appreciate all the technical and administrative staff of the Department.

I particularly appreciate the management and staff of the secondary schools visited, including Maude International School, Nana International School, Government Secondary School, Zaria (K/Kibo), Government Junior Secondary School, Kofar Doka, Government Secondary School, Kofar Kuyambana, Sheikh Ibrahim Arab Special Secondary School, Karau-Karau, Barewa and Alhudahuda Colleges all in Zaria, for allowing me to use their staff and facility during my research.

I owe so much gratitude to my brothers, Ibrahim Umar, Umar H. Umar, Isma'ila Bature and Sunusi Musa for their kind assistance and brotherly advise throughout the course of my study. May Allah reward them all, Ameen. My profound gratitude also goes to my friends and my family, especially my wife Bilkisu and kids for their patience during the study. May Allah reward and guide them, ameen.

Abstract

The study of individual variability in pain has become an area of research interest globally. Inter-individual variability in pain perception and response is believed to be due to multiple bio-psycho-social factors, including genetic influences. This study was designed to investigate the influence of sex, age and ethnicity on perception and response to experimentally induced pain among a Nigerian adult population in Zaria, Northern Nigeria. Participants included 161 adult volunteers of 20 years and above, drawn from the staff population of selected secondary schools under the Zaria zonal education authority. Experimental pain was induced using the cold pressor test, sub-maximal effort tourniquet model of ischaemic pain, as well as pressure algometry. About 5 ml of blood was collected for determination of blood group, haemoglobin (Hb) genotype, serum concentrations of glutamate, serotonin, electrolytes, Malodealdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD). Data collected were analysed using parametric (t-test, ANOVA) and non parametric (Mann-Whitney test, Kruskal-Wallis test) analysis. P value ≤ 0.05 was considered significant. Results of the study showed that there was no statistically significant differences in cold pressor pain outcomes and ischaemic pain tolerance between sexes ($p > 0.05$), but pressure and ischaemic pain thresholds were significantly higher in males than females ($p = 0.000$). Age difference on ischaemic pain threshold was statistically significant, with older subjects (≥ 50 years) having higher threshold than the younger subjects ($p = 0.029$). Ethnic differences on experimental pain outcomes showed that Yorubas have significantly higher pressure pain threshold than the Hausas ($p = 0.008$), while Fulanis have significantly higher ischaemic pain tolerance than those in the others group ($p = 0.034$). Subjects with normal body mass index (BMI) had a statistically significant higher ischaemic pain tolerance than their obese counterparts (*p*

= 0.012). Effect of handedness showed that right handed subjects have a higher cold pressor pain threshold than left handed ones (p = 0.001). Subjects with the AA haemoglobin genotype were found to have significantly lower cold pressor pain tolerance (p = 0.028) and ischaemic pain threshold (p = 0.043) than the AS subjects. The O^- blood group subjects were found to have significantly lower cold pressor pain tolerance when compared to those with O^+ (p = 0.025) and A^+ (p = 0.019) blood groups, while AB^+ subjects have lower ischaemic pain threshold than those with O^+ (p = 0.027) and A^+ (p = 0.035) blood groups. Result of pain catastrophizing showed that male subjects have a lower pain catastrophizing score than females (p = 0.041). Male subjects have significantly lower serum glutamate concentration than females (p = 0.033). There was significantly higher serum sodium concentration among participants in the 20 – 30 age group than those in the 41 – 50 (p = 0.001) and above 50 groups (p = 0.018), while ethnic variation showed lower serum sodium among Hausas and Fulanis compared to the other ethnic groups. The oxidative stress markers (MDA, GSH and SOD) showed no statistically significant variations by sex, age or ethnicity in the present study. In conclusion, this study showed that perception and response to experimentally induced pain, as well as pain catastrophizing among adult Nigerian subjects in Zaria showed variation by sex, age and ethnicity. Factors such as BMI, blood group, Hb genotype and handedness were also found to influence the outcomes of some experimental pain models.

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List of Abbreviations

5-HT	5-hydroxytryptamine (serotonin)
ACC	Anterior Cingulate Cortex
AEA	Arachidonoylethanolamide
AMPA	Alpha amino-3-hydroxy-5-methyl-4-isoxazolyl propanoic acid
ASIC	Acid sensing ion channel
ATD	Acute Tryptophan Depletion
Aδ	A delta nerve fibres
BDNF	Brain Derived Neurotropic Factor
BMI	Body mass index
$CACNA1_A$	Calcium Voltage-Gated Channel Subunit Alpha1 A
CB	Cannabinoid
CGRP	Calcitonin Gene Related Peptide
CIPA	Congenital insensitivity to pain with anhidrosis
COMT	Catechol-O-methyltransferase
CpG	Cytosine-guanine binding region
CREB	Cyclic AMP response element binding protein
CYP2D6	Cytochrome P 2D 6
DMR	Differentially methylated region
DRG	Dorsal Root Ganglion
ENaC	Epithelial Sodium Ion Channel
EPSC	Excitatory Post Synaptic Current
ER	Estrogen receptor
FD	Familial dysautonomia
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma Amino Butyric Acid
GAD	Glutamic acid decarboxylase

GluR	Glutamate receptor
GSH	Reduced glutathione
GWAS	Genome Wide Association Studies
Hb	Haemoglobin
HDAC	Histone deacetylase
HSAN	Hereditary sensory and autonomic neuropathy
IASP	International Association for the Study of Pain
IENF	Intra-epidermal nerve fibres
IKBKAP	Inhibitor of kappa B kinase complex associated protein
IL-1β	Inter-leukin 1 beta
LTP	Long term potentiation
MAPK	Mitogen activated protein kinase
MDA	Malondealdehyde
MOR	mu opioid receptor
NGF	Nerve Growth Factor
NHAMCS	National Hospital Ambulatory Medical Care Survey
NIH	National Institute of Health
NMDA	N-methyl-D-aspartic acid
NTRK1	NGF tyrosine kinase 1 receptor
PEA	N-palmitoylethanolamide
PEPD	Paroxysmal Extreme Pain Disorder
PFC	Pre-frontal cortex
PKC	Protein kinase C
PMP	Pain Management Programme
PPT	Pressure pain threshold
SCG	Superior cervical ganglia
SCN9A	Sodium voltage gated channel alpha subunit 9

SNP	Single Nucleotide Polymorphism
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SPT	Serine palmitoyl transferase
SPTLC1	Serine palmitoyltransferase long chain subunit 1
SSRIs	Selective Serotonin Reuptake Inhibitors
TG	Trigeminal ganglion
TLR	Toll like receptor
TNFα	Tumor necrosis factor alpha
TrkA	Tyrosine kinase A
TRP	Transient receptor potential
TRPA1	Transient receptor potential ankyrin 1
TRPM8	Transient receptor potential melastatin 8
TRPV1	Transient receptor potential vanilloid 1
TTX	Tetrodotoxin
WDR	Wide Dynamic Range Neurons

Abstract

The study of individual variability in pain has become an area of research interest globally. Inter-individual variability in pain perception and response is believed to be due to multiple bio-psycho-social factors, including genetic influences. This study was designed to investigate the influence of sex, age and ethnicity on perception and response to experimentally induced pain among a Nigerian adult population in Zaria, Northern Nigeria. Participants included 161 adult volunteers of 20 years and above, drawn from the staff population of selected secondary schools under the Zaria zonal education authority. Experimental pain was induced using the cold pressor test, sub-maximal effort tourniquet model of ischaemic pain, as well as pressure algometry. About 5 ml of blood was collected for determination of blood group, haemoglobin (Hb) genotype, serum concentrations of glutamate, serotonin, electrolytes, Malodealdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD). Data collected were analysed using parametric (t-test, ANOVA) and non parametric (Mann-Whitney test, Kruskal-Wallis test) analysis. P value ≤ 0.05 was considered significant. Results of the study showed that there was no statistically significant differences in cold pressor pain outcomes and ischaemic pain tolerance between sexes ($p > 0.05$), but pressure and ischaemic pain thresholds were significantly higher in males than females ($p = 0.000$). Age difference on ischaemic pain threshold was statistically significant, with older subjects (≥ 50 years) having higher threshold than the younger subjects ($p = 0.029$). Ethnic differences on experimental pain outcomes showed that Yorubas have significantly higher pressure pain threshold than the Hausas ($p = 0.008$), while Fulanis have significantly higher ischaemic pain tolerance than those in the others group ($p = 0.034$). Subjects with normal body mass index (BMI) had a statistically significant higher ischaemic pain tolerance than their obese counterparts ($p = 0.012$). Effect of handedness showed that right handed subjects have a higher cold pressor pain threshold than left handed ones ($p = 0.001$). Subjects with the AA haemoglobin genotype were found to have significantly lower cold pressor pain tolerance ($p = 0.028$) and ischaemic pain threshold ($p = 0.043$) than the AS subjects. The O^- blood group subjects were found to have significantly lower cold pressor pain tolerance when compared to those with O^+ ($p = 0.025$) and A^+ ($p = 0.019$) blood groups, while AB^+ subjects have lower ischaemic pain threshold than those with O^+ ($p = 0.027$) and A^+ ($p = 0.035$) blood groups. Result of pain catastrophizing showed that male subjects have a lower pain catastrophizing score than females ($p = 0.041$). Male subjects have significantly lower serum glutamate concentration than females ($p = 0.033$). There was significantly higher serum sodium concentration among participants in the 20 – 30 age group than those in the 41 – 50 ($p = 0.001$) and above 50 groups ($p = 0.018$), while ethnic variation showed lower serum sodium among Hausas and Fulanis compared to the other ethnic groups. The oxidative stress markers (MDA, GSH and SOD) showed no statistically significant variations by sex, age or ethnicity in the present study. In conclusion, this study showed that perception and response to experimentally induced pain, as well as pain catastrophizing among adult Nigerian subjects in Zaria showed variation by sex, age and ethnicity. Factors such as BMI,

blood group, Hb genotype and handedness were also found to influence the outcomes of some experimental pain models.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The study of inter-individual variability, as an area of research interest, focuses on studying differences in bio-psychosocial outcomes between subjects across diverse situations. Personality traits and cognitive abilities have specially received the most attention in this field (Williams *et al.,* 2008). Thus, study of individual differences in physiological parameters is of paramount importance. Sex and gender differences in pain sensitivity response may be due to biological factors such as blood pressure, gonadal hormones and body size, and psychological factors such as fear of pain, catastrophizing, depression and anxiety (Alabas *et al.,* 2012). Males report lower pain intensity and unpleasantness, and greater pain tolerance than females (Forsythe *et al.,* 2011).

The perception and response to pain depends on areas of the brain, such as the limbic system, and is influenced by ethno-cultural beliefs. It is believed that a person's expectation of how much pain they can cope with determines the amount of pain they actually feel. It implies that people who go into a painful procedure (such as labour) with a very strong belief in their ability to handle the pain may end up feeling much less pain than those with a tendency to ''catastrophize''. In Fulani culture, daughters are raised to not only believe in their ability to cope with the pain of labour, but that to scream or cry or lose control is absolutely beneath them. Mothers teach their daughters that if they handle the first childbirth very well (without screaming and going wild), subsequent experiences will be handled very well too and vice versa (Anonymous, 2012).

It is believed that people can learn to feel different amounts of pain just by viewing other people. Fulani women grow up around people with very high pain tolerance. They grow up seeing their mothers, aunts, senior sisters and in-laws going through labour as if it was just some boring and mildly irritating routine. So as they grow to maturity, so does their pain threshold (Anonymous, 2012). Furthermore, among the pain inducing Fulani cultural practice is *sharo/shadi*, a public flogging that is a test of manhood, where a youth is expected to undergo severe flogging in public without flinching. During this festival, the young man to be flogged comes out and strikes a defiant pose. Another young man of about the same age and size approaches, wielding a strong, supple cane about a half inch thick and moves around the victim taking careful aim. Without warning, he lands the whip heavily on the other's ribs, sometimes drawing blood. The victim does not flinch but shows utter indifference to pain and even sneers at his attacker. If he is able to achieve this, his family and friends surround him with joy, offering gifts and congratulations. He has now displayed his manhood and is considered worthy of a wife (Ndukwe, 1996).

Pain, a multi-dimensional sensory experience, is intrinsically unpleasant and associated with hurting and soreness. It varies in intensity (mild, moderate or severe), quality (sharp, burning or dull), duration (acute or chronic), referral (superficial or deep), and locality (localised or diffused). Pain can be adaptive and maladaptive. While adaptive pain contributes to survival by protecting the individual from injury or promoting healing when injury has occurred (symptomatic pain), maladaptive pain, on the other hand, is an expression of the pathologic operation of the nervous system (pathologic pain) (Woolf, 2004).

There is a high degree of individual variability in pain, very likely due to complex environmental and multiple genetic factors (James, 2013). A number of genes play critical role in determining pain sensitivity, pain reporting and susceptibility to developing chronic pain. Advances in 'genome wide association studies' (GWAS), 'single nucleotide polymorphism' (SNPs) and role of epigenetics in modulation of pain has established the role of genetics in pain (James, 2013). Association and linkage human studies have identified a number of genes responsible for heritable conditions involving dramatic alterations in pain perception (Cregg *et al.,* 2013). Pain is a subjective, multidimensional human experience, with complex interactions between sensory and emotional components. The management of pain may need an inter-disciplinary approach, thereby making therapeutic strategies to require the use of both pharmacological and non-pharmacological interventions (O'Brien and Kane, 2014).

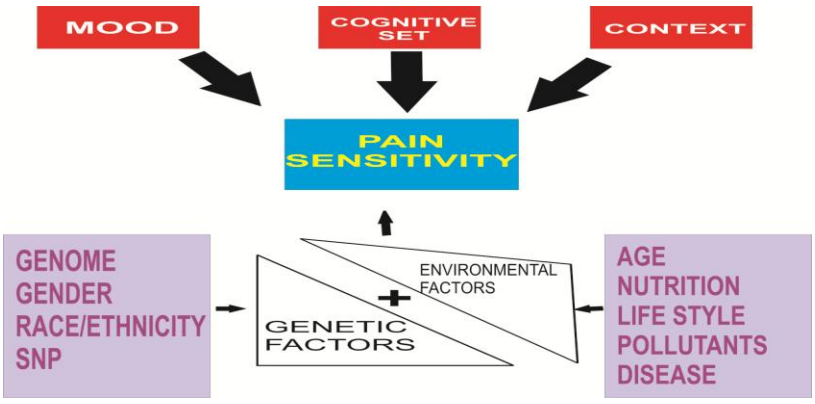


Figure 1.1: Factors that influence pain sensitivity (Fillingim *et al.*, 2008).

Nigeria is a country made up of 36 states and covers a land mass of about 923,768 km^2. It is a multi-ethnic nation with cultural differences between its component ethnic groups.

From the north to the coast, the range in types of social system, dress, diet and languages far exceeds that to be found elsewhere in the world (Salawu and Hassan, 2011).

1.2 Statement of Research Problem

Pain is associated with decreased health-related quality of life, as well as with negative mood and increased disability (Carr and Pujol, 2010). Although physicians now have effective pain treatments at their disposal, pain remains one of the most poorly assessed and treated physical symptoms. Lack of knowledge of adverse effects of pain medications and susceptibility to addiction continue to be significant barriers to good pain management and contribute unnecessarily to patient and family debilitation and suffering (Emanuel *et al.*, 1999). Pain, as a burden to individual and the society, is universally understood as a signal of disease, and is the most common symptom that brings a patient to a physician's attention (Fields and Martin, 2005). There is a significant global burden of chronic pain that directly influences quality of life (Macea *et al.*, 2010). Pain is biologically adaptive because it signals actual or potential tissue damage, which evokes withdrawal and/or recuperative behaviours (LaCroix-Fralish and Mogil, 2009). In some instances and in some people, however, pain becomes chronic and exquisitely maladaptive. Chronic pain conditions are highly prevalent and represent a huge economic burden on society, and in addition to causing unimaginable suffering, pain also contribute to mortality (Macfarlane *et al.*, 2001).

Persistent pain impedes employment prospects and negatively influences social interactions and emotional well-being (Brown *et al.*, 2014). The negative impact of

chronic pain breaches multiple aspects of life, including physical, neurological, psychological, social and spiritual concerns (Dargan *et al.,* 2014). In a comprehensive review of the National Hospital Ambulatory Medical Care Survey (NHAMCS) in United States, it was reported that pain related problems accounted for 42% of emergency room visit. It also reported that White patients with pain were significantly more likely to receive an opioid analgesic than Black, Hispanic, or Asian/other patients. These disparities did not change significantly over time and were evident for children as well as adults (Anderson *et al.,* 2009). In Nigeria, differences in pain perception among parturients have been shown to depend on factors such as culture, social status, prenatal education, counselling, support during labour and medical intervention (Aduloju, 2013). In many clinical conditions, biological markers of disease activity or tissue damage contribute only modestly to reported clinical pain, and it seems plausible that individual differences in nociceptive processing could influence clinical pain severity (Green *et al.,* 2003a). Advances in genotyping has revolutionised understanding of the influence of genetic predisposition and environmental variables, such as diet and general state of health, in individual's drug responses (Cregg *et al.,* 2013). A poor tolerance of pain among family members of subjects who themselves report pain has also been demonstrated (Norbury *et al.,* 2007).

Opioids are routinely used in the treatment of pain. However, there is a wide variation in opioid analgesic efficacy and side effects, the reasons for which may partly be genetic (Cregg *et al.,* 2013). Biochemical and psychosocial factors have been shown to have

influence on the development and duration of pain related disability (Ogunlana *et al.*, 2012).

1.3 Justification

Experience of pain is characterised by robust inter-individual variability, which is modified by interactions among numerous bio-psychosocial factors, including genetic influences. Dispositional characteristics such as sex, age, race/ethnicity and personality have been associated with pain responses (Fillingim *et al.*, 2008).

Disparities in pain assessment and treatment exists for all types of pain (acute, chronic and cancer) across life span (Anderson *et al.*, 2009). Studies of pain management in United States reported that African Americans, Hispanics and others reported more severe pain, pain related interferences, and were more likely than White patients to be prescribed analgesics that were not adequate (McNeill *et al.*, 2004). Another study by Everslay *et al.* (2005) reported that African-American and Latina women who underwent surgery for breast cancer had greater post-surgical pain and related symptoms than White women. Retrospective studies evaluated chronic pain population and revealed increased pain severity for Blacks when compared with Whites, regardless of age and gender (Green *et al.*, 2003b; McCracken *et al.*, 2001). There is an interface between pain and ethnicity, and both are shaped by culture, behavioural and attitudinal norms and systems of meaning (Rahim-Williams *et al.*, 2007).

A study by Akinpelu *et al.* (2013) showed that prevalence of low back pain among Nigerian adolescents increase with age, and is higher in males than in females. Ayanniyi *et al.* (2011) also reported significant age differences in prevalence of low back pain among Nigerian adolescents, but no sex difference. Omoke and Amaraegbulam (2016) reported that the incidence of low back pain among Nigerians was significantly higher in females than males. Significant difference on pain intensity between Nigerian males and females for cold pressor test has also been reported, though there was no significant difference on pain threshold and pain tolerance (Ogedengbe *et al.,* 2015).

Study among patients with chronic pain have demonstrated an association between experimental pain responses and reported clinical pain, such that greater experimental pain sensitivity predicts higher levels of clinical pain (Green *et al.,* 2003a). Individual differences in clinical pain responses can be influenced by many factors, such as disease severity and access to pain medications. Experimental pain studies, however, provide unique insight into the dimensions of pain and into individual differences in pain responsiveness by controlling different aspects of pain-eliciting stimuli and pain measures (Lacourt *et al.,* 2012). Studies of experimental pain are free from the confound of disease progression, but can be highly relevant to clinical pain states. Understanding factors that determine experimental pain sensitivity in healthy people will be of value in understanding some chronic pain states. Experimental pain affords the ability to incorporate pain stimuli with different temporal and sensory characteristics, and can provide more specific information regarding the nature of individual differences in pain (Green *et al.,* 2003a). Though understanding the influence of age, sex and ethnicity on inter-individual

variability in pain perception will go a long way in helping physicians to prescribe the appropriate and adequate pain medication, yet, there is paucity of information on the effect of these factors on inter-individual variability in experimental pain responses among Nigerians. This study was designed to evaluate sex, age and ethnic differences in experimental pain perception among a healthy Nigerian adult population in Zaria.

1.4 Aim and Objectives

1.4.1 Aim

This study aimed to investigate the effect of sex, age and ethnicity on inter-individual variability in experimental pain responses among a healthy Nigerian adult population in Zaria, Northern Nigeria.

1.4.2 Objectives

The following were the specific objectives:

i. To evaluate differences in pain perception among healthy adults in Zaria, Nigeria, using experimental pain models.
ii. To study effect of sex, age and ethnicity on pain catastrophizing.
iii. To investigate role of some physiological parameters such as BMI, Hb genotype, blood groups and handedness on inter-individual variability in pain perception among healthy adults in Zaria, Nigeria.
iv. To determine relationship between serum glutamate and serotonin concentrations with experimental pain responses in a healthy adult population of Zaria, Nigeria.

v. To evaluate sex, age and ethnic differences in serum concentration of malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD) among healthy adults in Zaria, Nigeria.

1.5 Research Hypothesis

Sex, age and ethnicity do not influence experimental pain responses among healthy Nigerian adults in Zaria.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Studies of experimental pain provide information into individual differences in pain responsiveness by controlling different aspects of pain-eliciting stimuli and pain measures. In experimental pain studies, measures based on the stimulus intensity are frequently used, such as pain threshold and pain tolerance. Pain responsiveness can also be assessed by pain ratings (e.g. using visual analogue scale). Different studies use different modalities, such as temperature, electrocutaneous stimuli and pressure, which do not elicit the same pain responses and outcome measures between them are not always related (Lacourt *et al.*, 2012).

The aim of any medical treatment is to provide effective therapy, boost efficacy and minimise potential toxicity. Response to many common medications, including analgesics, are influenced by some type of genetic variation, knowledge of which could be useful to prescribers. Population-based genetic association studies, which aim to correlate genotype to phenotype in pain and analgesic responses, have had variable success. Twin studies demonstrate that up to 60% of the observed variability in response to painful stimuli is genetically determined. However, genetic and environmental factors known to contribute to pain experience are only moderately correlated across different pain modalities, which suggest that different genes influence different types of pain (Cregg *et al.*, 2013). The Human Genome projects, together with advances in high throughput genotyping, has revolutionised the understanding of the importance of genetic predisposition and

environmental variables, such as diet and general state of health, in individual drug responses.

Pain medicine presents many clinical challenges, particularly in the accurate diagnosis of complex pain syndromes and in establishing the efficacy of pharmacological interventions, interventional pain procedures, surgical procedures and psychological therapies. Significant strides have been made in the specialty of pain medicine in the past few decades, but consistently effective treatment for some of the more complex pain syndromes still proves to be elusive (Goulden, 2013). Chronic pain has the potential to negatively impact patients across multiple domains. It breaches multiple aspects of the self, including physical, neurological, psychological, social and spiritual concerns. As chronic pain is among the most prevalent of chronic conditions, there has been an increase in the availability of 'Pain Management Programmes' (PMPs) which attempt to offer accessible understanding, education and support to the significant number of people living with chronic pain (Dargan *et al.*, 2014).

Growing complexity has been associated with individual's response to opioids, as well as the differential response that individuals may have to different opioids. The complexity of an individual's response to opioids is influenced not only by genetic and molecular factors but also by phenotypic determinants such as psychosocial factors, gender and pain sensitivity (Gretton and Droney, 2014). There is need to acquire information that can contribute to a clinically meaningful treatment method for personalising the prescription of

pain medicine, which would help to guide prescription, so that patients receive treatments that are most suited to them.

2.2 Pain

Pain, a complex sensory and emotional response to injury, is an unpleasant sensation associated with actual or potential tissue damage. It is a complex phenomenon derived from sensory stimuli or neurologic injury and modified by individual memory, expectations, and emotions. Accurate, reliable and sufficient evidence for presence and intensity of pain can usually be obtained by an individual's description and self report (Ferrell *et al.,* 2002). Pain is the most common symptom with which patients seek medical consultation and its management involves tremendous health care expenditure (Ghai and Dureja, 2004).

Life expectancy is reduced as a result of lack of pain in patients with congenital insensitivity to pain due to mutation of the nerve growth factor tyrosine kinase A receptor (Woolf, 2004). Pain is a multifaceted and highly personal experience and is whatever the experiencing person says it is and exists whenever he/she says it does. It causes significant distress to patients and has adverse effects on the endocrine and immune functions. Identification of the predictive factors for pain would facilitate early intervention and better pain management (Ip *et al.,* 2009)

Persistent/chronic pain has become a label associated with negative images and stereotypes often associated with longstanding psychiatric problems, futility in treatment,

malingering, or drug-seeking behaviour. It is well recognized that medical and surgical procedures may lead to persistent post procedural pain, yet, predicting patients that are at risk of developing chronic pain is still a challenge. Reliable and timely identification of individuals who are at risk may allow for the use of preventative interventions (MacLachlan *et al.*, 2016).

Though genetics and neurobiology are considered as important factors, several experimental or physiological sensory tests have emerged, often using extremes of temperature to measure a participant's resilience to pain. Preoperative results of these tests have been shown to predict the severity of acute postoperative pain. Preoperative assessment of an individual's capacity to withstand pain could provide clues as to their risk of developing a prolonged pain state postoperatively. Experimental stimulation methods include: the cold pressor test, heat immersion test, brief phasic or tonic heat stimulation, cutaneous electrical stimulation, pressure algometry, punctate mechanical stimulation and induction of an inflammatory injury (MacLachlan *et al.*, 2016).

2.2.1 Theories of pain

As a result of the discovery of neurones and how they conduct signals, including pain, various theories have been proposed as to the causes of pain and its role or function. Many theories have been postulated to describe mechanisms underlying pain perception (Moayedi and Davis, 2013). Specialized peripheral sense organs (nociceptors) detect tissue-damageing events, and their activities initiate pain. Their signals have been shown

to be partially segregated in transmission to multiple brain centres including the thalamus and the cerebral cortex (Perl, 2007).

2.2.1.1 Specificity theory of pain

The specificity theory, proposed based on the earlier work of Rene Descartes, a 17th century philosopher, and supported by Max von Frey (Chen, 2011) states that pain is a specific sensation, with its own sensory apparatus independent of touch and other sensations. It is one of the first modern theories of pain and holds that specific pain receptors transmit signals to a "pain centre" in the brain that produces the perception of pain. According to this theory, each modality has a specific receptor and associated sensory fibre that is sensitive to one specific stimulus. The theory proposes that non-noxious mechanical stimuli are encoded by low-threshold mechanoreceptors, which are associated with dedicated primary afferents that project to specific second-order neurones in the spinal cord. Similarly, noxious stimuli would activate a nociceptor, which would project to higher “pain” centres through a pain fibre. These ideas have been emerging over several millennia but were experimentally tested and formally postulated as a theory by physiologists in the 19th century (Moayedi and Davis, 2013).

2.2.1.2 Intensity theory of pain

The intensity theory, first conceptualised by Plato in the fourth century, defines pain, not as a unique sensory experience but rather, as an emotion that occurs when a stimulus is stronger than usual. The theory was supported by the German neurologist, Wilhelm Erb in 1874, who claimed that pain was produced by stronger activation of nerves by an intense

stimulus, while a weak stimulus produced non-painful sensation (Chen, 2011). The intensive (or Summation) theory was further advanced by Arthur Goldscheider and been postulated at several different times throughout history. The theory defines pain, not as a unique sensory experience but rather, as an emotion that occurs when a stimulus is stronger than usual (Moayedi and Davis, 2013). This theory competed with the specificity theory, but later lost support with Sherrington's evolutionary framework for the specificity theory and also postulated the existence of sensory receptors that are specialized to respond to noxious stimuli, for which he coined the term "nociceptor" (Moayedi and Davis, 2013).

2.2.1.3 Pattern theory of pain

The pattern theory of pain is a quantitative theory of feeling formulated by American Psychologist, John Paul Nafe in 1929. It stated that any somaesthetic sensation occurred by a specific and particular pattern of neural firing and that the spatial and temporal profile of firing of the peripheral nerves encoded the stimulus type and intensity (Moayedi and Davis, 2013). The pattern theory was mostly dependent upon the development of the cathode-ray oscilloscope and the use of electrophysiological recordings to identify and classify single sensory afferent fibres according to size and conduction velocity by Joseph Erlanger and Herbert Spencer Gasser (Chen, 2011).

2.2.1.4 Gate control theory of pain

The transmission of information about injury from the periphery to the first central cells is under control, and the setting of this control or gate is influenced by peripheral afferents,

other than those which signalled the injury (Wall, 1978). The gate theory of pain, proposed by Ronald Melzack (a Canadian psychologist) and Patrick David Wall (British neurologist) in 1965, remains the most recognised theory of pain perception.

According to the gate theory, a gate exists at the first synaptic relay between primary afferent neurons in lamina II (substantia gelatinosa) of the spinal dorsal horn. When the activity mediated by nociceptive afferents prevails, it exacerbates pain via deactivation of the inhibitory substantia gelatinosa interneurones. When neural activity mediated by large non-nociceptive afferent fibres prevails, it inhibits the activity of small nociceptive afferent fibres by activating the inhibitory substantia gelatinosa interneurones, resulting in hypoalgesia or analgesia. The gate is also modulated by central control of supraspinal (descending) or segmental origin (Chen, 2011). The gate in the spinal cord is the substantia gelatinosa in the dorsal horn, which modulates the transmission of sensory information from the primary afferent neurones to transmission cells in the spinal cord. The gating mechanism is controlled by the activity in the large and small fibres. Large-fibre activity inhibits (closes) the gate, whereas small-fibre activity facilitates (opens) the gate. Activity from descending fibres that originate in supraspinal regions and project to the dorsal horn could also modulate the gate. When nociceptive information reaches a threshold that exceeds the inhibition elicited, it opens the gate and activates pathways that lead to the experience of pain and its related behaviours (Moayedi and Davis, 2013).

The gate control theory of pain is based on the propositions that the transmission of nerve impulses from afferent fibers to spinal cord is modulated by a spinal gating mechanism in

the dorsal horn. The spinal gating mechanism is influenced by the relative amount of activity in large-diameter and small-diameter fibres, where activity in large fibres inhibit transmission (close the gate) while activity in small fibres facilitate transmission (open the gate). The spinal gating mechanism is influenced by nerve impulses that descend from the brain. A specialized system of large-diameter, rapidly conducting fibres (the central control trigger) activates selective cognitive processes that then influence, by way of descending fibres, the modulating properties of the spinal gating mechanism. When the output of the spinal cord transmission exceeds a critical level, it activates the action system that underlies the complex and sequential patterns of behaviour and experience characteristic of pain (Melzack, 1996).

2.2.2 Pain pathways

Nociceptors (pain receptors) are free nerve endings that respond to noxious stimuli. They transmit information to the brain and are stimulated by noxious electrical, thermal, mechanical, and chemical stimuli. Pain perception occurs when these stimuli are transmitted to the spinal cord and then to the brain. Pain impulses travel to the dorsal horn of the spine, where they synapse with dorsal horn neurones in the substantia gelatinosa and then ascend to the brain. The basic sensation of pain occurs at the thalamus. It continues to the limbic system (emotional center) and the cerebral cortex, where pain is perceived and interpreted (Helms and Barone, 2008).

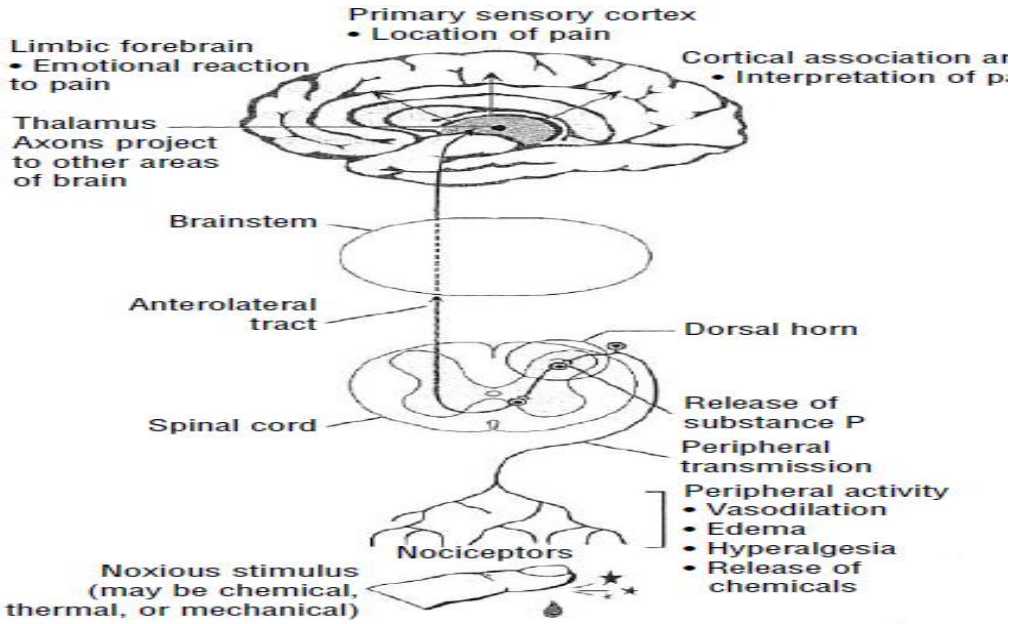


Figure 2.1: The pain pathway (Helms and Barone, 2008).

The nociceptors, located in the dorsal root ganglia (DRG), are high threshold and non adapting receptors that are stimulated only by stimuli of noxious intensity. Primary afferent fibres involved in pain transmission include a population of large diameter myelinated Aδ fibres and small diameter non-myelinated C fibres. The large Aδ fibres transmit sharp well-defined pain, called "fast pain" or "first pain," (e.g. by a cut, an electrical shock), while the smaller C fibers transmit dull burning or aching sensations, known as "slow pain" (Helms and Barone, 2008). Aδ nociceptors project to lamina I and V, while C fibre nociceptors project more superficially to laminae I and II. While peptidergic C fibres terminate within lamina I and dorsal part of lamina II, non-peptidergic C fibres terminate in the mid-region of lamina II. The most ventral part of lamina II is characterised by the presence of excitatory inter-neurones that express the gamma isoform of protein kinase C (PKCγ), which has been implicated in injury-induced persistent pain (Basbaum *et al.*, 2009).

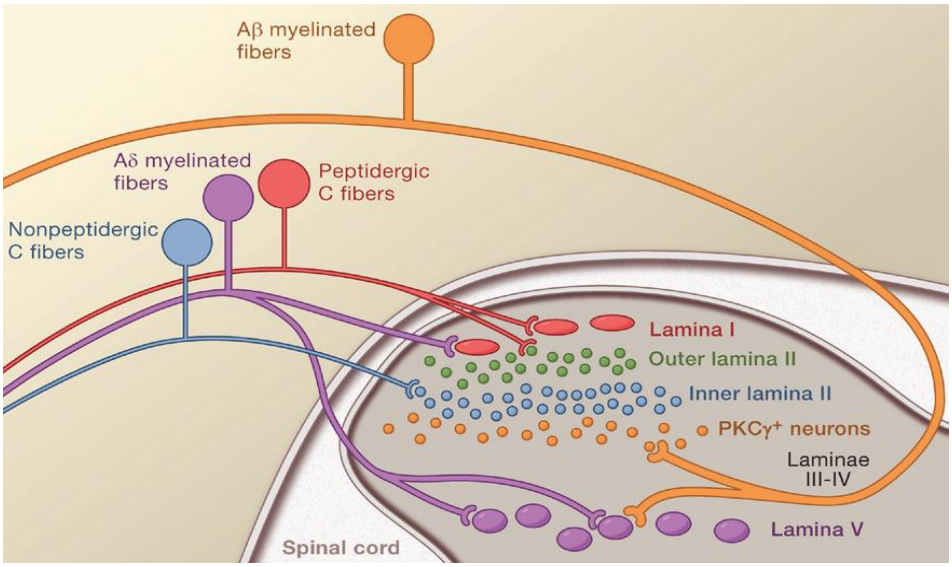


Figure 2.2: Afferent pain fibres – spinal cord connection (Basbaum *et al.*, 2009)

Sensory nerves are pseudounipolar in structures, with one cytoplasmic extension moving to the periphery and the other to the spinal cord from the cell body located in the dorsal root ganglion (DRG). Myelinated large diameter Aα and Aβ fibres act as afferents from low-threshold tissue mechanoreceptors. Small calibre myelinated Aδ and unmyelinated C fibres transmit nociceptive information. Unmyelinated fibres cross the epidermal–dermal junction (basement membrane) into the epidermis, forming intra-epidermal nerve fibres (IENFs). These nociceptors respond to thermal, mechanical and chemical stimuli. A diverse array of receptors and signalling molecules are present in these nociceptor terminals (Brown *et al.*, 2014).

The PKCγ layer is targeted predominantly by myelinated non-nociceptive afferents. Electrophysiological analyses demonstrate that spinal cord neurones within lamina I are

generally responsive to noxious stimulation (via Aδ and C fibers), while neurones in laminae III and IV are primarily responsive to innocuous stimulation (via Aβ). Lamina V neurones (also called wide dynamic range (WDR) neurones, in that they respond to a broad range of stimulus intensities) receive a convergent non-noxious and noxious input via direct (monosynaptic) Aδ and Aβ inputs and indirect (polysynaptic) C fiber inputs. There is also commonly a visceral input to WDR neurones, such that the resultant convergence of somatic and visceral inputs likely contributes to the phenomenon of referred pain (Basbaum *et al.*, 2009).

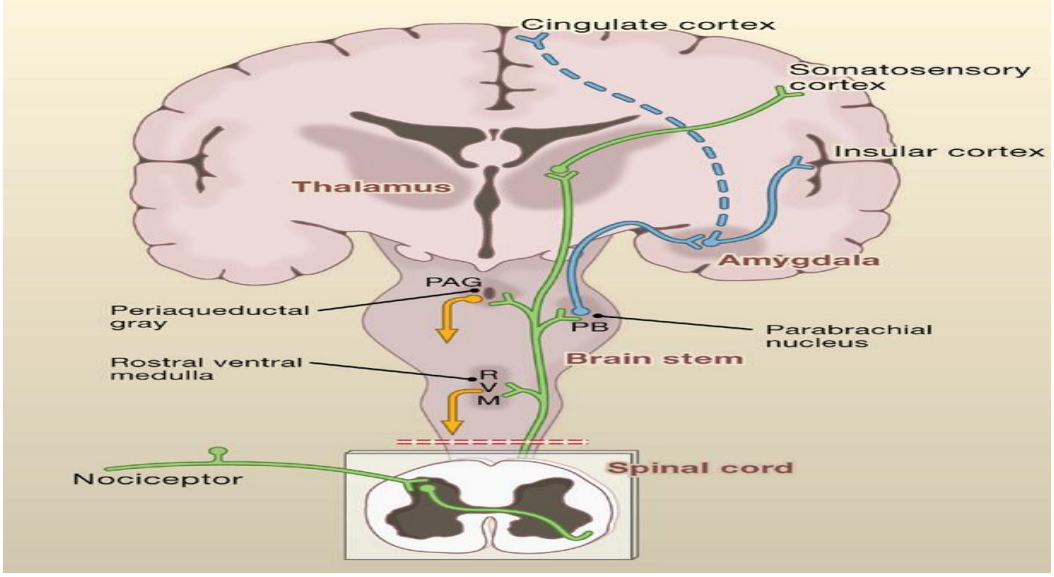


Figure 2.3: Ascending and descending pain pathway (Basbaum *et al.*, 2009).

2.2.3 Molecular and chemical pain mediators

As a result of tissue damage, chemical substances that modulate the transmission of pain are released into the extracellular fluid and activate the pain receptors by irritating nerve endings. These chemical mediators include histamine, substance P, bradykinin,

acetylcholine, leukotrienes, and prostaglandins (Helms and Barone, 2008). Persistent pain is associated with the production and release of many nociceptive and inflammatory mediators. Important roles in the pain inflammatory process have been suggested for circulatory neurochemical biomarkers, including endocannabinoids, endogenous opioids, and serotonin. Endogenous opioids (dynorphins, endorphins, enkephalins) have also been implicated in pain modulation, both directly and through the placebo response. Opioids act via central and peripheral opiate μ, κ and δ receptors to produce analgesic effects. Endogenous opioids also regulate inflammation through opioid receptors found on immune cells at the site of inflammation (Degenhardt *et al.,* 2007).

Serotonin (5-hydroxytryptamine [5-HT]), a major neurotransmitter component of the inflammatory chemical milieu and a potent stimulant for nociceptive nerve endings in the peripheral nervous system, is found in platelets and basophils, where it can be released under conditions of injury, and acts on variety of 5HT receptors, of which 5-HT1A, 5-HT2A, 5-HT3, and 5-HT4 have the greatest relevance in nociception. Serotonin is found in high concentrations in the blood products of individuals with chronic painful inflammatory conditions, such as fibromyalgia and rheumatoid arthritis (Degenhardt *et al.,* 2007).

Cannabinoids, such as Δ^9-tetrahydrocannabinol, act via cannabinoid-1 (CB1) receptors within peripheral, spinal, and central pathways to produce analgesic effects. Cannabinoid-2 (CB2) receptors are sparse in the central nervous system but are prominent in cells of the immune system, playing an important role in the mediation of inflammatory pain. The

endocannabinoid anandamide (arachidonoylethanolamide [AEA]) has been shown to cause strong analgesic and anti-inflammatory effects in animal models. Although not an endocannabinoid, an endogenous analogue of AEA called N-palmitoylethanolamide (PEA) also possesses potent analgesic and anti-inflammatory properties. Studies have shown elevated PEA levels in humans with inflammatory and neuropathic conditions. B-endorphin, AEA, and PEA were reported to significantly change at 30 minutes and 24 hours after osteopathic manipulative treatment (Degenhardt *et al.*, 2007).

2.2.4 Nociceptor activation

2.2.4.1 Thermal activation

There is a clear demarcation between the perception of innocuous warmth and noxious heat, which enables humans to recognize and avoid temperatures capable of causing tissue damage. The pain threshold, which typically rests around 43°C, parallels the heat sensitivity of C and type II Aδ pain fibres. Cultured neurones from dissociated dorsal root ganglia show similar heat sensitivity. Molecular insights into the process of heat sensation came from the cloning and functional characterization of the receptor for capsaicin, the main pungent ingredient in “hot” chili peppers. Capsaicin and related vanilloid compounds produce burning pain by depolarizing specific subsets of C and Aδ nociceptors through activation of the capsaicin (or vanilloid) receptor, TRPV1, a member of the transient receptor potential (TRP) ion channel family. The cloned TRPV1 channel is also gated by increases in ambient temperature, with a thermal activation threshold of about 43°C (Basbaum *et al.*, 2009).

Most cold sensitive neurones respond to natural cooling agents, such as menthol, and they display a thermal activation threshold of about 25°C. TRPM8 is a cold- and menthol-sensitive channel and TRPM8-deficient mice show a significant loss of menthol and cold-evoked responses. TRPM8-deficient mice are not completely insensitive to cold. There is a group of cold sensitive, menthol-insensitive neurones that have a low threshold of activation, approximately 12°C. These may account for the residual cold sensitivity seen in behavioral tests, wherein TRPM8-deficient animals can still avoid extremely cold surfaces below 10°C (Basbaum *et al.,* 2009).

2.2.4.2 Mechanical activation

High-threshold mechanoreceptors include C fibres and slowly adapting Aδ mechanoreceptors, both of which terminate as free nerve endings in the skin. Low-threshold mechanoreceptors include Aδ D hair fibers that terminate on down hairs in the skin and detect light touch. Finally, Aβ fibers that innervate Merkel cells, Pacinian corpuscles, and hair follicles detect texture, vibration, and light pressure (Basbaum *et al.,* 2009).

2.2.4.3 Chemical activation

Chemo-nociceptive mechanisms trigger aversive responses to a variety of environmental irritants. TRP channels which function as receptors for plant-derived irritants, including capsaicin (TRPV1), menthol (TRPM8), isothiocyanates, and thiosulfinates (TRPA1) have prominent roles in chemo-nociception. Acrolein (2-propenal, a highly reactive α,β-unsaturated aldehyde present in tear gas, vehicle exhaust, or smoke from burning

vegetation, i.e., forest fires and cigarettes), and other volatile irritants (such as hypochlorite, hydrogen peroxide, formalin, and isocyanates) activate sensory neurones that innervate the eyes and airways, producing pain and inflammation. Mice lacking TRPA1 show greatly reduced sensitivity to such agents, indicating the critical role of TRPA1 as a sensory detector of reactive environmental irritants. In addition to these environmental toxins, the channel is targeted by some general anesthetics (such as isofluorane) or metabolic by-products of chemotherapeutic agents (such as cyclophosphamide), which likely underlies some of the adverse side effects of these drugs, including acute pain and robust neuro-inflammation (Basbaum *et al.*, 2009).

Acid sensing ion channels (ASICs) are acid-sensitive ion channels that serve as receptors for extracellular protons (tissue acidosis) produced during ischaemia. Although these channels are expressed by both low- and high-threshold mechanosensitive neurones, genetic studies do not uniformly support an essential role in mechanotransduction. ASICs are members of the degenerin/epithelial Na channel (DEG/ENaC) family that are activated by acidification and thus represent another important site for the action of extracellular protons produced as a consequence of tissue injury or metabolic stress. ASIC3 are specifically expressed by nociceptors and especially well represented in fibers that innervate skeletal and cardiac muscle. In these tissues, anaerobic metabolism leads to build-up of lactic acid and protons, which generate musculoskeletal or cardiac pain. ASIC3-containing channels detect and integrate signals specifically associated with muscle ischaemia (Basbaum *et al.,* 2009).

2.2.5 Pain sensitisation

A wide range of thermal, mechanical as well as chemical stimuli are detected and interpreted by the nervous system. At noxious thresholds, these stimuli generate pain. In the setting of persistent injury, the peripheral and central nervous system components of the pain transmission pathway exhibit tremendous changes (plasticity), thus enhancing pain signals and producing hypersensitivity. Alterations of the pain pathway lead to hypersensitivity, such that pain outlives its usefulness as an acute warning system and instead becomes chronic and debilitating. Plasticity can be beneficial when it facilitates protective reflexes, but if the changes persist, it may lead to a chronic pain condition (Basbaum *et al.,* 2009).

Learning as well as motivational models of pain postulates that in addition to respondent conditioning, operant conditioning, including responses to rewarding outcomes, is important in the development and maintenance of chronic pain. Individual responsiveness to reward was reported to correlate with the magnitude of analgesia during acute pain. Pain and reward are interacting processes which are related to partly similar brain regions, such as the striatum, the prefrontal cortex (PFC), the anterior cingulated cortex (ACC), the orbitofrontal cortex, or the amygdala, that are also found being altered in chronic pain. In particular, individual variation in the nucleus accumbens response to reward anticipation was highlighted as significant predictor of placebo analgesia and thus pain reduction, accounting for one-third of the explained variance. Moreover, for the transition from acute to chronic pain, higher connectivity in corticostriatal regions predicts pain severity and persistence as well as affective aspects of pain (Nees *et al.*, 2017).

2.2.5.1 Peripheral mechanism of pain sensitization

Sensitization of the pain signaling system is a key process in the development chronic pain states. Mediators generated and released at different levels of the neuroaxis, e.g from peripheral tissues affected by injury, causes sensitization and also tonic activation in the pain signaling pathway. As a result of tissue injury, resident cells (including mast cells, dendritic cells, and resident macrophages) and recruited cells (most prominently circulating macrophages, neutrophils, and T cells) release proalgesic mediators, including prostanoids, TNFα and IL-1β, nerve growth factor (NGF), and a number of chemokines. Such proalgesic mediators activate and sensitize the terminals of nociceptors, making them spontaneously active and more readily activated, probably by altered neurotrophic support, in the form of increased factors such as NGF from inflamed tissues, which will act on a subpopulation of DRG neurones to effect release of further neurotrophins such as BDNF (Denk and McMohan, 2012).

Peripheral sensitization results from inflammation-associated changes in the chemical environment of the nerve fibre. Tissue injury is often accompanied by the accumulation of endogenous factors released either from activated nociceptors or non-neural cells that reside within or infiltrate into the injured area (including mast cells, basophils, platelets, macrophages, neutrophils, endothelial cells, keratinocytes, and fibroblasts). Collectively, these inflammatory soup represent a wide array of signaling molecules, including neurotransmitters, peptides (substance P, CGRP, bradykinin), eicosinoids and related lipids (prostaglandins, thromboxanes, leukotrienes, endocannabinoids), neurotrophins, cytokines, and chemokines, as well as extracellular proteases and protons. Remarkably,

nociceptors express one or more cell-surface receptors capable of recognizing and responding to each of these pro-inflammatory or pro-algesic agents. Such interactions enhance excitability of the nerve fibre, thereby heightening its sensitivity to temperature or touch (Basbaum *et al.*, 2009).

2.2.5.2 Central mechanism of pain sensitization

Amplification of pain-related signals also occurs within the central nervous system (central Sensitization). Projection neurones in the spinal cord that carry sensory information to the brain become more responsive to both noxious and innocuous inputs. Central sensitization is a form of synaptic plasticity and is precipitated by repetitive activity in nociceptors and depends critically on recruitment of NMDA receptors. fMRI studies have shown alteration in the cortical representation of somatotopic areas generating pain, a shift in their connectivity, and dynamic changes in gray and white matter density in brains of chronic pain patients. Brains of chronic pain patients also exerts altered descending control on the spinal cord (Denk and McMohan, 2012).

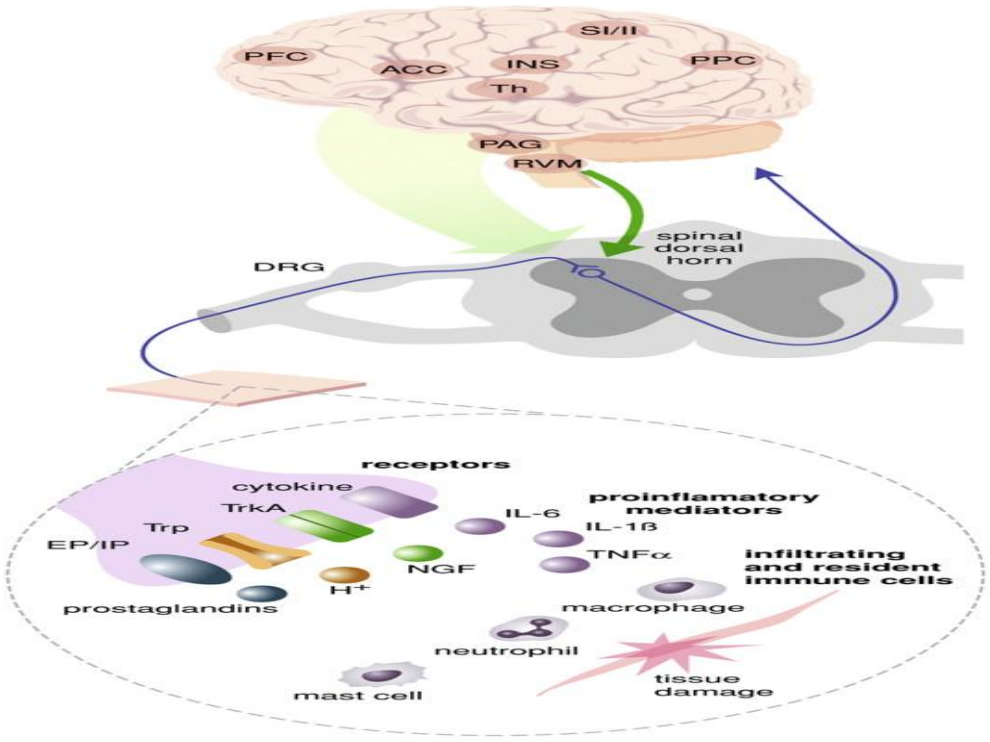


Figure 2.4: Peripheral and central pain sensitization (Denk and McMohan, 2012)

2.2.5.3 Glutamate mediated sensitization

Acute pain is signalled by the release of glutamate from the central terminals of nociceptors. The released glutamate act via AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propanoic acid) and kinate ionotropic glutamate receptor to generate excitatory postsynaptic currents (EPSCs) in second order dorsal horn neurones. In the setting of injury, increased release of neurotransmitters from nociceptors will sufficiently depolarize postsynaptic neurones to activate quiescent NMDA (N-methyl-D-aspartic acid) receptors. Increase in calcium influx strengthens synaptic connections between nociceptors and dorsal horn pain transmission neurones, which in turn will exacerbate responses to noxious stimuli (hyperalgesia) (Basbaum *et al.*, 2009).

Glutamate mediates fast excitatory synaptic neurotransmission via ionotropic and metabotropic receptors. Activation of NMDA receptors by glutamate has been hypothesised to play a role in primary headache disorders. Plasma glutamate levels monitoring in migraine patients might serve as a biomarker of response to treatments and as an objective measure of disease status (Surbaki *et al.*, 2017b). Elevation of interstitial glutamate concentration in the masseter muscle was found to significantly increase the intensity of spontaneous pain in Temporo-mandibular disorder patients (Shimada *et al.*, 2016). Higher level of glutamate within the right posterior insula has been associated with lower pressure pain thresholds (Harris *et al.*, 2009). Hyperalgesia is associated with higher levels of glutamate in the posterior insula (known to play a prominent role in pain and interoceptive sensory processing) than the anterior insula (involved in the affective processing of pain and other subjective feelings) (Harris *et al.*, 2009). Serum glutamate concentration was reported to be significantly higher in multiple sclerosis patients when compared with the healthy control (Al Gawwam and Sharquie, 2017).

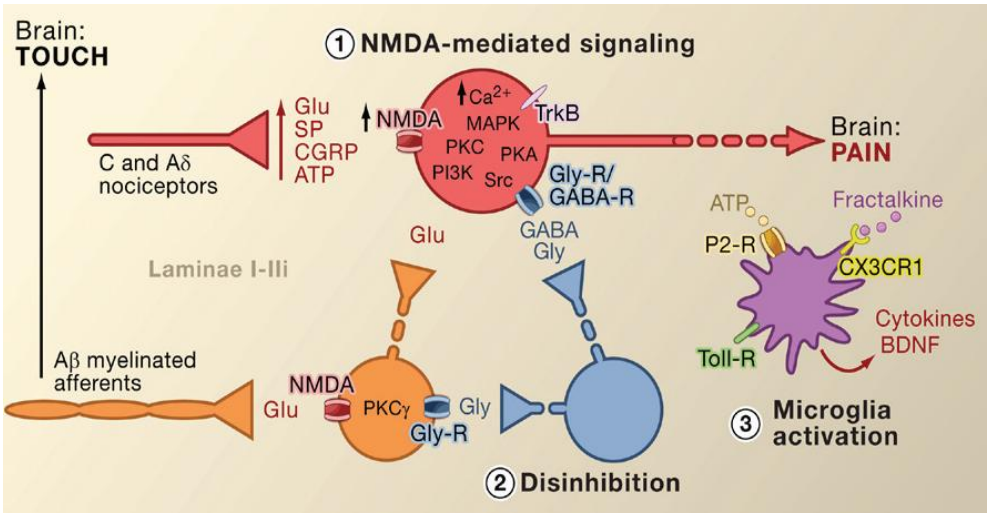


Figure 2.5: Central sensitisation signalling (Basbaum *et al.*, 2009)

The kainate receptor, a member of the ionotropic glutamate receptor (iGluR) family, has been reported to function as mediator and modulator of synaptic transmission and plasticity by regulating postsynaptic currents and presynaptic neurotransmitter release. Increasing evidence has shown activation and modulation of kainate receptors by nociceptive stimuli. Kainate receptors are present in dorsal root ganglia, trigeminal ganglion (TG), and in both pre and postsynaptic sites in superficial laminae of the spinal cord and caudal brain stem. Non-selective AMPA/kainate receptor antagonists have been shown to produce anti-nociceptive effects in both animal and human studies. Competitive glutamate kinate receptor (GluK1) selective receptor antagonists were reported to be effective in blocking trigeminal activation after stimulation of the TG. In a double-blind controlled study, GluK1 receptor antagonist was reported to be effective in relieving pain in acute migraine. Topiramate, a partial kainate receptor antagonist, was reported to be clinically effective migraine preventive and has been shown to reduce trigeminovascular and thalamic activation and to inhibit cortical spreading depression in the mammalian cortex. The use of microiontophoretic application of a kainate specific antagonist demonstrated a bidirectional regulation of trigeminovascular nociceptive transmission in the trigemino-cervical complex (TCC) mediated by kainate receptors (Andreou *et al.*, 2015).

2.2.5.4 Loss of GABAergic and glycinergic controls

GABAergic or glycinergic inhibitory interneurones are densely distributed in the superficial dorsal horn and are at the basis of the gate control theory of pain. Loss of function of these inhibitory interneurones (disinhibition) results in increased pain

sensitivity. Spinal administration of GABA (bicuculline) or glycine (strychnine) receptor antagonists has been reported to produces behavioral hypersensitivity in rodents that resemble that observed after peripheral injury. Peripheral nerve injury leads to a decrease in inhibitory postsynaptic currents in superficial dorsal horn neurones, which results from peripheral nerve injury-induced death of GABAergic interneurones. The resulting decreased tonic inhibition enhances depolarization and excitation of projection neurones.

In NMDA-mediated central sensitization, disinhibition enhances spinal cord output in response to painful and nonpainful stimulation, contributing to mechanical allodynia. Deletion of the gene encoding PKCγ in the mouse leads to a marked decrease in nerve injury-evoked mechanical hypersensitivity, thus, addressing the involvement of these neurones in the disinhibitory process. After blockade of glycinergic inhibition with strychnine, innocuous brushing of the hindpaw activates PKCγ-positive interneurones in lamina II, as well as projection neurones in lamina I. Because PKCγ-positive neurones in the spinal cord are located only in the innermost part of lamina II, it follows that these neurones are essential for the expression of nerve injury-evoked persistent pain, and that disinhibitory mechanisms lead to their hyperactivation (Basbaum *et al.,* 2009).

Several members of the Toll-like receptors (TLRs) family have been implicated in the activation of microglia following nerve injury. TLRs are transmembrane signalling proteins expressed in peripheral immune cells and glia. As part of the innate immune system, they recognize molecules that are broadly shared by pathogens. Genetic or pharmacological inhibition of TLR2, TLR3, or TLR4 function in mice results in decreased

microglial activation, and also reduces the hypersensitivity triggered by peripheral nerve injury. Candidate endogenous ligands that activate TLR2-4 after nerve injury include mRNAs or heat shock proteins that could leak from the damaged primary afferent neurones and diffuse into the extracellular milieu of the spinal cord.

Astrocytes are induced in the spinal cord after nerve injury. Activation of astrocyte is generally delayed and persists much longer, up to several months, thus, indicating that astrocytes are more critical in the maintenance, rather than induction of central sensitization and persistent pain (Basbaum *et al.,* 2009).

Peripheral injury activates glia not only in the spinal cord, but also in the brainstem, where they contribute to supraspinal facilitatory influences on the processing of pain messages in the spinal cord (descending facilitation). Such facilitation is especially prominent in the setting of injury and appears to counteract the feedback inhibitory controls that concurrently arise from various brainstem loci (Basbaum *et al.,* 2009).

2.2.6 Conducting acute pain signal

Transduction of thermal and mechanical signals by primary afferent terminal leads to activation of a variety of voltage-gated ion channels by the receptor potential. Voltage-gated sodium and potassium channels are critical to the generation of action potentials that convey nociceptor signals to synapses in the dorsal horn. Voltage-gated calcium channels play a key role in neurotransmitter release from central or peripheral nociceptor terminals to generate pain or neurogenic inflammation, respectively (Basbaum *et al.,* 2009).

2.2.6.1 Role of voltage-gated sodium channels

A variety of sodium channels are expressed in somatosensory neurones, including the tetrodotoxin (TTX)-sensitive channels Nav1.1, 1.6, and 1.7 and the TTX-resistant channels Nav1.8 and 1.9. Among these channels, Nav1.7 has received much attention, as it's altered activity leads to a variety of pain disorders. Loss of function mutations within the SCN9A gene (that encode for Nav1.7) leads to inability to detect noxious stimuli, while it's gain of function mutation is associated with erythromelalgia (a rare condition that primarily affects the extremities, characterised by intense burning pain, severe redness (erythema), and increased skin temperature) and paroxysmal extreme pain disorder (PEPD/familial rectal pain syndrome; a rare disorder characterised by pain in the mandibular, ocular and rectal areas as well as flushing), both of which cause intense burning sensations. Animal studies have demonstrated that Nav1.7 is highly upregulated in a variety of inflammatory pain models.

2.2.6.2 Role of voltage-gated calcium channels

A variety of voltage-gated calcium channels are expressed in nociceptors. N-type (neutral/non-L), P-type (purkinje)/Q-type, and T-type (transient) calcium channels have received the most attention. P/Q-type channels are expressed at synaptic terminals in laminae II–IV of the dorsal horn and mutations in these channels have been linked to familial hemiplegic migraine. N and T-type calcium channels are also expressed by C fibres and are upregulated under pathophysiological states, e.g. in diabetic neuropathy or after other forms of nerve injury. Animals lacking Cav2.2 (N-type) or Cav3.2 (T-type)

channels show reduced sensitization to mechanical or thermal stimuli after inflammation or nerve injury, respectively (Basbaum *et al.*, 2009).

2.3 Inter-individual Variability in Pain

The experience of pain is one of the fundamental human senses and most ancient protective survival skills. However, the ways in which people express and treat pain change across time and origin and are influenced by cultural and social factors. Ethnic effects may potentially interact with other important variables, such as gender and age, to influence pain perception (Belfer, 2013). The human pain experience is composed of sensory, emotional and cognitive components. In both the expression and management of pain, biological, psychological and social factors interact in complex ways (Rollman, 1998).

The experience of pain is as old as history itself. However, variations have been present in the ways in which people conceptualize and treat pain over time. It has been suggested that nociception reflects anatomy and physiology, but cultural and social factors are the foundation for the expression and treatment of pain. The broad sociopolitical context of pain has changed over time. Ministries, departments and organizations in many countries have urged a proactive approach to pain control. The notion that pain is a biopsychosocial phenomenon that often requires interdisciplinary care has been emphasized. Research into the biology and neurobiology of pain has given new ways to think about and manage pain, and is paralleled by research into the cultural, psychological, and social factors related to

the experience of pain and its expression, behavioral responses, health care seeking, and receptivity to and adherence to treatment (IASP, 2002).

It has been reported that life style and habits (e.g., exercising, smoking, and drinking) also contribute to human pain perception. Exercise can be both a treatment and a stimulus of pain, so that too much exercise increases pain, while too little exercise may worsen pain through multiple mechanisms (e.g., pain posturing, deconditioned muscle microtrauma, and neuroendocrine responses). Exercise activates endogenous analgesia (in healthy individuals and clinical patients) by triggering the release of beta-endorphins from the pituitary gland and the hypothalamus, which in turn enables analgesic effects by activating μ-opioid receptors (Belfer, 2013).

Cigarette smoking and alcohol drinking also have a complex relationship with pain. Both nicotine and alcohol have analgesic properties. In general, nicotine administration via nasal spray or transdermal patches reduces pain sensitivity in both smokers and non-smokers likely resulting from effects at both central and peripheral nicotine acetylcholine receptors. Also smoking a cigarette decreases awareness of and increases tolerance to some experimental pain stimuli. A cross-sectional study of young twins demonstrated that smoking, alcohol consumption, and overweight in adolescence correlate with low back pain, and the follow up prospective study revealed that smoking also predicted the risk of future low back pain. Thus, life style modification may have long-term effect on adult pain (Belfer, 2013). Ayeni *et al.* (2014) reported that people who consumed alcohol were about 3 times more likely to develop low back pain.

2.3.1 Sex, gender and pain

The existence of differences between men and women in the experience of pain has been shown in epidemiological investigation, as well as in the laboratory, where pain was induced experimentally (Garcia *et al.,* 2007). Potential mechanisms in sex differences in pain include sex hormones, differences in the brain and spinal cord in men and women, genetics, sociocultural roles, stress, and neuroactive agents (Helms and Barone, 2008).

Though contradictory reports exist, sex differences in the perception of noxious experimental stimuli have been reported by various groups, with women exhibiting higher pain sensitivity than men. Variation in female sex hormone levels across the menstrual cycle has been reported to be responsible for sex related discrepancies in pain, as various studies did not specify the menstrual phase of female subjects at the time of conducting the experiments. Pain sensitivity was also found to be highest on days 7 and 14 of the menstrual cycle. Leutinizing hormone has been reported to cause increase in pain sensitivity in rats. Oestradiol and progesterone have been stated to produce analgesia that is modulated through the spinal cord k-opiate receptor analgesic system. Also, greater opioid mediated stress induced analgesia has been reported in male rats compared with female rats, and oestrogen is believed to cause this difference by suppressing stress-induced analgesia (Kumar *et al.,* 2010). Oestrogen receptors (ERα and ERβ) are found to be present in small-diameter DRG neurones, presumably nociceptors (Chaban, 2012).

A study of electronic medical records containing at least one disease-associated pain score analyzed differences in disease-specific pain reported by men and women found

significant gender specificity. Women have been reported to feel pain more intensely than men across almost all disorders, and also exhibit greater sensitivity to noxious stimuli in the laboratory compared with men. Gender has been found to also affect analgesic response, as opioids were found to produce greater analgesic responses in women than men. Gender role also influences sensitivity to experimental pain. Mechanisms underlying gender effects include biological factors (contribution of gonadal hormones, endogenous and exogenous modulations of pain) and psychosocial factors (gender roles) as well as cognitive/affective variables (Belfer, 2013). Garcia *et al.* (2007) reported that women showed a lower mean pressure pain threshold than men at different evaluated points.

Studies have shown that experimenter's gender influences the results of experimental pain assessment. Male subjects have been reported to show significantly less cold pressor-evoked pain in front of a female experimenter than a male experimenter, while female subjects tended to report higher pain to the male experimenter. A significant interaction of experimenter gender versus subject gender on pain tolerance indicated that subjects tolerated pain longer when they were tested by an experimenter of the opposite sex (Belfer, 2013).

Sex-related difference in blood pressure is emerging as a potential biological explanation of sex-related differences in pain. Studies have reported continuous, inverse relationship between resting blood pressure and pain sensitivity, and women generally have lower resting blood pressure than men. After adjusting for resting blood pressure, sex-related differences in thermal pain report were found to be no longer statistically significant.

Resting blood pressure has been reported to be inversely related to ischaemic and thermal pain sensitivity in men but not women. Other studies found resting blood pressure to be inversely related to pain sensitivity in women but not men using thermal and electrical stimuli. Women were reported to exhibit lower systolic blood pressure and lower cold pressor pain threshold and tolerance than men (Myers *et al.*, 2001).

Epidemiologic data have consistently demonstrated gender differences in pain, with women reporting a higher frequency of several types of pain, as well as showing more sensitivity to experimental painful stimulation than men, yet, this information is neither universally nor largely accepted. In a study to evaluate the effect of gender on pain perception using visual analogue pain scale after intramuscular injections among a sample of south Indian adult men and women, moderately significant higher pain scores were observed in women than in men. Also significant higher pain scores were observed in women as compared to men in the age group of 21-30 years, suggesting that increased sensitivity to pain in women than in men appear to be greatest in middle age (Kusumadevi *et al.*, 2011).

2.3.2 Racial/ethnic disparities in pain

Health disparities, as defined by the National Institute of Health (NIH), is the differences in the incidence, prevalence, mortality, and burden of diseases and other adverse health conditions that exist among specific population groups. Literature documents racial and ethnic disparities in pain epidemiology, access to quality pain care, pain assessment and treatments, as well as pain-related outcomes. It is racial and ethnic differences in the

quality of health care that are not due to access-related factors or clinical needs, preferences, and appropriateness of intervention (Anderson *et al.,* 2009). In Nigeria, Obuna and Umeora (2014) reported that perception of labor pain by the Igbo women is influenced by their personality, culture and other factors such as maternal age, parity, weight and educational status.

Experimentally controlled pain studies show that the intensity at which most people perceive a sensation and the point at which it becomes painful are approximately the same, yet, members of some ethnic groups have a higher tolerance for pain than others and can endure increasing levels of a pain stimulus for longer periods, while other cultural groups tend to be more expressive about pain (Naranyan, 2010).

Research into relations between ethnicity and pain has yielded variable outcomes. Pain and ethnicity are multidimensional, malleable, and shaped by culture. Research into ethnic disparities in pain is associated with difficulty because different studies use different protocols as well as measures such as pain intensity, pain severity, pain report, pain perception, pain behaviour, pain expression, and pain response. Further difficulties are associated with consideration of crucial distinctions among acute pain, chronic pain, clinical pain, and experimental pain (IASP, 2001). In a study in the US, electric shocks were presented to a group of American born women belonging to different ethnic groups: Yankee, Irish, Italians and Jewish. No difference was observed in their pain threshold, but there were sizable differences in tolerance, with Yankees and Jews showing higher pain tolerance, and Italians showing least (Rollman, 1998).

Several studies have reported cultural differences in perception of experimentally induced noxious stimuli. In one study, pressure pain tolerance has been found to be greater among whites relative to African Americans. Also, greater pain tolerance has been observed among whites on the cold pressor test. Reduced heat pain thresholds and tolerances have also been reported among African American subjects in comparison to white subjects. In a sample of healthy college students, African American subjects demonstrated lower thermal pain tolerances and higher ratings of pain unpleasantness in response to noxious thermal stimuli than did white subjects. Ethnic differences in ratings of the unpleasantness of noxious thermal stimuli have also been reported, with African Americans rating the thermal stimuli as more unpleasant than whites. African Americans showed a lower ischaemic pain tolerance than whites using a standard experimental pain procedure (Edwards *et al.*, 2001).

Experimental pain measures are reported to be influenced by a wide variety of factors, including peripheral transduction mechanisms, central nervous system mechanisms of pain modulation, personality, sociodemographic characteristics, and psychological variables. Ethnic differences in pain responses have been suggested to emerge as a consequence of ethnic differences in one or more of these biopsychosocial variables. Other important variables such as coping, social learning, or attitude, are believed to influence outcomes, as study has suggested that use of specific coping strategies may differ across ethnic groups. Coping styles generally vary widely across cultures and cultural differences in use of pain coping strategies may be at least as important as differences in the prevalence or reported

severity of pain. Ethnic differences in attitudinal variables related to measurement of pain may also play a role in experimental outcomes (Edwards *et al.,* 2001).

Ethnic differences in central pain regulatory systems may be responsible for ethnic variability in pain. In response to stress, a subgroups of African American hypertensives demonstrated significantly lower β-endorphin levels than Whites. Thus, ethnic differences in pain related endogenous opioid release could be responsible for observed ethnic disparities in pain response. Pain, as a stressor, is associated with enhanced epinephrine release and cardiovascular reactivity, both of which are associated with increased report of pain. African Americans have been reported to demonstrate greater adrenergic and vascular responses to stress than whites, an effect which could potentially contribute to ethnic differences in clinical and experimental pain (Edwards *et al.,* 2001).

2.3.3 Effect of life style/physical activity on pain perception

Mikkelsen and Hoej (2012) found that the usage of shock absorbing insoles caused a tendency for enhanced comfort intensity as well as a significant reduction in pain among adolescent players, during physical activity and at rest. Changes in comfort and pain perception were also found to be associated with higher PPT after using shock absorbing insoles for three weeks. The PPTs of the erector spinae muscle, tibialis anterior muscle and 5^{th} metatarsal bone increased significantly, underling important changes in pain sensitivity after three weeks of insoles usage.

It has been reported that two-thirds of the patients that report low back pain has an elevated body mass index (BMI). Studies also indicate that an elevated BMI is associated with back pain and other musculoskeletal pain syndromes due to the presence of a chronic systemic inflammatory state (Seaman, 2013). Overweight has been shown to negatively affects the musculoskeletal system disorders (Hozumi *et al.,* 2016), and there is a direct relationship between level of obesity and the likelihood of developing low back pain (Atchison and Vincent, 2012). BMI was not found to be related to changes in pain and disability in patients with chronic low back pain after 8 weeks of exercise (Brooks *et al.,* 2013), but BMI at the age of 20 years predicted radiating low back pain later in life (Frilander *et al.,* 2017). Cross sectional studies of persons with radiographic knee osteoarthritis demonstrated a strong link between increased BMI and knee pain, indicating overweight and obesity as a potential cause of knee pain (Hawamdah and Ajlouni, 2013; Rogers and Wilder, 2008). Obesity is also associated with painful conditions, including low back pain, fibromyalgia headache, and osteoarthritis. It has been estimated that approximately 50% of people who are obese regularly experience pain (Astita *et al.,* 2015). Study in a Korean community residents shows that increased fat mass and fat/muscle mass ratio were significantly associated with musculoskeletal pain among women. Widespread pain was found to be significantly associated with a high fat/muscle mass ratio. Thus, understanding the relationship between fat mass and pain may provide insights into preventative measures and therapeutic strategies for musculoskeletal pain (Yoo *et al.,* 2014).

Although as clinical syndromes, pain and obesity are significantly associated with each other. Research evaluating the relationship between obesity and pain sensitivity has yielded conflicting results. This suggests that the relationship between obesity and pain is not a direct one but is mediated by various factors. Such factors include biomechanical/structural changes associated with obesity, inflammatory mediators, mood disturbance, poor sleep, and lifestyle issues (Okifuji and Hare, 2015).

2.3.4 Effect of age on pain perception

Persistent pain is common in older people and it has been estimated that 45% to 80% of nursing home residents have substantial pain that is undertreated. Studies of both the community-dwelling and nursing home populations have found that older people commonly have several sources of pain, as older patients commonly have multiple medical problems. Older people are more likely to suffer from arthritis, bone and joint disorders, back problems, and other chronic conditions. A survey found that 45% of patients who take pain medications regularly had seen three or more doctors for pain in 5 years. Studies have suggested that 25% to 50% of community-dwelling older people suffer important pain problems. A high prevalence of dementia, sensory impairments, and disability in old population make assessment and management of pain more difficult (Ferrell *et al.*, 2002). Neuroanatomic and neurochemical findings have shown that the perception of pain and its modulation in the central nervous system are very elaborate and complex. Alterations of transmission along A-delta and C nerve fibres may be associated with ageing. Experimental studies of pain sensitivity and pain tolerance across all ages (young and old

persons) have had mixed results. In the final analysis, age-related changes in pain perception are probably not clinically significant (Ferrell *et al.,* 2002).

Psychosocial factors affect and are affected by pain in older patients. It has been shown that older adults with good coping strategies have significantly lower pain. Depression is commonly associated with pain in the older patient as researchers have found a significant correlation between pain and depression among nursing home residents (Ferrell *et al.,* 2002).

Old age has been reported to profoundly contribute to experience of pain during pathological conditions. Studies have shown that elderly patients are more vulnerable to chronic opioid use in the postoperative period. This may be the result of a change in nociceptive threshold and/or efficacy of opioids in the elderly because of old age induced impaired metabolism leading to altered pharmacokinetics and pharmacodynamics. Alternative studies have suggested that postsurgical opioid consumption is influenced less by advanced age and more by other predictors such as chronic sleeping difficulties and previous pain history. Age could also have a negative correlation with postoperative pain intensity as multiple studies have been undertaken to determine the effect of ageing on baseline nociception threshold. Meta analysis of over 50 studies indicates that there is definitive evidence of an increase in baseline nociceptive threshold with advancing age (Mecklenburg *et al.,* 2017).

2.4 Pain Catastrophizing

The experience of pain is influenced by a number of biological, social, and psychological factors. Physical state, external support and beliefs about pain all work together to influence the intensity and quality of pain. One of the strongest psychological factors associated with pain is catastrophizing. Pain catastrophizing is a negative cognition related to the belief that the experienced pain will inevitably result in the worst possible outcome. It is believed to be a multidimensional construct comprising rumination, helplessness, and pessimism (Bishop *et al.,* 2016). Catastrophizing has been broadly conceived as an exaggerated negative "mental set" brought to bear during actual or anticipated pain experience, and is associated with heightened pain experience (Sullivan *et al.,* 2001), and involves an exaggerated negative orientation toward noxious stimuli (Sullivan *et al.,* 1995). The tendency to catastrophize in response to pain contributes to negative emotional and physical outcomes. Pain catastrophizing has been associated with several pain-related outcomes including more intense pain, heightened pain behavior, greater analgesic consumption, reduced involvement in daily activities, and occupational disability (Sullivan *et al.*, 2006).

Pain catastrophizing has been associated with a number of indices of pain sensitivity in the context of experimental pain testing paradigms, both among healthy participants and individuals with various chronic pain conditions. The term catastrophizing was formally introduced by Albert Ellis, and subsequently adapted by Aaron Beck to describe a mal-adaptive cognitive style employed by patients with anxiety and depressive disorders (Quartana *et al.,* 2009). Pain catastrophizing has been associated with wide range of health-related outcomes, including pain intensity, interference of pain with patients' life,

physical disability, and mental well-being. It also contributes unique variance to the prediction of health outcomes even when controlling for the effect of pain intensity (Suso-Ribera *et al.,* 2017).

Pain catastrophizing can be measured by the Pain Catastrophizing **Scale (PCS)**. Factor analysis suggests a hierarchical factor structure, with three individual subscales of the PCS (i.e., rumination, magnification, and pessimism) contributing to an overall factor of pain catastrophizing. The PCS offers multidimensional measurement of catastrophizing, whereas other common measures of catastrophizing (e.g., **Pain-Related Self-Statement Scale [PRSS]or Coping Strategies Questionnaire [CSQ]**) assess the construct unidimensionally (Bishop *et al.,* 2016).

2.4.1 The pain catastrophizing scale

The pain catastrophizing scale (PCS) is a 13 item scale developed in 1995 at the McGill University Centre for Research on Pain and Disability (Sullivan *et al.,* 1995). Factor analyses of the PCS have shown that catastrophizing can be viewed as a multidimensional construct comprising elements of rumination ("I can't stop thinking about how much it hurts"), magnification ("I worry that something serious may happen"), and helplessness ("There is nothing I can do to reduce the intensity of the pain"), which have been replicated in several investigations.

The PCS instructions ask participants to reflect on past painful experiences, and to indicate the degree to which they experienced each of 13 thoughts or feelings when experiencing

pain, on 5-point scales (0: not at all; 1: to a slight degree; 2: to a moderate degree; 3: to a great degree; 4: all the time). The items are made of statements as follows:

1. I worry all the time about whether the pain will end
2. I feel a can't go on
3. It's terrible and I think it is never going to get any better
4. It's awful and I feel that it overwhelms me
5. I feel I can't stand it anymore
6. I become afraid that the pain will get worse
7. I keep thinking of other painful events
8. I anxiously want the pain to go away
9. I can't seem to keep it out of my mind
10. I keep thinking about how much it hurts
11. I keep thinking about how badly I want the pain to stop
12. There is nothing I can do to reduce the intensity of the pain
13. I wonder whether something serious may happen

The PCS yields a total score and three subscale scores assessing rumination, magnification and helplessness. The total score, ranging from 0 – 52, is computed by summing responses to all 13 items.

Rumination: Sum of items 8, 9, 10, 11

Magnification: Sum of items 6, 7, 13

Helplessness: Sum of items 1, 2, 3, 4, 5, 12 (Sullivan *et al.*, 1995).

2.5 Experimental Pain Induction

Fast and efficient evaluations of pain treatments are made possible in healthy participants, making these techniques a useful and less expensive prerequisite to clinical studies. The precise quantitative control and the ability to manipulate the stimuli is considered to be a considerable research advantage (Mitchell *et al.*, 2004).

2.5.1 The cold pressor test

The cold pressor task (CPT), an experimental technique for inducing pain in humans, involves placing a hand or forearm in cold water, thus inducing a slowly mounting pain that disappears on withdrawal of the hand from the water. The CPT is free from the influence of potentially confounding factors related to illness and painful medical procedures (such as nausea, fatigue, and anxiety) often found in the clinical setting. It also allows greater control over details of the stimulus location, duration, and intensity (Baeyer *et al.*, 2005).

The cold pressor pain is suggested to be a method that mimics the effects of chronic conditions effectively because of its unpleasantness, and it has excellent reliability and validity. It has been used in studies investigating a wide range of pain management techniques such as acupuncture, hypnosis, neutral distraction, and cognitive preparation (Mitchell *et al.*, 2004).

Temperature and other environmental stressors are known to affect physiological responses. Sudden and increasingly painful cold stress causes massive sympathetic

discharge and release of norepinephrine, which triggers responses in the cardiovascular system, including arteriolar constriction, increased heart rate and increased cardiac contractility, with resultant increase in blood pressure. The cold pressor test provides a valid and low-risk test for evaluating pain in preoperative patients and has been routinely used in pain research. The test is extensively documented in pain research and in cardiac physiology. Preoperative cold pressor scores for time to pain threshold and tolerance appeared to predict the likelihood of experiencing prolonged pain. In a study of patients scheduled for lower third molar extraction, preoperative pain unpleasantness in the cold pressor test was found to be the best predictor of maximum post-operative pain intensity (MacLachlan *et al.*, 2016).

Cold pressor test has been used clinically as a stress test to assess left ventricular function, cardiac autonomic function and as an experimental pain stimulus. Several studies have indicated that the cardiovascular response to the cold pressor test can predict the future development of hypertension. Studies of black and white adults and children have indicated that black subjects, who are at increased risk for developing early hypertension, show stronger vascular reactions to the cold pressor test than do white subjects. The cold pressor test has been shown to dilate the coronary arteries of normal subjects but constrict the coronary arteries of hypertensive subjects. A study used cold pressor test-induced coronary vasoconstriction to successfully predict subsequent adverse cardiovascular events (e.g., stroke) in asymptomatic type 2 diabetic patients. Presumably, the vasoconstriction is associated with endothelial dysfunction that results in long-term cardiovascular pathology (Silverthorn and Michael, 2013).

The CPT involves dipping a hand in cold water, which acts as a pain stimulus and causes massive stimulation of the sympathetic nervous system. It is a widely used experimental technique for pain or stress induction. It is typically performed by immersing a subject's hand into ice water (1°to 5°C) for a short period of time (Banoo *et al.,* 2016). Nerve fibres stimulated by cold are myelinated A delta, related to pain sensation, and non-myelinated C, responsible for nociceptive stimuli perception. Controlled temperature between 0^{o}C and 2^{o}C has been reported to be able to induce pain. Pain as a result of cold exposure may be related to injury to blood vessels or to peripheral nervous structures responsible for thermoregulation. Injury to peripheral nerves leads to release of cell mediators acting on nerve fibres and changing the number and location of ion channels. These changes tend to increase the inflow of action potential generating ions and, as a result, cause a decrease in depolarization threshold of nociceptors. Thus, stimuli which do not activate nociceptors, reach pain threshold (Silva *et al.,* 2016).

2.5.2 Ischaemic pain test

Ischaemic pain procedure produces a deep, aching pain similar to many clinical pain syndromes and is thought to activate endogenous opioid pain regulatory systems (Sherman and LeResche, 2006). Ischaemic pain can be induced using a modified sub-maximal effort tourniquet procedure (Moore *et al.,* 1979). The left arm is exsanguinated by elevating it above heart level for 30 sec. The arm is occluded using segmental blood pressure cuff inflated to 240 mmHg. Subject is asked to perform 20 handgrip exercises of 2 seconds

duration at 4 seconds intervals to determine the time (in seconds) for pain threshold and pain tolerance.

2.5.3 Mechanical (pressure) pain

Pressure pain threshold (PPT) can be reliably measured using a handheld digital algometer in a clinical setting and appears to be more reliable than a self-reported pain score using a visual analogue scale. Variables that may influence PPT include sex, age and sleep quality (Walton *et al.*, 2011a). The concept of PPT and the measurement of the onset of pain sensation using the algometer have been the focus and attention of much research and interest among clinicians and researchers. The algometer has become widely used and accepted in the research literature as well as the clinical environment as a valid tool to evaluate aspect of pain. The progressive development of pressure by the application of the algometer head produces a gradual depression of the local skin surface. The PPT is the point where the amount of pressure being applied produces a local sensory shift from pressure to pain. Algometer has been shown to be an effective tool in the determination of PPT (Jones *et al.*, 2007). The intensity of acute postoperative pain in patients undergoing hysterectomy was reported to be associated with results from preoperative brush-evoked allodynia, pinprick hyperalgesia, and vaginal pressure pain threshold (MacLachlan *et al.*, 2016).

Pressure pain threshold is clinically accessible as a testing tool. It has been reported that lower PPT in distal, non-injured tissue in patients with Whiplash associated disorders may reflect increased sensitivity of the central nervous system to nociceptive input, and acute

global hypersensitivity subsequently places a patient at risk of developing centrally maintained persistent pain (Walton *et al.,* 2011b). Algometry has been used frequently for comparing pain perception between pain patients and healthy controls. The use of algometry in the diagnosis of rheumatic diseases indicates that pressure pain is considered a relevant experimental model for clinical pain experience. Other studies have demonstrated that pressure-pain perception measures are related to clinical pain in fibromyalgia patients and in women with a wide range of pain complaints, while other stimulus modalities are not (Lacourt *et al.,* 2012).

2.5.4 Electrical stimulation

Regarding the intensity of acute postoperative pain, pain threshold testing using electrical stimuli demonstrates great predictive power. The pain thresholds measured by preoperative electrical pain stimuli were reported to be significantly correlated with postoperative opioid consumption and pain control. Preoperative measurement of patients' electrical pain thresholds may also be useful as a screening tool to identify patients at high risk of postoperative pain, yet, it may be a less palatable option for many patients than mechanical or thermal stimuli, which may limit its usefulness in the context of routine screening (MacLachlan *et al.,* 2016).

2.5.5 Heat pain

Heat stimulation produces a brief, sharp, cutaneous pain that is attributable to both A-delta fiber activity and C-afferent activity (Sherman and LeResche, 2006). Contact heat stimuli can be delivered using a computerised Thermal Sensory Analyser. Intensity of supra-

threshold heat pain was reported to correlate most consistently with postoperative pain. A meta-analysis of heat pain in children found that boys had significantly higher tolerance than girls overall. Boys were reported to have significantly higher heat pain thresholds than girls (MacLachlan *et al.,* 2016). Heat pain can be induced by determining the maximally tolerated temperature at the volar side (palm side) of either forearm. Starting at a baseline temperature of 30°C, the thermode is heated at a rate of 2.0°C/s. The volunteer presses a button when he/she did not want the temperature to be further increased (pain tolerance = maximal temperature tolerated; cutoff limit is 52°C) (Luginbuh *et al.,* 2001).

2.6 Genetics, Epigenetics and Pain

Psychological factors also influence how people respond to and cope with pain (Abdulla *et al.,* 2013). A pain gene is a gene for which there are one or more polymorphisms that affect the expression or the functioning of its protein product in a way that affects pain response. A number of genes play critical role in determining pain sensitivity, pain reporting and susceptibility to developing chronic pain. Advances in 'genome wide association studies' (GWAS), 'single nucleotide polymorphism' (SNPs) and role of epigenetics in modulation of pain has established the role of genetics in pain (James, 2013). Association and linkage human studies have identified a number of genes responsible for heritable conditions involving dramatic alterations in pain perception (Cregg *et al.,* 2013).

Clinical literature has demonstrated that only a proportion of patients with a particular disease or injury go on to develop chronic pain. For example, only a minority of patients

with diabetic neuropathy report pain as one of their symptoms; a subset of individuals undergoing operations develop chronic pain (between 5 and 40% depending on the type of surgery); about a third of lower back pain sufferers go on to develop a persistent syndrome lasting for 12 months or more (Denk *et al.*, 2014).

2.6.1 Pain genetics

Pain is polygenic (controlled by the interaction of more than one gene) and environmental. Variation is implicated in pain development, characteristic, intensity, frequency, and analgesic responses. Genes that affect pain may be classified into mechanistic and susceptibility genes. The pain mechanistic genes are genes that are vital to mechanisms involved in mediation of pain.

Serine palmitoyltransferase long chain subunit 1 (SPTLC1) is a gene that encode for serine palmitoyl transferase (SPT), an endoplasmic reticulum bound and key rate determining enzyme in the complex sphingolipid metabolic pathway (Myers *et al.,* 2014). Mutations within SPTLC1 result in potential dysfunction and perturbations in sphingolipid synthesis and metabolism (Stimpson *et al.,* 2016), leading to Hereditary sensory and autonomic neuropathy type I (HSAN-I), characterised by progressive loss of pain and temperature sensation in the limbs (Annelies *et al.,* 2011). Affected individuals have increased *de novo* glucosylceramide synthesis and abnormal neuronal apoptosis (Klein *et al.,* 2016).

Hereditary sensory neuropathy type 2 (HSN2), attributed to mutations of the with-no-lysine (K)-1 (WNK1) gene, is a genetically heterogeneous disorder primarily affecting the

peripheral sensory system and characterised by sensory loss and insensitivity to pain. It is a very rare autosomal recessive disease (Yamada *et al.,* 2016) caused by mutations of HSN2 (Potulska-Chromik *et al.,* 2012).

IKBKAP: It is a gene that encodes the inhibitor of kappa B kinase complex associated protein (IKAP). Mutations cause an autosomal recessive disorder called familial dysautonomia (FD)/Riley–Day syndrome/hereditary sensory neuropathy type III (HSAN III), characterised by cardiovascular instability, decreased sensitivity to pain and temperature (Coli *et al.,* 2001). Individuals with FD suffer from abnormal or incomplete neuronal development as well as progressive neuronal degeneration (Dietrich *et al.,* 2011).

NTRK1 is responsible to encode tyrosine kinase 1 receptor for NGF, which is essential for the precise differentiation and maintenance of sympathetic ganglia and nociceptive sensory neurones. Nerve growth factor (NGF) binds to TrkA, a member of the Trk neurotrophin receptor family mainly expressed in the dorsal root ganglia (DRG), the superior cervical ganglia (SCG), and the trigeminal ganglia (Yu *et al.,* 2014). Mutations in NTRK1 cause congenital insensitivity to pain with anhidrosis (CIPA)/hereditary sensory and autonomic neuropathy IV (HSAN IV), characterised by loss of pain sensation mainly in extremities and tongue. Insensitivity to pain results from the degeneration/absence of primary afferent fibers; while loss of sweating (anhidrosis) is due to loss of sympathetic postganglionic neurones that innervate the sweat glands (Sawal *et al.,* 2016).

Pain susceptibility genes are polymorphic genes that contribute to variation in responses to experimental painful stimuli and pathological conditions. Small changes in pain susceptibility genes translate to alterations in pain sensitivity. The COMT gene encodes for Catechol-O-methyltransferase (COMT), an enzyme involved in the degradation of catecholamine neurotransmitters after their release in the synaptic cleft (Loggia *et al.*, 2011). Studies have shown that Val/Met SNP in COMT causes chronic widespread pain and influence opioid analgesic efficacy. It is associated with higher pain sensitivity, diminished regional mu-opioid system responses to pain, as well as a higher mu-opioid receptor binding potential. fMRI responses to experimental pain stimuli in subjects genotyped for the COMT val/met polymorphism demonstrate that individuals with the met/met genotype exhibit stronger pain-evoked signals in a number of cortical and sub-cortical structures after repeated noxious stimulation (Loggia *et al.*, 2011).

Transient receptor potential Vanilloid subfamily member 1 (TRPV1) is a noxious heat–activated channel with a thermal activation threshold of about 43°C. Genetic and pharmacologic studies have shown that TRPV1 is an essential component of the cellular signaling mechanisms through which injury produces thermal hyperalgesia and pain hypersensitivity (Julius, 2013). Laboratory study have shown that TRPV1 null mice are significantly less sensitive to acute noxious heat stimulation; $TRPV1^{(-/-)}$ mice exhibit significantly longer withdrawal latencies in response to noxious heat in the hotplate assay than their wild-type littermates (Trevisani and Szallasi, 2010).

OPRM1 is a gene that encodes for the mu opioid receptor (MOR). Animal studies have indicated that OPRM1 knockout mice present morphine-induced analgesic inefficacy. In humans, variations in the MOR gene also affect opiate-related analgesia. OPRM1 polymorphism has been found to be associated with higher pain rating and higher opioid consumption when compared to normal (Ren *et al.*, 2015).

The *CACNA1A* gene encodes the alpha-1 subunit of voltage dependent calcium channel CaV2.1, which are widely expressed throughout the CNS. Missense mutations in the CACNA1A gene has been shown to be associated with Familial Hemiplegic Migraine type 1 (FHM1) (Silberstein and Dodick, 2013).

CYP2D6 is a unique functional protein in the CYP2D subfamily of the CYP450 families of liver enzymes. Because of genetic polymorphism, allelic variants of CYP2D6 have been divided into poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM), and ultrarapid metabolizer (UM). Distinct CYP2D6 variants play differential roles in opiate metabolism. E.g it has been shown that codeine had no effect on analgesia in PM patients, whereas UM patients achieve immediate pain relief but have severe side effects. CYP2D6 catalyzes codeine to more active morphine (Ren *et al.,* 2015).

Voltage-gated sodium channels (Nav) are crucial in the process of nociceptor excitability and signal transmission. SCN9A encodes the Nav1.7 voltage-gated sodium channel which is mainly expressed in dorsal root and sympathetic ganglion neurones. Studies have shown

that an SCN9A SNP is associated with the incidence of congenital insensitivity to pain (Ren *et al.,* 2015).

2.6.2 Pain epigenetics

The epigenome is viewed as a collection of regulatory layers that control when, where, and how genes are turned on and off (Gokhman *et al.,* 2016). Epigenetics is a term derived from the Greek prefix “epi”, that literally means “above” or “in addition to” genetics. It involves processes that physically occur with or on genes, and involves the physical support of the chromatin. Epigenetics refers to various dynamic features that modify the genome's functionality under exogenous influence and also provide a molecular substrate that allows for the stable propagation of gene expression states from one generation of cells to the next (Muka *et al.,* 2016). Epigenetics describe the link between the ‘gene’ and the ‘environment’. Factors such as age, nutrition, environmental chemicals, social context and so on are implicated. Epigenetic mechanisms can silence the expression of pro- or anti-nociceptive genes. Processes such as histone modifications and DNA methylation have been known to be associated with altering many neural functions, including synaptic plasticity, memory and learning. Epigenetic mechanisms have also been implicated in the transition from acute to chronic pain, as well as the development of chronic post-surgical neuropathic pain. Epigenetic as well as genetic mechanisms have been implicated for diverse pain conditions (James, 2013).

Epigenetics, which refers to transmissible (somatic and/or trans-generational) changes in phenotype/gene expression without changes in the gene sequence, is a phenomenon that

has been demonstrated in a wide variety of organisms including plants and animals. It affects diverse processes such as development, evolution, response to drugs and chemicals and cause diseases (Martin *et al.,* 2013). Epigenetics was defined by the developmental biologist Conrad Hal Waddington (1905–1975) as "the interactions of genes with their environment which bring the phenotype into being". They are chemical modifications to chromatin that modulate gene activity without altering the DNA sequence (Geranton, 2012; Graff and Mansuy, 2008). Alterations in patterns of epigenetic modifications are associated with several pathological conditions, including cancer, autoimmune diseases and viral infections (Alberghini *et al.,* 2015).

Epigenetic processes have been associated with many neural functions, including synaptic plasticity, learning, and memory (Denk and McMohan, 2012), and the importance of epigenetic modulations in the control of pain states has been predicted. Epigenetic changes are required for long-lasting neuronal plasticity that is essential for the development of chronic pain states. Also, neurotrophins such as NGF and BDNF, and signalling pathways such as MAPK/ERK, that contributes to pain signalling, have been found to induce epigenetic modifications (Geranton, 2012).

Studies have shown that induction of the δ-opioid receptor by NGF in PC12 cells – an NGF-responsive model for the study of gene expression during neuronal differentiation and development– temporally correlates with acetylation of H3K9 and reversal of H3K9 tri-methylation, and that the level of expression of the μ-opioid receptor in mice brain correlates with changes in histone modifications and DNA methylation at the μ-opioid

receptor promoter, and is dependent on methyl CpG binding protein (MeCP2) activity (Geranton, 2012). In another examination of genome-wide DNA methylation, whole blood DNA methylation was characterised to identify differentially methylated regions (DMRs) associated with high or low pain sensitivity. Nine meta-analysis pain DMRs show robust evidence for association with the strongest signal in the pain gene TRPA1 (Bell *et al.*, 2014).

2.6.2.1 Epigenetic Mechanisms in Chronic Neuropathic Pain

Intrathecal treatment with two different class I HDACIs (MS-275 and MGCD0103) has been reported to increase spinal acetylation of histones. Using Western blot, global acetylation of lysine residue 9 was found to be significantly increased in spinally injured dorsal lumbar cord after drug administration (Denk *et al.*, 2013).

Histone acetylation of lysine residues, such as lysine residue 9 (H3K9ac), was reported to cause relaxation in chromatin structure, recruits and stabilizes chromatin remodeling enzymes, including transcription factors, and promotes transcriptional elongation. Sodium valproate, which interferes with HDACs as well as the GABAergic system, has been shown to be an effective analgesic under some circumstances. In a study by Denk *et al.* (2013), intrathecal administrations MS-275 and MGCD0103 has been found to significantly attenuate mechanical hypersensitivity after nerve injury by 40% to 50% (Fig 2.6). HDACI treatment also improved thermal hypersensitivity after neuropathic injury. In another experiment, pretreatment with intrathecal MS-275 was found to increase withdrawal thresholds after nerve injury on a radiant heat paw withdrawal assay.

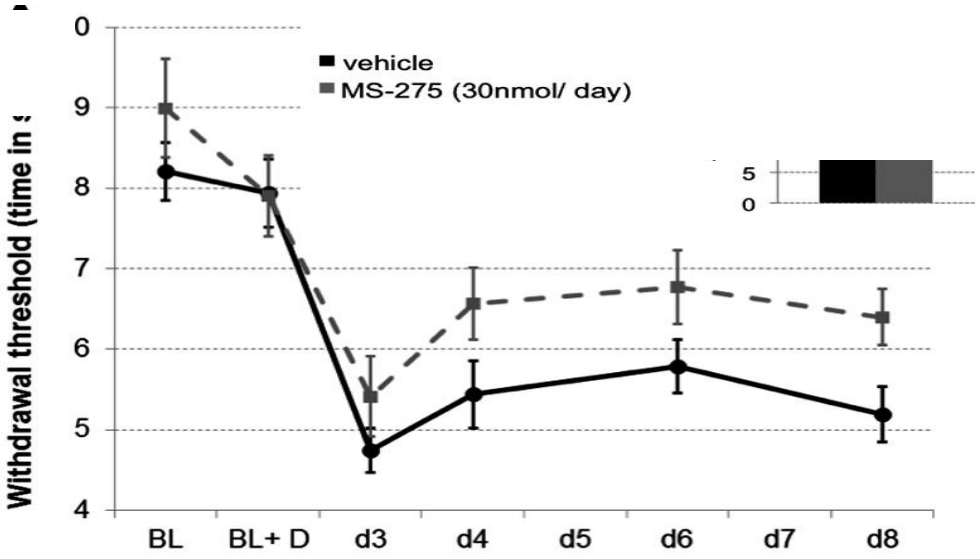


Figure 2.6: Paw withdrawal assay after intra-thecal MS-275 treatment; BL: baseline; D: drug (Denk *et al.*, 2013)

Histone acetylation may also be involved in the generation of nontraumatic peripheral neuropathy. A study found that pretreatment and long term administration of HDACI significantly increased hind limb withdrawal thresholds to punctate mechanical stimuli compared with vehicle controls. Intrathecal HDACI (MS-275 at 30 nmol/d) treatment attenuated mechanical hypersensitivity in a more clinically relevant model of antiretroviral (d4T)–induced neuropathy.

2.6.2.2 Epigenetic Regulation of Peripheral Inflammation

Significant clinical benefits of an HDAC inhibitor have been observed against both arthritic and painful components of juvenile idiopathic arthritis. The effects of these compounds are believed to be mediated in part through suppression of cytokines, with their administration having been shown to reduce expression of many crucial proinflammatory mediators, including IL-1β and TNFα. Binding of these proinflammatory

factors to their receptors can also harness epigenetic processes. Thus, interleukin and TNFα receptor activation results in H4 hyperacetylation of many other inflammatory promoters through the action of the transcription factor NF-kB (Denk and McMahon, 2012).

2.6.2.3 Epigenetic gene regulation in pain processing

Histone acetylation and DNA methylation have been described as important epigenetic factors that can influence expression of nociceptive genes in chronic pain states. Pharmacological interference with the process of histone acetylation can affect pain behavior. Systemic and intrathecal administration of HDAC inhibitors have shown analgesic effects in models of inflammatory pain, an effect believed to be mediated by changes in expression of the mGluR2 receptor in both DRG and spinal cord. Conversely, a pathological pain state may be able to induce changes in histone acetylation at relevant pronociceptive genes. Injection of CFA into the paws of rats was shown to lead to transcriptional downregulation of GAD65 in the dorsal raphe nucleus coupled with hypoacetylation at its promoter (Denk and McMahon, 2012).

2.6.2.4 Epigenetic involvement in cortical pain processing

Central sensitization of spinal neurones relies on molecular processes very similar to those underlying associative learning, in particular the formation of LTP. Both forms of plasticity crucially involve NDMA receptor function, protein kinase pathways, CREB activation, and can be influenced by BDNF release. In the hippocampus, those signaling pathways have now all been shown to be epigenetically regulated, and in turn control or

influence epigenetic processes. In the brain, multiple areas undergo changes in neural connectivity as a result of chronic pain, including the anterior cingulate cortex and the amygdala. Cortical epigenetic processes have been hypothesized to be modulators of chronic back pain to account for shifts in event related EEG peaks over relevant brain regions (Denk and McMahon, 2012).

2.7 Assessment of Pain

Pain is often regarded as the fifth vital sign in regard to healthcare because it is accepted now in healthcare that pain, like other vital signs, is an objective sensation rather than subjective, and its assessment is the cornerstone to optimal pain management (Fink, 2000). Most pain assessments are done using a scale which is explained to the patient, and then the patient chooses a score. A rating is taken before administering any medication and after the specified time frame to rate the efficacy of treatment. Formal assessment of pain is important to initiate and evaluate the effectiveness of pain treatments, and subjectivity can be reduced by using an assessment tool. Assessment tools may include self-report and observational or behavioural for people who cannot self-report (Gregory and Richardson, 2014).

2.7.1 Self report

Self assessment of pain may be carried out using the WILDA (word, intensity, location, duration and aggravating factors) approach.

2.7.1.1 Word

It involves asking the patients to describe their pain using words, which will guide clinicians to the appropriate interventions for specific pain types. Questions like "What does your pain feel like?" are used to in order for the patient to describe the type of pain being experienced. Patients may respond with words like burning, shooting, tingling, radiating, lancinating, or numbness to describe neuropathic pain, achy, throbbing, or dull for somatic pain, and squeezing, pressure, cramping for visceral pain (Fink, 2000).

2.7.1.2 Intensity

The ability to quantify the intensity of pain is essential when caring for persons with acute and chronic pain. The universal adoption of a 0 to 10 scale for clinical assessment of pain intensity in adult patients has been advocated (Fink, 2000). Pain intensity assessment tools include the Visual Analogue Scale (VAS), Numerical Rating Scale (NRS), Verbal Descriptor Scale (VDS) and the Wong Baker smiley faces (Gregory and Richardson, 2014). The verbal categories mild, moderate, and severe pain may correspond to different values on the VAS in the same patient on different occasion (Breivik *et al.,* 2008).

2.7.1.3 Location

Some patients may have 2 or more sites of pain. Thus, it is important to ask patients, "Where is your pain?" or "Do you have pain in more than one area?" (Fink, 2000).

2.7.1.4 Duration

Patients need to be asked, “Is your pain always there, or does it come and go?” or “Do you have both chronic and breakthrough pain?” Pain descriptors, intensity, and location are important to obtain not only on breakthrough pain but on stable (continuous) pain as well (Fink, 2000).

2.7.1.5 Aggravating/alleviating factors

Asking the patient to describe the factors that aggravate or alleviate the pain will help plan interventions (Fink, 2000).

2.7.2 Neurophysiological Assessment

Pain can be assessed by neuro and electro physiological techniques such as Magnetic Resonance Imageing (MRI/fMRI), which assess brain structure and activity, Single photon emission computed tomography (SPECT) and Positron Emission Tomography (PET), nuclear imageing techniques that can trace radiolabeled molecules injected into the bloodstream, electroencephalography (EEG), a method which assess electrical activity in the brain generated by firing between neurones, or Magnetoencephalography (MEG), a technique for mapping brain activity by recording magnetic fields produced by electrical currents in the brain (Olesen *et al.*, 2012).

2.7.3 Behavioural pain assessment

A number of behavioural pain assessment tools have been devised for people who can’t self-report pain, e.g. children, critical care patients and people with dementia. The Critical

Care Pain Observation Tool (CPOT), consists of four items and scores range from 0-8. Tools designed to assess pain in people with dementia have tended to use some or all of the six behaviours identified by the American Geriatric Society [AGS], verbalisation, facial expression, body movements, changes in interaction, and changes in activities of living and mental status changes (Gregory and Richardson, 2014). Pain in children can be assessed using the FLACC scale (face, leg, activity, cry and consolability).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Materials used for the study included a 66LB/30KG baseline pressure algometer (Fabrication Enterprises. Inc. NW, USA), with a $9cm^2$ rubber tip that was placed on the skin, Rayto microplate reader (RT-2100C, Rayto Life and Analytical Science Co. Ltd, Shenzen, China), a visual analogue scale (VAS), stopwatch, mercury-in-glass thermometer, ice cubes, digital balance, stadiometer, 5mL syringes, EDTA and plain sample bottles.

3.2 Subjects and Sampling

3.2.1 Study location

The research was carried out in nine randomly selected secondary schools under the Zaria Zonal Education Authority between October, 2015 and February, 2016. Zaria is a town in Northern Nigeria, located at latitude 11.07 – 11.4 N, and longitude 7.71 – 7.42 E (GPS co-ordinate: 11.111, 7.723). It has a population of 975,228 (2015 projected), about 0.535% of Nigeria's population, and covers an area of 563 km^2 (UNDESA, 2014). It has an average temperature of 37^0C and about 31% relative humidity, with an altitude of 675 m above sea level. Secondary schools visited include Maude International School, Nana International School, Sheikh Ibrahim Arab Special Secondary School, Karau-Karau (A), Government Secondary School, Kofar Kibo, Government Secondary School, Kofar Kuyambana, Government Secondary School, Kofar Doka, Alhuda-huda College and Barewa College.

3.2.2 Study design

Observational cross-sectional study was used for this research. Schools visited were selected randomly among the secondary schools under Zaria Zonal Education Authority. Responses of individual subjects to pain stimuli were noted and recorded. Collected blood samples were used for laboratory analysis.

3.2.3 Subjects

One hundred and sixty one (161) apparently healthy volunteers aged 20 years and above, comprising 91 males and 70 females, were used for the study. The subjects were recruited mostly from secondary schools staff population under the Zaria Zonal Education Authority 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 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 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.

3.2.4 Exclusion criteria

Subjects were excluded if they reported significant psychiatric disorder, 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.

3.2.5 Inclusion criteria

i. Aged ≥ 20 years

ii. Does not smoke or drink alcohol

iii. Is not under any analgesic

iv. Is not suffering any inflammatory condition.

3.2.6 Sample size determination

Using a standard deviation of 7.42 obtained from a conducted pilot study, and according to the formula described by Eng (2003), the sample size was calculated as follows:

$$N = \frac{4S^2(Zcrit)^2}{D^2}$$

N = total sample size

S = standard deviation

Zcrit = Z statistic for a level of confidence,

D = expected difference between means

$$N = 4\frac{(7.42)^2 * (1.645)^2}{(2)^2}$$

$$N = 148.98$$

$$N \approx 148$$

3.3 Experimental Procedure

The subjects were introduced to completion of questionnaire and a brief interview. Cold-pressor pain was conducted first, followed by a 10-minute rest period, and then pressure and ischaemic pain procedures were conducted last and in separate sessions, to avoid

carry-over effect. Experimental pain assessment was carried out by a trained personnel who is blind to the experimental outcome.

3.3.1 Cold pressor test

Cold pressor pain is a method that mimics the effects of chronic conditions effectively because of its unpleasantness, and it has excellent reliability and validity. The cold pressor apparatus consisted of a container filled with ice water, monitored by a mercury-in-glass thermometer. Participants remained comfortably seated, with the non-dominant arm supported along the body and the dominant arm rested on the table. Cold pressor pain was assessed using the method of Hines and Brown (1936). Prior to the test, each participant was asked to put his or her hand in container filled with water at a temperature between 22-25°C so that every participant started with the same baseline hand temperature. Subjects were asked to immerse their non dominant hand (to about 5cm above the wrist) in a container of cold water. The subjects were asked to keep their hand in the water for as long as possible, until the pain becomes intolerable, where they could remove their hand at any time (Rahim-Williams *et al.,* 2007). Water was maintained at 2 to 4^0C using ice. Pain threshold (time after which subject reported feeling pain) and tolerance (time for which the subject tolerated the pain) were measured in seconds using two separate stop watches (Kumar *et al.,* 2010). Cut-off time was set at 5 minutes.

3.3.2 Pressure pain

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 (Chesterton *et al.,* 2007). Tenderness of soft tissues causes sensitization of peripheral nociceptive nerves in the affected area, which is commonly associated with painful conditions resulting from repetitive trauma, inflammation, excessive strain or psychosocial stress (Ylinen *et al.,* 2005). Pressure pain was assessed by applying pressure at the first dorsal interosseous muscle (Chesterton *et al.,* 2007), using pressure algometer, at the rate of 1 kg/sec. The pressure at which pain was first reported was recorded (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.



Plate I: Baseline pressure algometer (66LB/30KG; Fabrication Enterprises. Inc. NW, USA)

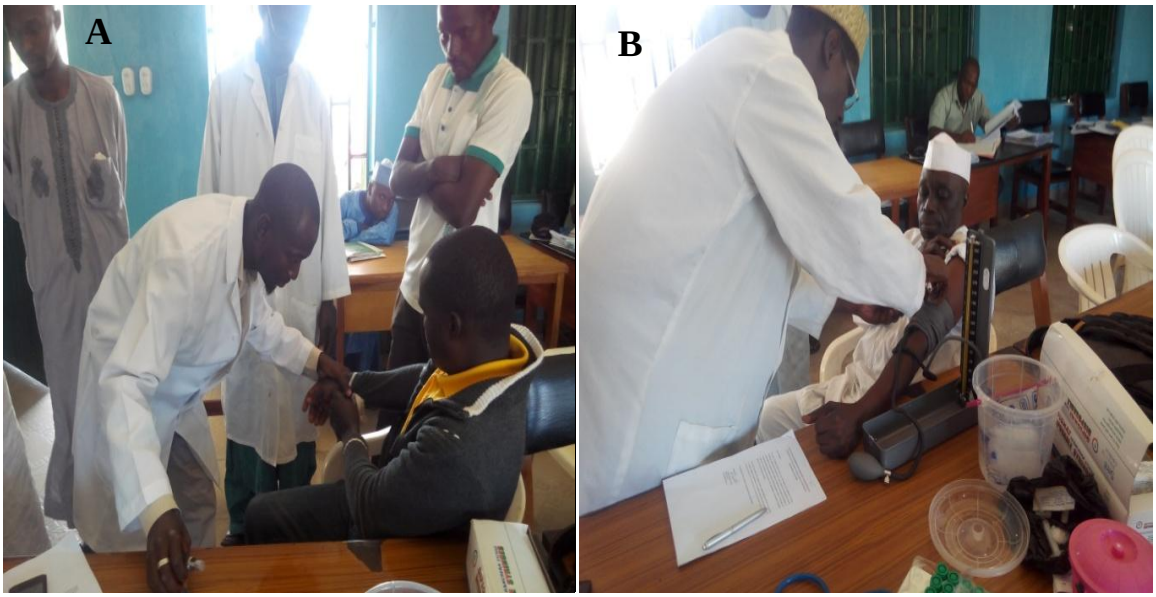


Plate II (A): Using the Baseline pressure algometer during an experimental session. (B): Submaximal effort tourniquet procedure for ischaemic pain



Plate III: RT-2100C microplate reader

3.3.3 Ischaemic pain procedure

Ischaemic pain procedure produces a deep, aching pain similar to many clinical pain syndromes and is thought to activate endogenous opioid pain regulatory systems (Sherman and LeResche, 2006). Ischaemic pain was induced using the sub-maximal effort tourniquet procedure (Moore *et al.*, 1979). 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 (Edwards *et al.*, 2004). The time for pain threshold and pain tolerance were recorded in seconds.

3.3.4 Pain catastrophizing

Pain catastrophizing was assessed using the visual Analogue Scale, a 10cm line anchored by verbal descriptors (no pain to worst imaginable pain). The subjects were asked to indicate their pain intensity from 0 to 10 (Williamson and Hoggart, 2005).



Plate IV: Visual analog scale used for assessment of pain catastrophizing

3.4 Blood Collection and Analysis

About 5 ml of venous blood was collected using 5 ml syringe. One millilitre of the collected blood was stored in EDTA sample bottle for determination of blood group and Hb genotype. The remaining 4 ml was placed in plain bottle, allowed to clot and centrifuged using bench centrifuge at 1000 x *g* for five minutes, and serum collected for analysis of glutamate, serotonin, electrolytes and oxidative stress. Analysis was carried out at Kayomeg Medical Diagnostic Centre, No. 4, Ribadu Road, off Kofan Gamji, Anguwan Rimi, Kaduna, Nigeria.

3.4.1 Determination of blood group

Blood group was determined using the haem agglutination method as described by Regan (2011). A white tile, divided into three chambers labelled A, B and D was used. About 1ml of isotonic saline was placed in each chamber, followed by a drop of the blood sample. One drop of corresponding antisera (anti-A, anti-B and anti-D) were placed in each chamber, and the contents gently mixed using applicator stick. The slide was tilted slightly to ensure proper mixing and mixture was observed for ten minutes. Blood groups were determined by the presence or absence of agglutination.

3.4.2 Determination of haemoglobin genotype

Haemoglobin genotype was determined by alkaline cellulose acetate haemoglobin electrophoresis method of Dacie and Lewis, (1986), as described by TIDI *et al.* (2013) and Cheesbrough (2006) – an electrophoresis in an alkaline buffer at pH 8.4–8.6 using a cellulose acetate membrane. The blood sample was centrifuged and the plasma was

removed. Red blood cells were washed three times in physiological saline and centrifuged at 1000 *g* for 5 minutes. Haemolysate was prepared by adding 50µl of the washed cells into khan tubes containing 50µl of 0.1% white saponin. The mixture was shaked thoroughly. The haemolysate was then centrifuged at 1000 *g* for 20 minutes (to remove debris). The supernatant was used for the test. Cellulose acetate papers were soaked and bloted. HbA, HbF, HbS and HbC were used as controls. 100 ml of Tris-EDTA and boric acid buffer was added into each of the outer section of the electrophoresis chamber. 1µl of each haemolysate sample (tests and controls) was transferred into the well plate. Using an applicator, 0.5µl of the haemolysate (samples and controls) was applied onto the cellulose acetate paper leaving about 0.5 cm gap for each sample. The cellulose paper was placed on a cathode bridge of the electrophoresis chamber containing Tris-EDTA and boric acid buffer. The chamber was connected to power supply and the plate electrophorased for 15 minutes, and the results recorded.

3.4.3 Determination of serum glutamate concentration

Serum glutamate concentration was determined according to the method described by Koochekpour *et al.* (2012), using human glutamate Enzyme Linked Immunosorbent Assay (ELISA) kit (GA-E5446HM, GenAsia Biotech Co. Ltd., Shanghai, China) according to manufacturers' instructions. 25 µl of the prepared standards, controls and samples were pipette 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 pipette 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 pipette 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).

3.4.4 Determination of serum serotonin concentration

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 pipette 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).

3.4.5 Determination of serum electrolytes

3.4.5.1 Serum sodium concentration

This was determined by colorimetric method of Maruna (1951) and Trinders (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.

3.4.5.2 Estimation of serum potassium

This was determined by colorimetric method of Henry (1974) and Tietz (1968). 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.

3.4.5.3 Serum chloride concentration

This 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.

3.4.6 Determination of serum MDA, SOD and GSH

3.4.6.1 Malondialdehyde (MDA)

Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA, thus, MDA level is commonly known as a marker of oxidative stress and the antioxidant status (Gawel *et al.,* 2004). 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. The concentration of MDA in the sample was obtained by plotting the absorbance against the standard graph (in nmol/mL).

3.4.6.2 Superoxide dismutase (SOD) activity

Superoxide dismutase 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). The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of sample and 0.03 ml of epinephrine, 0.005 N HCL was used to initiate the reaction. The reference sample contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 minutes.

3.4.6.3 Reduced glutathione (GSH) concentration

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 (19.8mg of 5, 5-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate). The mixture was incubated for 10 minutes and the absorbance was read at 412 nm using spectrophotometer. GSH concentration was determined from standard curve.

3.5 Statistical Analyses

All analyses were carried out using SPSS version 23 software for windows (SPSS Inc, Chicago) and data obtained were presented as mean ± SEM. Sex differences were analysed by Independent-Samples *t*-test. Age and ethnic differences were analysed by analysis of variance (ANOVA), followed by Tukey's *post-hoc* tests to indicate significance (if any). Pain catastrophizing was analyzed by Mann-Whitney test for sex differences and Kruskal-Wallis test (followed by pairwise multiple comparisons) for age and tribe. Association between serum glutamate and serotonin concentrations with experimental pain outcomes were determined using Pearson's correlation. Values of $p \leq 0.05$ were considered to be statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-Demographic Characteristics

Though a total of four hundred (400) questionnaires were distributed, only two hundred and seventy nine (279) turned up for the study. A total of one hundred and eighteen (118) subjects were excluded due to possession of one or more 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%). 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. A total of 152 subjects (94.4%) were teachers, while 2 subjects (1.2%) were administrators (Tables 4.1 and 4.2).

Table 4.1: Socio-demographic characteristics, 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 4.2: Socio-economic characteristics, showing frequency and percentage distribution for marital status, educational level and occupation 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**
Occupation	Teacher	152	94.4
	Administrator	2	1.2
	Others	5	3.1
	Total	**159**	**98.8**

4.2 Physiological Characteristics

About 81.4% of the studied population have the AA genotype, 18.0% AS, and 0.6% AC. Blood group analysis shows that 47.2% of the subjects are O^+, 18% A^+, 26.1% B^+, 3.7% AB^+, 4.3% O^- and 0.6% AB^-. On the other hand, 63.4 % of studied subjects are dark skinned, while 26.1% are light skinned, while 17 (11%) subjects did not report their skin colour. Handedness analysis showed that 60.9% of the subjects are right handed, while 7.5% are left handed (Table 4.3).

Table 4.3: Physiological characteristics showing genotype, blood group, skin colour and handedness in an adult Nigerian population in Zaria

Variable	Description	Frequency	Percentage
Genotype	AA	131	81.4
	AS	29	18.0
	AC	1	0.6
	Total	**161**	**100**
Blood group	O+	76	47.2
	A+	29	18.0
	B+	42	26.1
	AB+	6	3.7
	O-	7	4.3
	AB-	1	0.6
	Total	**161**	**100**
Skin colour	Dark	102	63.4
	Light	42	26.1
	Total	**144**	**89.5**
Handedness	Right	98	60.9
	Left	12	7.5
	Total	**110**	**68.4**

4.3 Sex Differences in Anthropometric Characteristics

After subjecting the data to independent sample t-test analysis, the result showed that male subjects have significantly higher mean weight (70.45 ± 13.02; p = 0.04) than females. Mean height was also significantly higher in males (1.69) than in females (1.60) (p = 0.00), while BMI was higher in females (25.47 ± 5.03) than in males (24.55 ± 4.51), though the difference in BMI was not statistically significant. Mean systolic and diastolic blood pressures were also found to be significantly higher in males (119.12 ± 19.89; 79.25 ± 13.33) than in females (112.65 ± 15.22; 74.19 ± 10.17) (p = 0.02; p = 0.01) (Table 4.4).

Table 4.4: Sex differences in mean anthropometric characteristics in an adult Nigerian population in Zaria

Characteristic/sex	**Males (Mean±SD)**	**Females (Mean±SD)**	***p* value (t)**
Weight (Kg)	70.45±13.02	65.99±14.23	0.04 (2.07)
Height (m)	1.69±0.07	1.60±0.06	0.00 (7.67)
BMI (Kg/m^2)	24.55±4.51	25.47±5.03	0.22 (-1.22)
SBP (mmHg)	119.12±19.89	112.65±15.22	0.02 (2.23)
DBP (mmHg)	79.25±13.33	74.19±10.17	0.01 (2.61)

t-test, $p < 0.05$ = significant, df = 157. SBP (systolic blood pressure); DBP (diastolic blood pressure)

4.4 Age Differences in Anthropometric Characteristics

Participants in 20 – 30 age group were found to have significantly (p = 0.00) less weight (61.29 ± 10.42) when compared to the other age groups, though the differences in height were not statistically significant across the age groups. The BMI values showed statistically significant (p = 0.00) variations across the age groups, with the oldest group (above 50 years) having the highest BMI (28.40 ± 5.38) and the youngest group (20 – 30 years) having the lowest BMI (22.46 ± 3.47). Systolic and diastolic blood pressures also showed significant (p = 0.00) increase with age across the studied population (Table 4.5).

Table 4.5: Result of age differences in mean anthropometric characteristics in an adult Nigerian population in Zaria

Characteristic/age (yrs)	**20 – 30 (Mean±SD)**	**31 – 40 (Mean±SD)**	**41 – 50 (Mean±SD)**	**Above 50 (Mean±SD)**	***p* value (F)**
Weight (Kg)	61.29±10.42[b]	71.02±14.06	73.16±10.19	76.68±15.39	0.00 (11.85)
Height (m)	1.65±0.09	1.67±0.08	1.66±0.09	1.64±0.08	0.63 (0.56)
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]	0.00 (13.46)
SBP (mmHg)	107.58±14.55[a]	114.79±9.84[a,b]	122.96±26.28[b,c]	133.54±15.07[c]	0.00 (16.52)
DBP (mmHg)	72.15±10.47[a]	76.26±7.61[a,b]	79.63±16.46[b]	88.12±11.87[c]	0.00 (12.06)

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).

4.5 Ethnic Differences in Anthropometric Characteristics

From the result of ethnic differences in anthropometric characteristics, BMI showed significant (p = 0.01) variation, with the Yorubas having lower BMI (23.05 ± 3.82) compared to others tribe (26.73 ± 3.83). Hausas and Fulanis have higher BMI (24.93 ± 4.82 and 24.86 ± 5.82 respectively) than the Yorubas. Fulanis have lower height (1.63 ± 0.11) than the Hausas and Yorubas (1.66 ± 0.08 and 1.65 ± 0.07 respectively) (Table 4.6).

Table 4.6: Ethnic differences in mean anthropometric characteristics in an adult Nigerian population in Zaria

Characteristic/ethnicity	**Hausa (Mean±SD)**	**Yoruba (Mean±SD)**	**Fulani (Mean±SD)**	**Others (Mean±SD)**	***p* value (F)**
Weight (Kg)	69.04±13.59	63.39±11.76	66.19±15.33	70.62±10.56	0.19 (1.60)
Height (m)	1.66±0.08	1.65±0.07	1.63±0.11	1.62±0.08	0.20 (1.55)
BMI (Kg/m^2)	$24.93±4.82^{a,b}$	$23.05±3.82^{a}$	$24.86±5.82^{a,b}$	$26.73±3.83^{b}$	0.01 (2.45)
SBP (mmHg)	116.94±19.52	115.41±16.14	110.77±14.98	117.62±17.29	0.69 (0.48)
DBP (mmHg)	78.06±12.71	76.25±12.09	73.46±11.79	75.48±11.39	0.54 (0.73)

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)

4.6 Sex Differences on Experimental Pain Responses

Mean cold pressor pain threshold (in seconds) for male and female (23.67 ± 1.23 and 23.45 ± 1.37 respectively) was found to be statistically similar (p = 0.931; t = 0.114), though the males have slightly higher threshold when compared to the females. The male were found to have a higher cold pressor pain tolerance (17.26 ± 1.58) than their female counterparts (17.05 ± 1.70), but the difference was also not statistically significant (p = 0.985; t = 0.087) (Figure 4.1). Male participants had higher ischaemic pain threshold (34.77 ± 1.55) when compared to the females (26.73 ± 1.47), and the difference was statistically significant (p = 0.000; t = 3.671). On the other hand, ischaemic pain tolerance did not significantly vary, though males had slightly higher tolerance (20.38 ± 1.24) than the females (18.91 ± 1.46) (p = 0.995; t = 0.772) (Figure 4.1). Perception of pressure pain was found to be significantly lower in males than in females, as indicated by higher mean pressure pain threshold in males (6.90 ± 0.14) compared to females (6.00 ± 0.17) (p = 0.00; t = 4.095) (Figure 4.1).

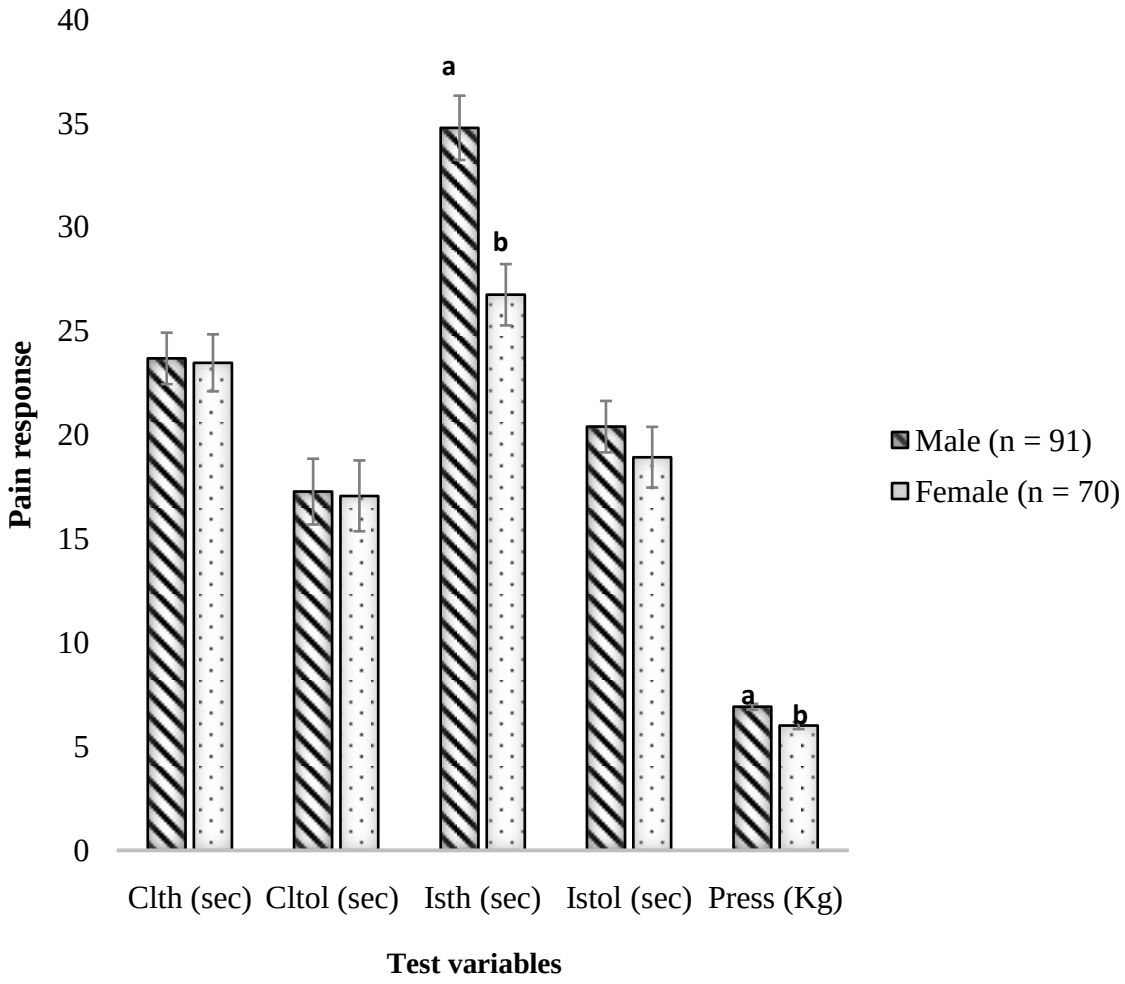


Figure 4.1: Sex differences on experimental pain responses among healthy adult Nigerian population in Zaria

[a,b]Mean difference is statistically significant ($p<0.05$) for corresponding variable between male and female (t-test). Clth (cold pressor pain threshold); Cltol (cold pressor pain tolerance); Isth (ischaemic pain threshold); Istol (ischaemic pain tolerance); Press (pressure pain threshold). Unit of parameters indicated in parenthesis

4.7 Age Differences on Experimental Pain Responses

Age differences on perception of cold pressor pain showed no statistical variation (ANOVA) among healthy Nigerian adults in Zaria. Participants in the highest age group considered (above 50 years) had the least pain perception as indicated by their high threshold (28.42 ± 2.91), while those in the lowest age group (20 – 30 years) had the highest pain perception as shown by their low threshold (21.69 ± 1.18), though the differences do not statistically vary (p = 0.127; F= 1.930). Response to cold pressor pain stimuli (tolerance) also showed no statistically significant variation among the age groups (p = 0.305; F = 1.218). Participants belonging to the 41 – 50 age group (middle age) reported the highest pain tolerance (21.47 ± 3.65), while those in the oldest age group (>50) reported the least (14.39 ± 2.17) (Figure 4.2). Ischaemic pain threshold showed statistically significant variation by age group among healthy Nigerian adult population in Zaria. Participants in the oldest age group (above 50 years) had significantly higher ischaemic pain threshold (38.75 ± 2.87) compared to the 20 to 30 age groups (29.26 ± 1.71; p = 0.029), as well as when compared to the 41 to 50 group (27.13 ± 1.99; p = 0.018). Participants in the 41 – 50 age group where found to have the highest mean ischaemic pain tolerance (22.65 ± 2.42), though one way ANOVA also showed that the differences were not statistically significant (p = 0.957; F = 0.415) (Figure 4.2). Perception of pressure 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; $p < 0.05$). The result also showed that participants that fall in the youngest age group (20 to 30 years) had the highest threshold (6.67 ± 0.19), while those in the oldest age group (>50) had a threshold of 6.61(0.29). Participants in the age range of 31 to 40 years group have a threshold of 6.63(0.21) (Figure 4.2).

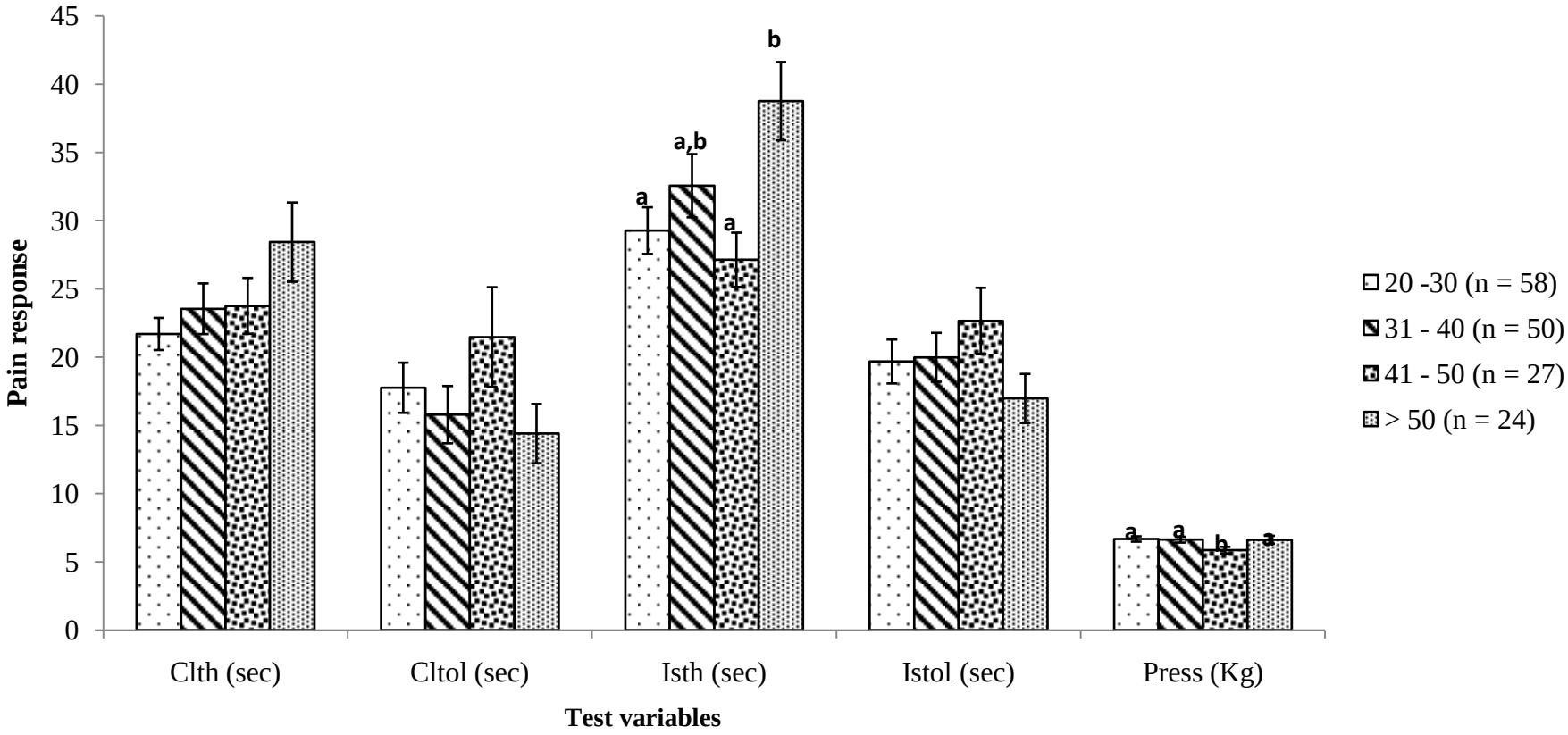


Figure 4.2: Age differences on Experimental Pain Responses among healthy Nigerian adult population in Zaria

[a,b]Mean difference is statistically significant ($p < 0.05$) for corresponding variable between the groups (ANOVA). Clth (cold pressor pain threshold); Cltol (cold pressor pain tolerance); Isth (ischaemic pain threshold); Istol (ischaemic pain tolerance); Press (pressure pain threshold). Unit of parameters indicated in parenthesis

4.8 Influence of Ethnicity on Experimental Pain Responses

Ethnic differences on cold pressor pain threshold and tolerance showed no statistically significant variation among healthy Nigerian adult population of Zaria ($p = 0.343$ and $p = 0.408$ respectively). Participants belonging to the Fulani ethnic group were found to have the highest pain threshold (28.45 ± 4.44) and the lowest pain tolerance (14.14 ± 2.76), while Yorubas had the lowest pain threshold (22.38 ± 2.83). The highest pain tolerance was found among participants belonging to others ethnic group (21.90 ± 4.16) (Figure 4.3). When data were compared by tribe using ANOVA, ischaemic pain threshold showed no significant variation. Though participants belonging to the Yoruba tribal group had the highest ischaemic pain threshold (34.10 ± 3.81), while those in the Hausa tribal group had the lowest (29.74 ± 1.35), the differences do not statistically vary across all the tribal groups ($p = 0.462$; F = 0.862). Ischaemic pain tolerance, on the other hand, showed that the Fulani tribal group had significantly higher ischaemic pain tolerance (27.84 ± 3.02) when compared to all the other groups ($p < 0.05$) (Figure 4.3). Perception of pressure pain showed statistically significant variation by tribe among healthy Nigerian adult population in Zaria, Northern Nigeria, 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 4.3).

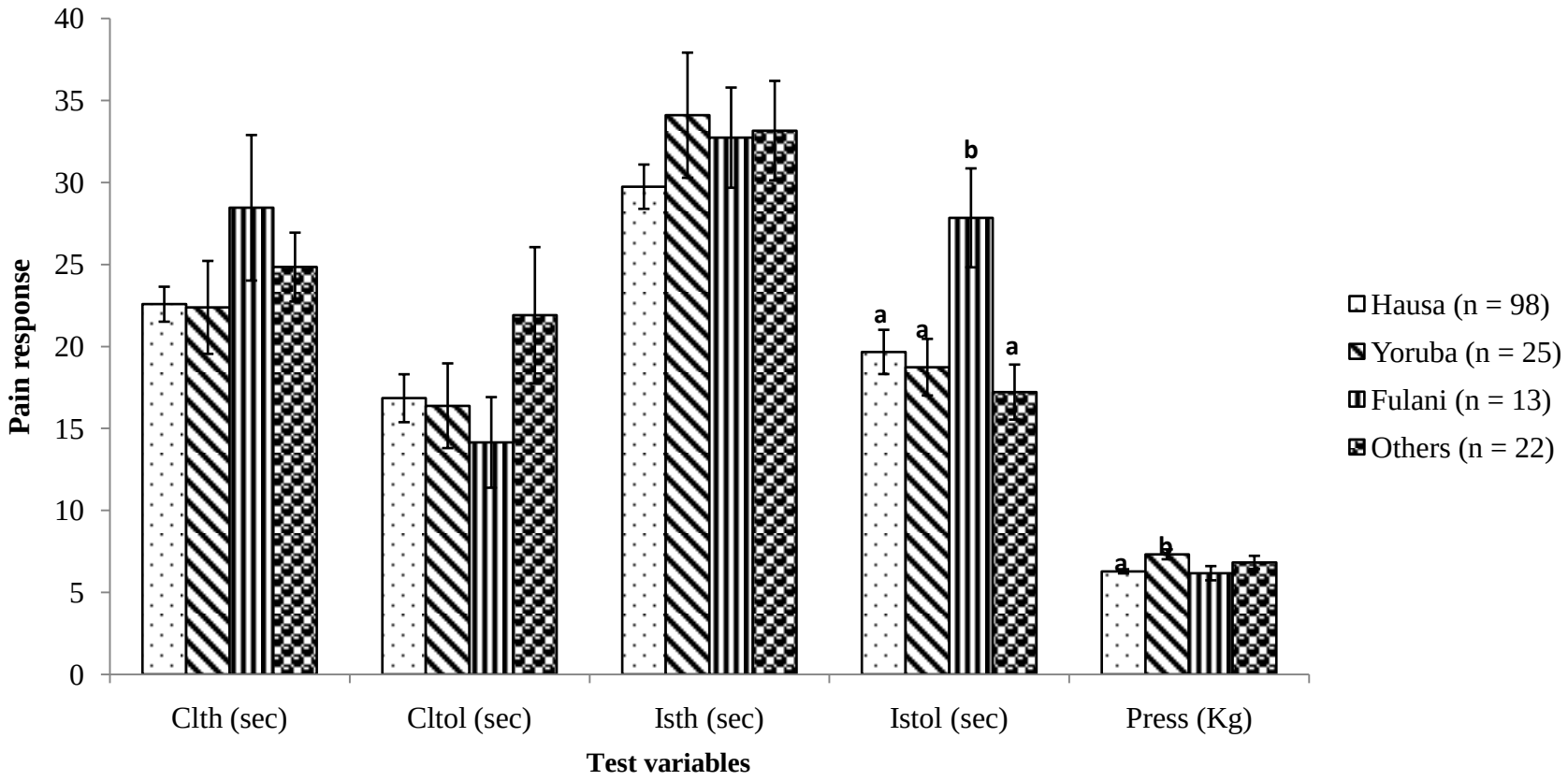


Figure 4.3: Ethnic differences on Experimental Pain Responses among healthy Nigerian adult population in Zaria

[a,b]Mean difference is statistically significant ($p < 0.05$) for corresponding variable between the groups (ANOVA). Clth (cold pressor pain threshold); Cltol (cold pressor pain tolerance); Isth (ischaemic pain threshold); Istol (ischaemic pain tolerance); Press (pressure pain threshold). Unit of parameters indicated in parenthesis

4.9 Effect of Body Mass Index on Experimental Pain Responses

Obese subjects were found to have the highest pain threshold (26.01 ± 2.51), whereas underweight subjects had the lowest pain threshold (21.66 ± 4.46), though the differences were not statistically significant across all the studied groups ($p = 0.677$; F = 0.509). Cold pressor pain tolerance, on the other hand, showed that overweight subjects have significantly higher cold pressor pain tolerance (21.15 ± 2.92) when compared to all the other groups ($p < 0.05$). The underweight subjects had the least tolerance (13.00±1.68), but the differences were not statistically significant ($p = 0.170$; F = 1.698) (Figure 4.4). Result of BMI effect on ischaemic pain threshold among healthy adult population in Zaria, Northern Nigeria showed no statistically significant variation. Subjects with normal BMI were found to have the highest ischaemic pain threshold (31.47 ± 1.51), while underweight subjects have the lowest (30.32 ± 8.65), though the differences do not statistically vary across all the groups ($p = 0.994$; F = 0.026). Underweight participants were found to have the highest ischaemic pain tolerance (22.29 ± 8.56), while those that were obese have the least (15.18 ± 1.22). Subjects with normal BMI were found to have a statistically significant higher ischaemic pain tolerance (21.06 ± 1.38) than their obese counterparts ($p = 0.012$; F = 1.767) (Figure 4.4). The result showed that participants with normal BMI have the highest pressure pain threshold (6.61 ± 0.16), while obese participants were found to have the least pain threshold (6.29 ± 0.23), though effect of BMI on pressure pain does not show statistically significant differences across all the groups studied ($p = 0.770$; F = 0.376) (Figure 4.4).

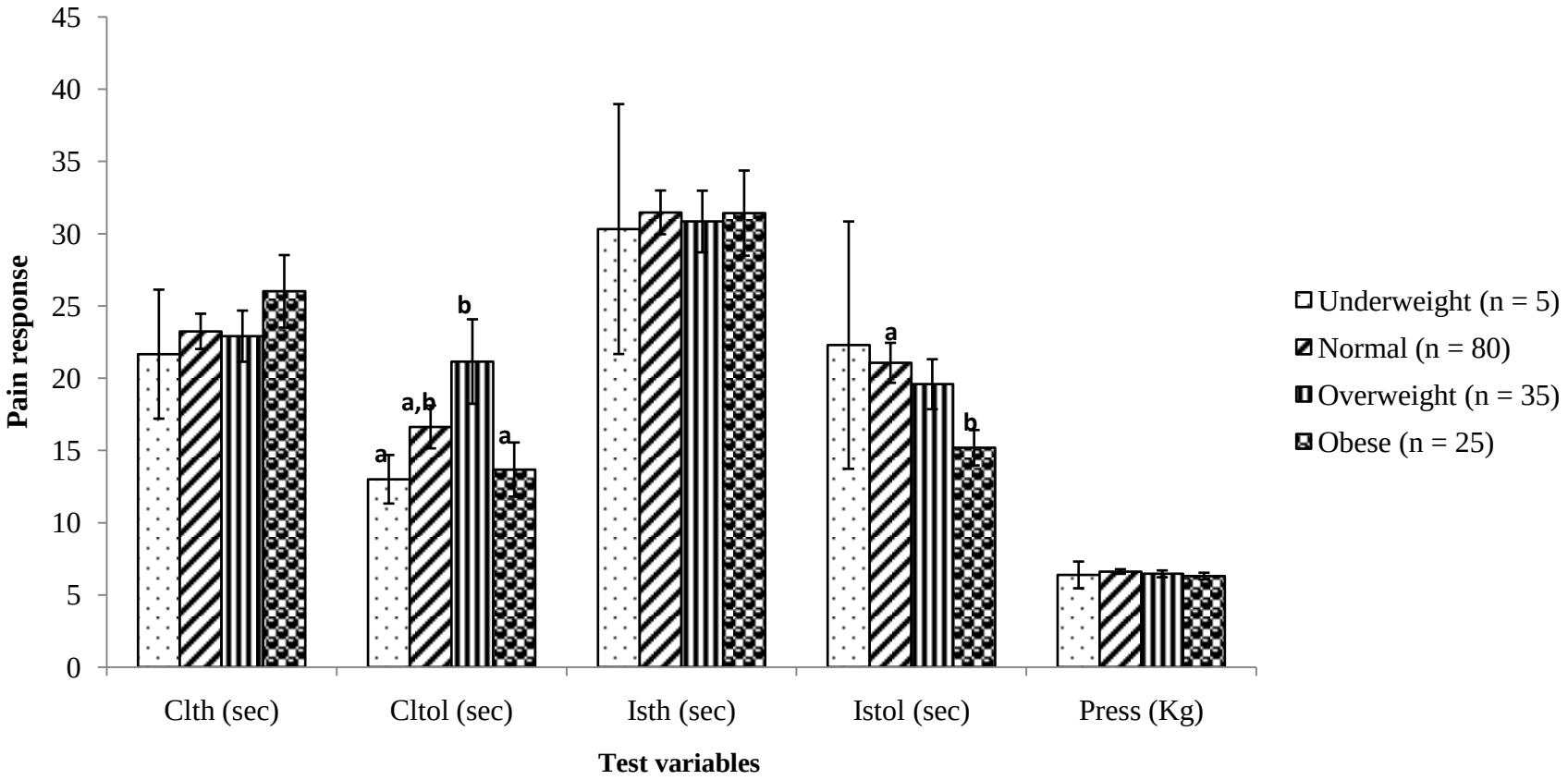


Figure 4.4: Effect of BMI on experimental pain responses among healthy adult Nigerian population in Zaria

[a,b]Mean difference is significant (p<0.05) for corresponding variable between the groups (ANOVA). Clth (cold pressor pain threshold); Cltol (cold pressor pain tolerance); Isth (ischaemic pain threshold); Istol (ischaemic pain tolerance); Press (pressure pain threshold). Unit of parameters indicated in parenthesis

4.10 Effect of Handedness on Experimental Pain Responses

After subjecting the data to independent sample *t* test, with handedness as the grouping variable, result shows that participants that are right handed had higher cold pressor pain threshold (25.09 ± 1.34) than their left handed counterparts (17.74 ± 1.51), and the difference was highly statistically significant (p = 0.001; t = 3.630). Result of cold pressor pain tolerance does not show statistically significant variation (p = 0.169; t = 1.419), though right handed subjects had higher tolerance (18.82 ± 1.66) than the left handed ones (14.70 ± 2.38) (Figure 4.5). Result of ischaemic pain test showed that participants that are right handed had higher ischaemic pain threshold (31.12 ± 1.37) than left handed participants (28.59 ± 4.43), but the difference was not statistically significant (p = 0.551; t = 0.598). The result of ischaemic pain tolerance does not also show statistically significant variation (p = 0.555; t = -0.593), though right handed subjects had lower pain tolerance (20.00 ± 1.29) than the left handed subjects (22.36 ± 4.34) (Figure 4.5). Effect of handedness on pressure pain threshold showed that right handed subjects had a lower pressure pain threshold (6.15 ± 0.12) than the left handed ones (6.62 ± 0.28), though the difference was not statistically significant (p = 0.235; t = -1.284) (Figure 4.5).

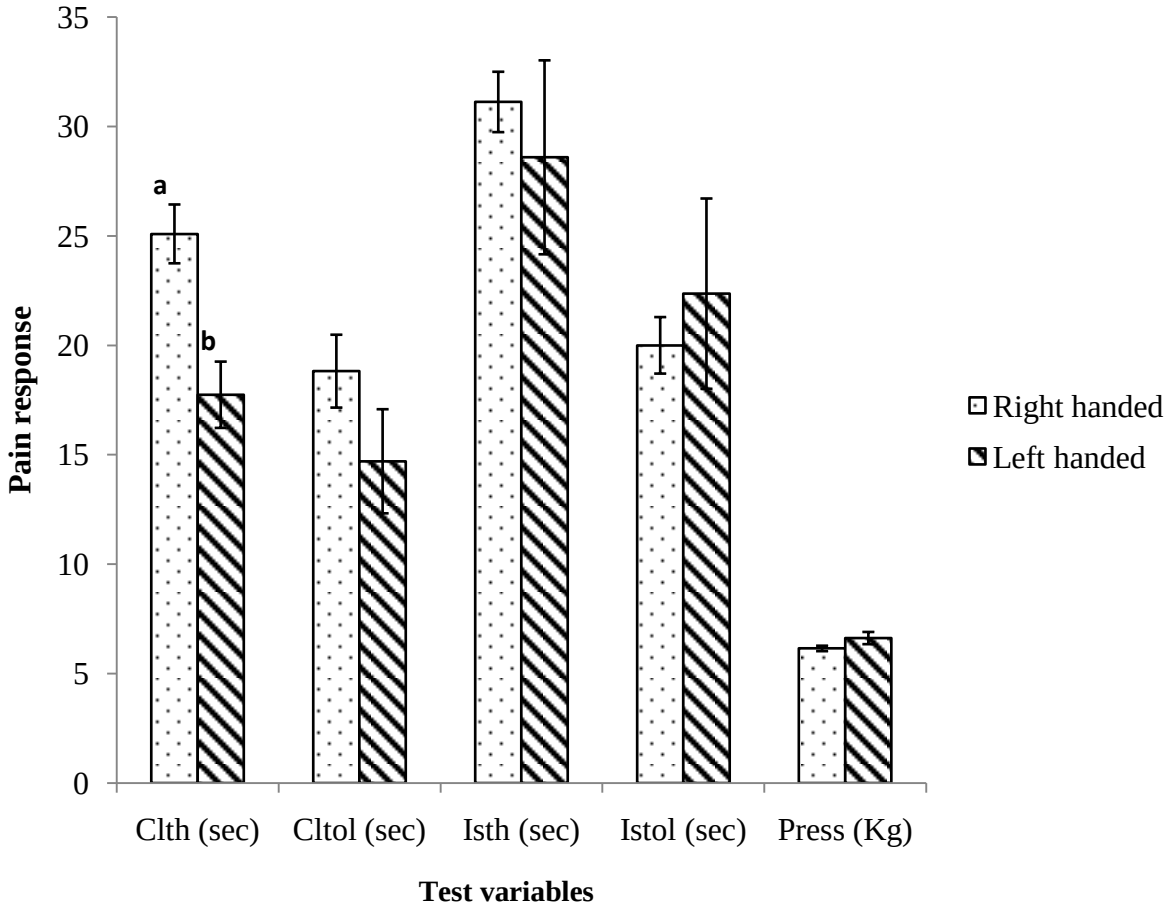


Figure 4.5: Effect of handedness on experimental pain responses among healthy adult Nigerian population in Zaria

[a,b]Mean difference is statistically significant ($p < 0.05$) for corresponding variable between the groups (t-test). Clth (cold pressor pain threshold); Cltol (cold pressor pain tolerance); Isth (ischaemic pain threshold); Istol (ischaemic pain tolerance); Press (pressure pain threshold). Unit of parameters indicated in parenthesis

4.11 Effect of Hb Genotype on Experimental Pain Responses

Independent sample *t* test analysis shows that there is no statistically significant variation in cold pressor pain threshold between subjects with AA and AS genotypes (p = 0.710; t = -0.170), though AA genotyped participants had lower cold pain threshold (23.53 ± 1.01) than AS (23.94 ± 2.35). Cold pressor pain tolerance show statistically significant variation among the participants (p = 0.028; t = -1.313), with AA genotyped subjects having lower pain tolerance (16.38 ± 1.16) than the AS subjects (20.29 ± 3.64). The result also shows that ischaemic pain threshold significantly vary by Hb genotype (p = 0.043; t =-0.783), with AA genotyped participants having lower ischaemic pain threshold (30.89 ± 1.33) than their AS counterparts (33.19 ± 2.04). Ischaemic pain tolerance, however, did not show statistically significant variation by Hb genotype (p = 0.064; t =1.886), though participants with AA genotype have higher ischaemic pain tolerance (20.37 ± 1.10) than those with AS (16.68 ± 1.61). Pressure pain perception did not also show statistically significant variation between the groups (p = 0.087; t = -1.723), though AA genotyped subjects have lower pressure pain threshold (6.43 ± 0.13) than the AS group (6.94 ± 0.26) (Figure 4.16).

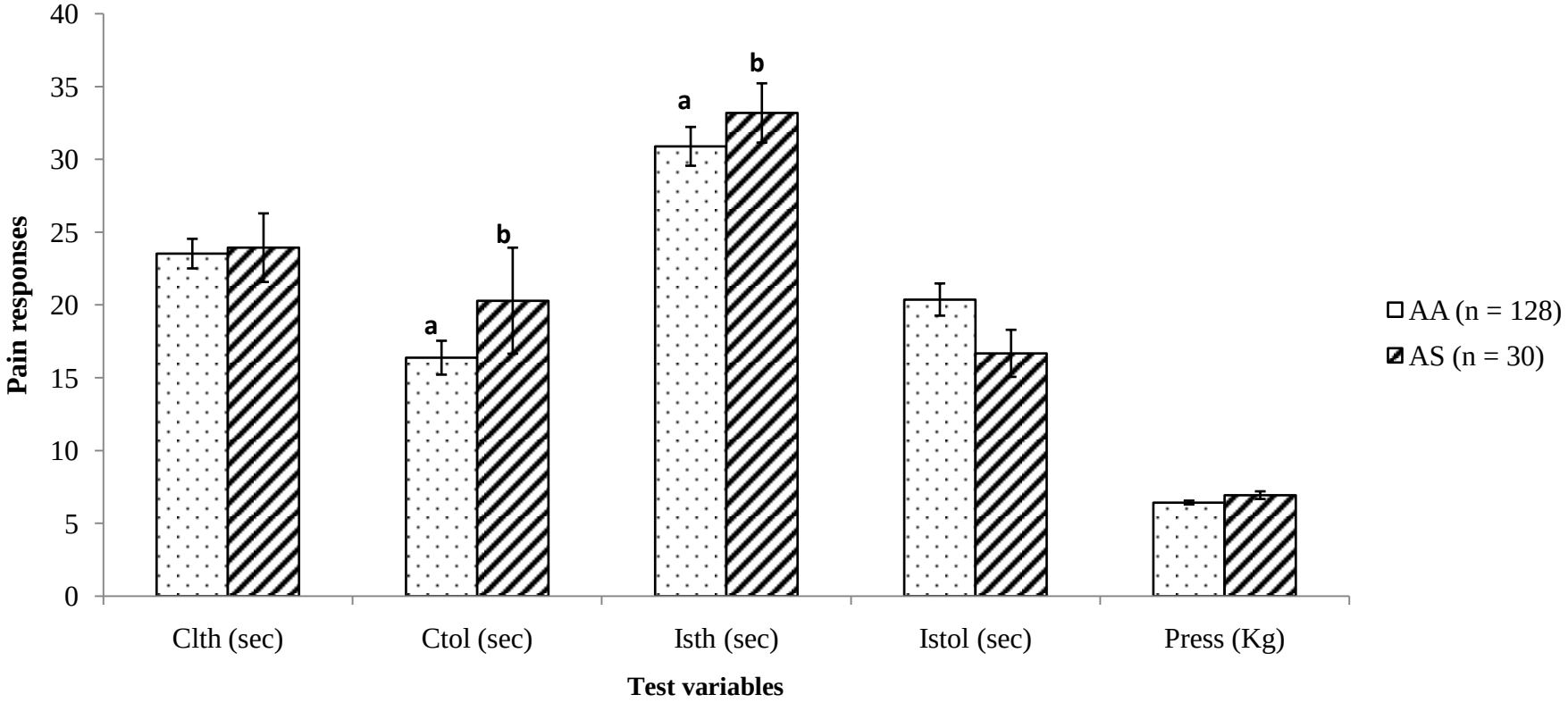


Figure 4.6: Effect of Hb genotype on experimental pain responses among healthy Nigerian adult population in Zaria

Mean difference between bars with different superscript letters[a,b] are statistically significant (*t*-test, $p < 0.05$) for corresponding test between AA and AS subjects. Clth (cold pressor pain threshold); Cltol (cold pressor pain tolerance); Isth (ischaemic pain threshold); Istol (ischaemic pain tolerance); Press (pressure pain threshold). Unit of parameters indicated in parenthesis

4.12 Effect of Blood Group on Experimental Pain Responses

The data from this study showed that cold pressor pain threshold does not statistically vary between subjects with different blood groups (p = 0.915, F(4, 150) = 0.240), though participants with A^+ blood group had the highest threshold (25.50 ± 2.19), while those with O^- group had the lowest (21.82 ± 1.92). Cold pressor pain tolerance, on the other hand, was significantly lower among the O^- group subjects (10.19 ± 1.15) when compared to O^+ and A^+ groups (16.03 ± 1.33 {p = 0.025}; 24.46 ± 4.04 {p = 0.019}; F(4, 150) = 2.589). Participants with O^+ blood group and those with A^+ had significantly higher ischaemic pain threshold (32.36 ± 1.83 {p = 0.027} and 33.44 ± 2.69 {p = 0.035}; respectively) than those with AB^+ group (22.88 ± 1.91) (F(4, 150) = 0.896). Result of ischaemic pain tolerance showed that the O^- group had significantly lower tolerance (12.95 ± 2.99) than all the other groups ($p < 0.05$). The A^+ group had the highest ischaemic pain tolerance (22.33 ± 2.69). For the pressure pain response, participants belonging to the AB^+ blood group had the highest pressure pain threshold (6.95 ± 1.38), while those in the A^+ group had the lowest (6.39 ± 1.39), though the mean differences do not significantly vary between all the groups (p = 0.312, F = 1.175) (Figure 4.7).

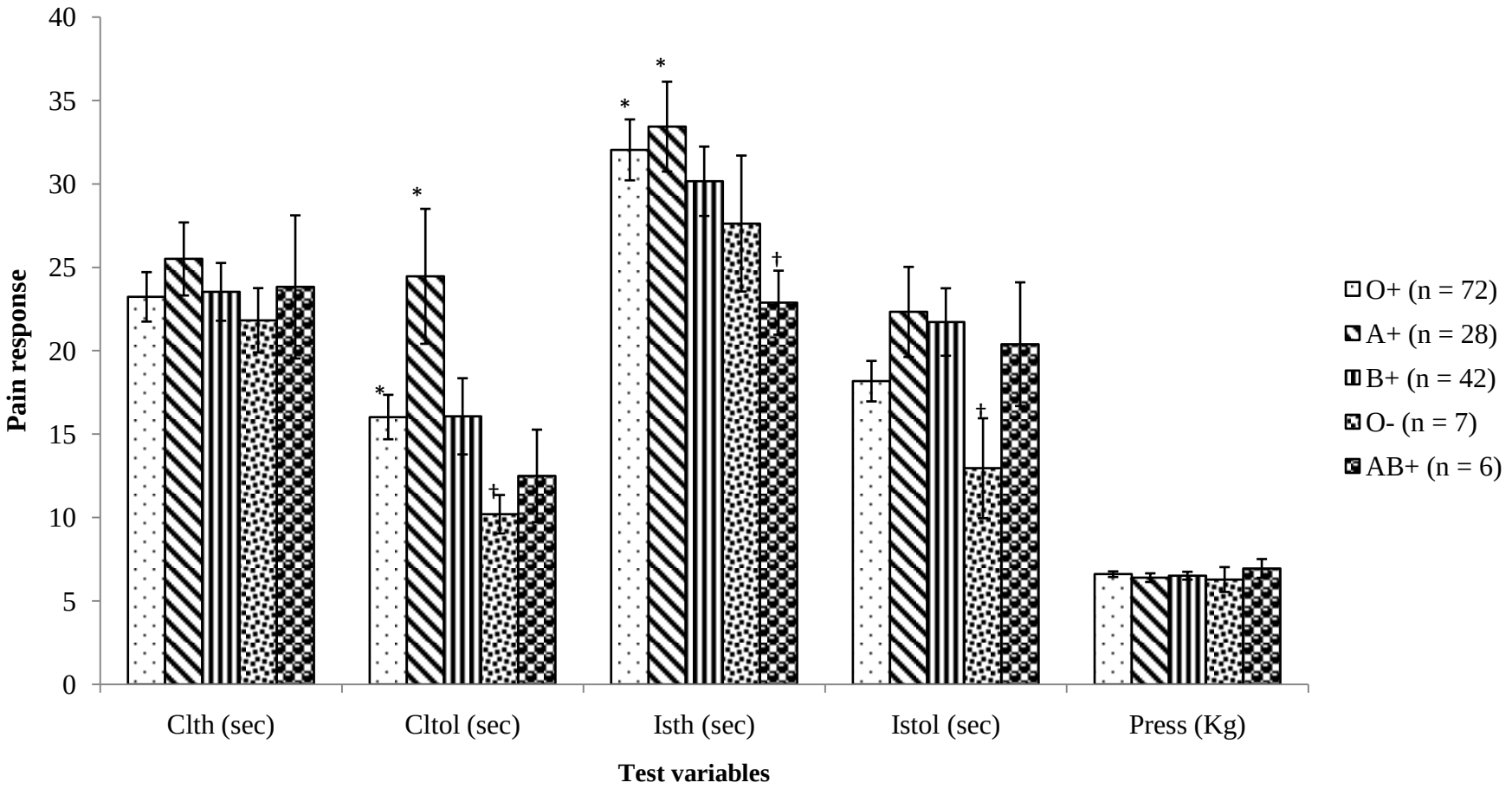


Figure 4.7: Effect of Blood Group on Experimental Pain Responses among Healthy Nigerian Adult Population in Zaria, Nigeria.

*† Mean difference is statistically significant ($p < 0.05$) for corresponding variable between the groups (ANOVA). Clth (cold pressor pain threshold); Cltol (cold pressor pain tolerance); Isth (ischaemic pain threshold); Istol (ischaemic pain tolerance); Press (pressure pain threshold). Unit of parameters indicated in parenthesis

4.13 Sex Differences on Pain Catastrophizing

Result of pain catastrophizing using the visual analogue scale showed that median difference in pain catastrophizing by sex is statistically significant, with male participants having less pain catastrophizing score (4.68) when compared to the females (4.90) (p = 0.041) (Figure 4.8).

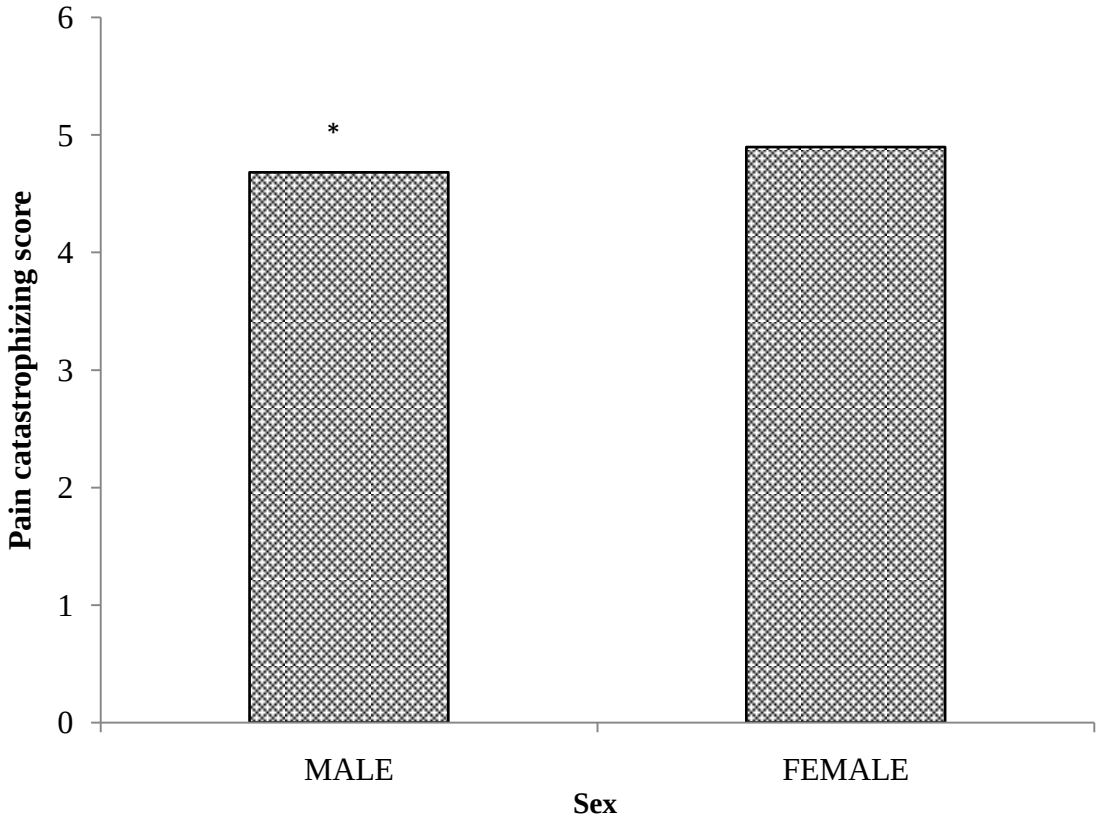


Figure 4.8: Median sex difference in pain catastrophizing (VAS) among healthy Nigerian adult population in Zaria

*Difference is statistically significant (p = 0.041) between males and females (t-test, df = 161)

4.14 Effect of age on Pain Catastrophizing among Healthy Nigerian Adults in Zaria

Age differences on pain catastrophizing did not statistically vary across the studied population groups. The highest catastrophizing was found with participants in the 41 – 50 age group (5.5), while participants belonging to the 31 – 40 age group, with a median VAS reading of 4.54, were found to catastrophize less than all the other age groups. The youngest age group of 20 – 30 had median VAS reading of 4.66, while the oldest age group of 50 years and above had a reading of 5.00 (Figure 4.9).

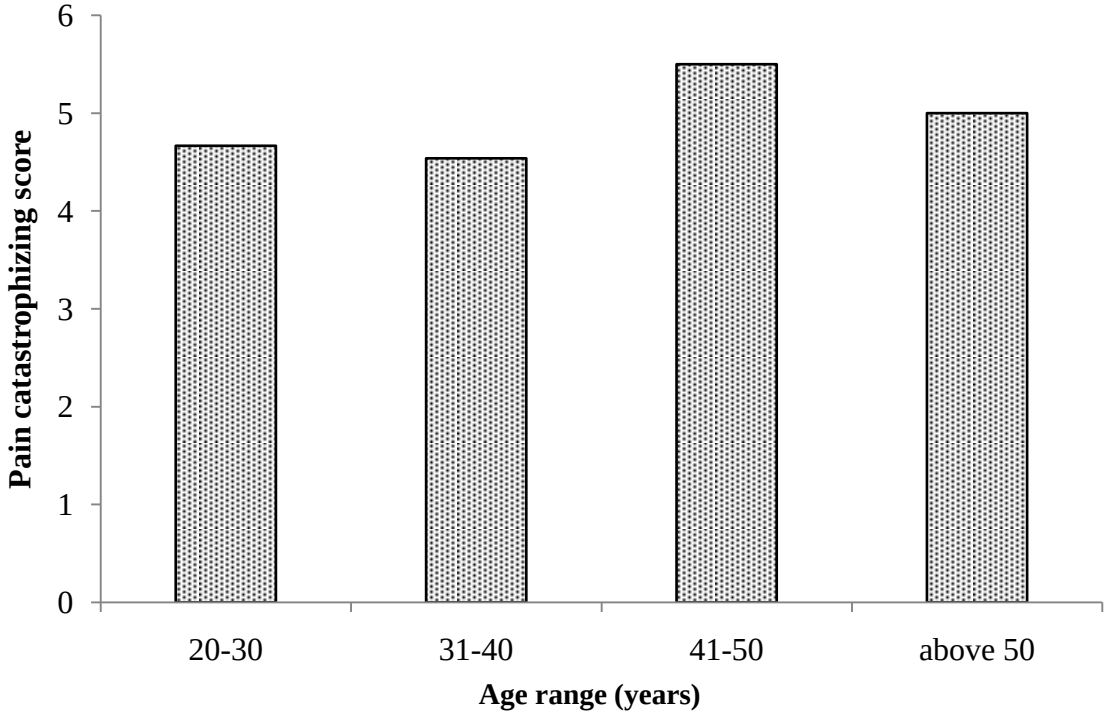


Figure 4.9: Effect of age on pain catastrophizing (VAS) in a healthy Nigerian adult population in Zaria

4.15 Effect of Ethnicity on Pain Catastrophizing among Healthy Nigerian Adults in

Ethnic differences on pain catastrophizing do not statistically vary in this study. Though surprisingly, participants belonging to the Fulani ethnic group were found to have the highest VAS reading of 5.14 (Highest pain catastrophizing), while those in the Others group had the least (4.33). The Hausas also had a higher VAS reading than the Yorubas (4.95 and 4.50 respectively) (Figure 4.10).

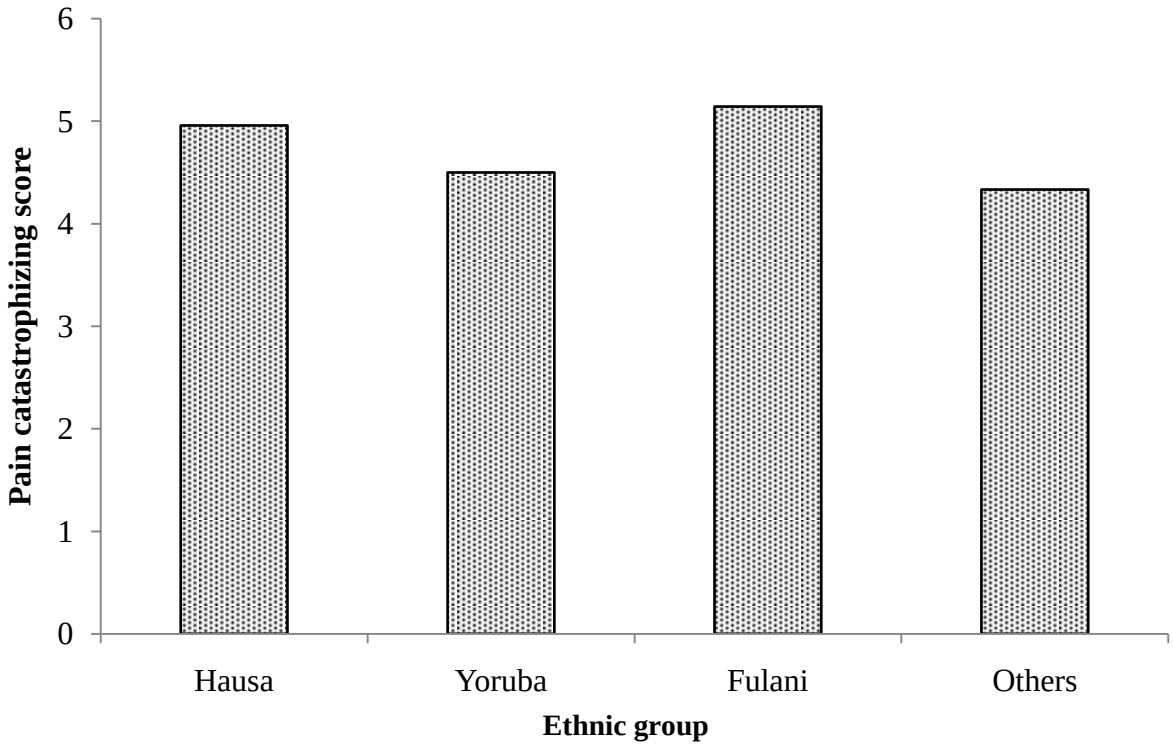


Figure 4.10: Median ethnic difference on pain catastrophizing (VAS) in a healthy Nigerian adult population in Zaria

4.16 Effect of Sex on Serum Glutamate and Serotonin Concentration

Male subjects were found to have lower serum glutamate concentration (24.64 ± 2.47) compared to the female subjects (33.76 ± 3.39), and the difference was statistically significant (p = 0.033; t = -2.160). Serum serotonin concentration was found to be lower in males (134.79 ± 11.11) than in females (151.92 ± 12.30), but the difference was not statistically significant (p = 0.304; t = -1.034) (Figure 4.11).

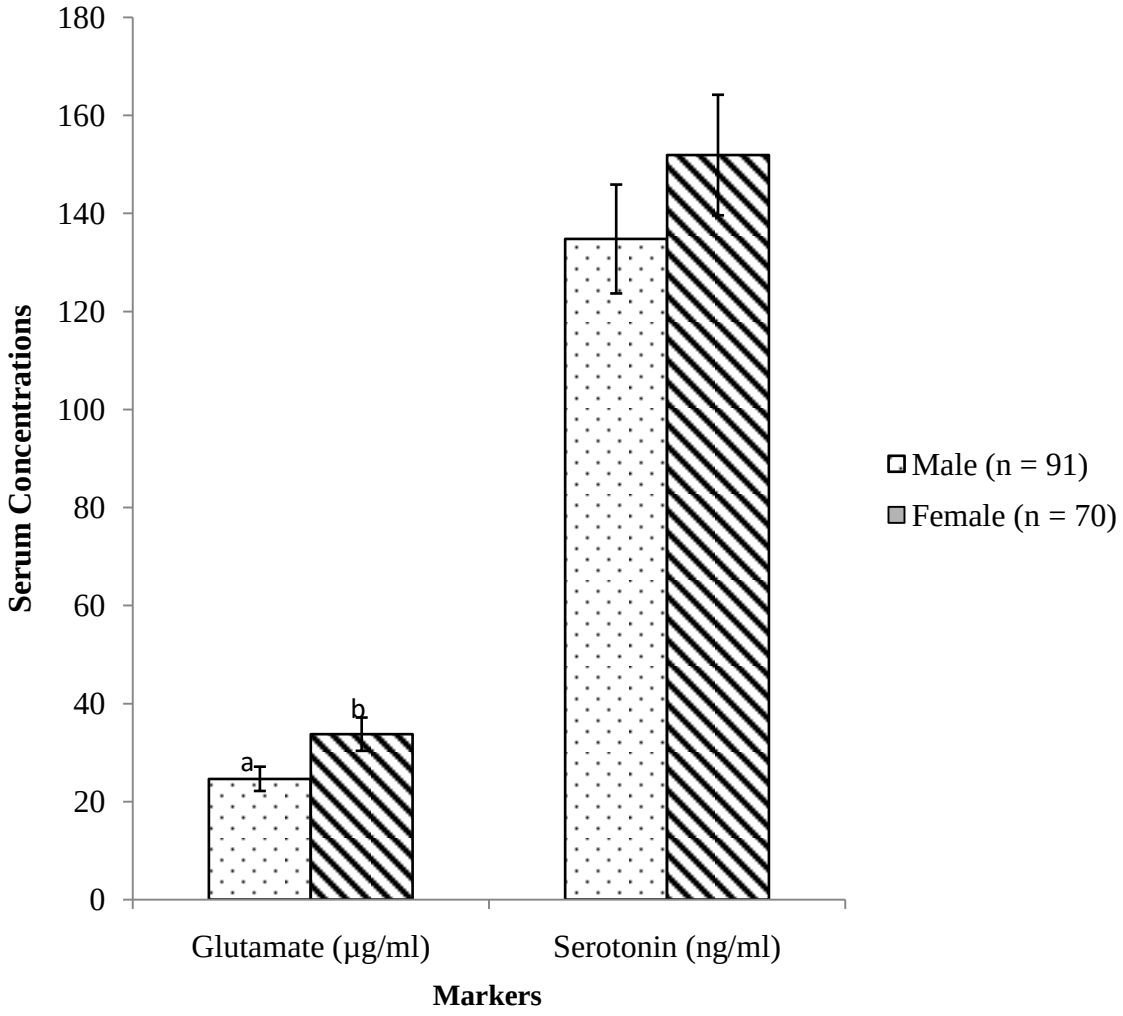


Figure 4.11: Sex differences in serum glutamate and serotonin concentrations among healthy Nigerian adult population in Zaria

[a,b]Mean difference is statistically significant (p = 0.033) between males and females (independent sample t-test) for the corresponding parameter. Unit of parameters indicated in parenthesis

4.17 Age Difference in Serum Glutamate and Serotonin Concentration

Result of age differences in blood glutamate and serotonin concentrations showed no statistically significant variation. Serum glutamate concentration was found to decrease with age, as participants in the 20 – 30 age group have the highest serum glutamate concentration (34.57 ± 3.68), while those in the above 50 age group have the lowest (20.44 ± 4.44), though the differences were not statistically significant (p = 0.138; F = 1.889). On the other hand, serum serotonin concentration was found to be lowest among subjects in the 20 – 30 age group (138.11 ± 12.16), and highest among subjects in the 41 – 50 age group (170.19 ± 24.41), but the differences were also not statistically significant (p = 0.577; F = 0.663) (Figure 4.12).

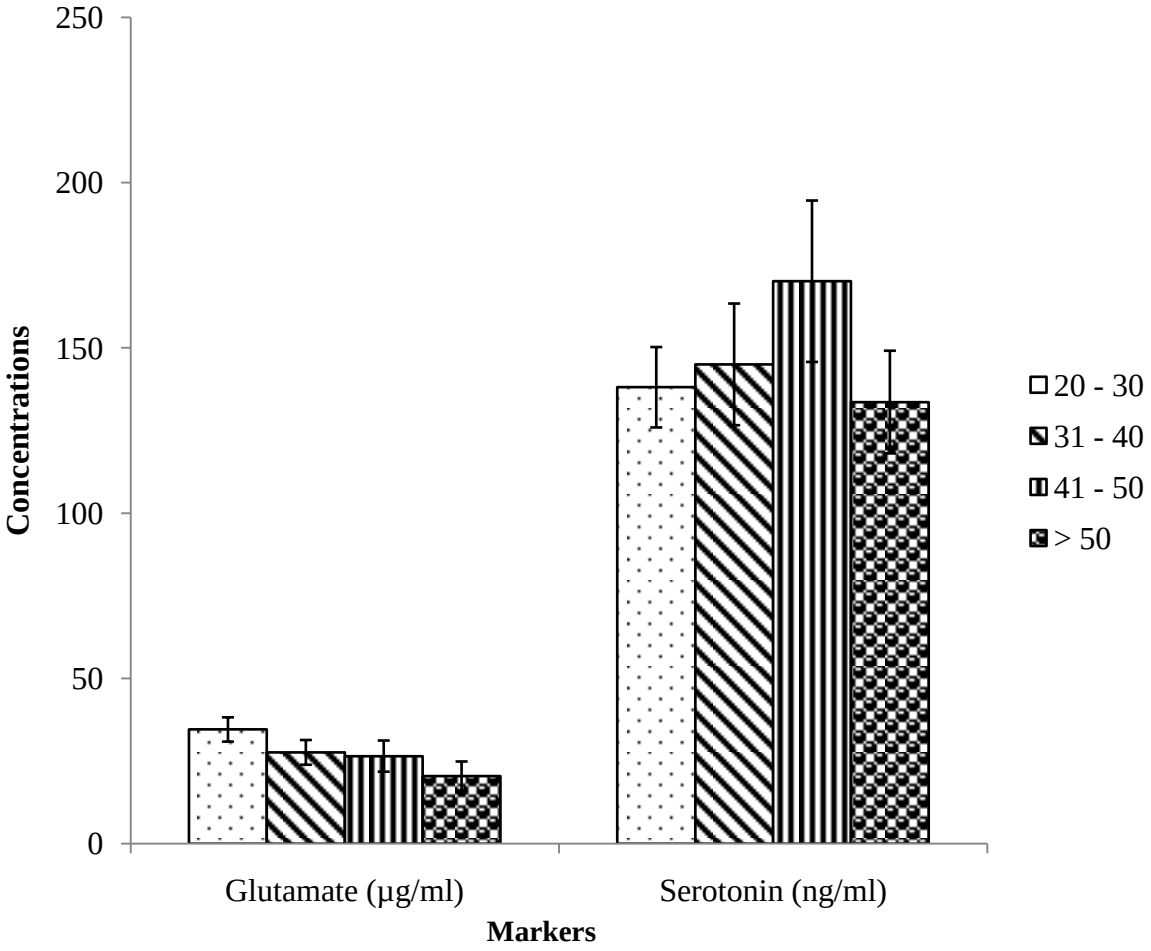


Figure 4.12: Effect of age on serum glutamate and serotonin concentrations in a healthy Nigerian adult population in Zaria. Unit of parameters indicated in parenthesis

The mean differences did not significantly vary between the age groups (ANOVA, $p >$ 0.05)

4.18 Ethnic Differences in Serum Glutamate and Serotonin Concentration

Result of ethnic differences in blood glutamate and serotonin concentrations showed no statistically significant ethnic variation among the studied population. Mean serum glutamate concentration was found to be highest among participants belonging to the others ethnic groups (38.21 ± 4.97), and lowest among the Hausa ethnic group (24.42 ± 2.53), though the differences were not statistically significant (p = 0.100; F = 2.151). Mean serum serotonin concentration was found to also be highest in the others ethnic group participants (173.79 ± 19.53), and lowest among the Yoruba ethnic group (127.28 ± 17.00), but the difference was also not statistically significant (p = 0.198; F = 1.590) (Figure 4.13).

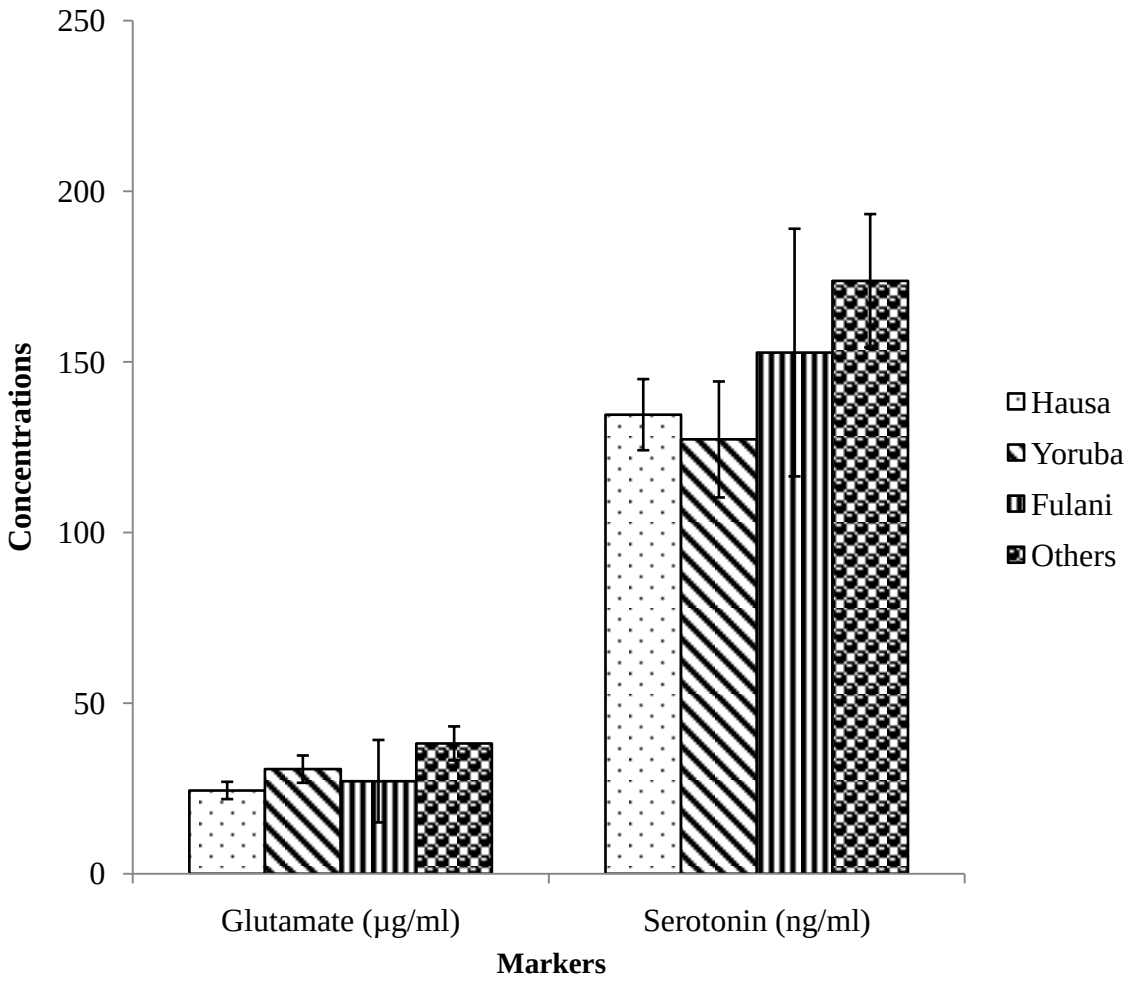


Figure 4.13: Ethnic differences in serum glutamate and serotonin concentrations in a healthy Nigerian adult population in Zaria

The mean differences do not significantly vary between the age groups (ANOVA, $p >$ 0.05)

4.19 Relationship between Serum Glutamate and Experimental Pain Response

Pearson's correlation analysis of serum glutamate concentration with experimental pain response showed that there is a negative correlation between serum glutamate concentration and cold pressor pain threshold ($R^2 = 0.0125$), but a positive correlation with cold pressor pain tolerance ($R^2 = 0.0192$; $p = 0.198$) though not significant ($p = 0.300$). Serum glutamate concentration showed a statistically insignificant positive correlation with ischaemic pain threshold ($R^2 = 0.0021$; $p = 0.673$), and negative correlation with ischaemic pain tolerance ($R^2 = 0.0003$; $p = 0.875$). On the other hand, serum glutamate concentration showed a statistically significant positive correlation with pressure pain threshold ($R^2 = 0.0549$; $p = 0.027$) (Figure 4.14).

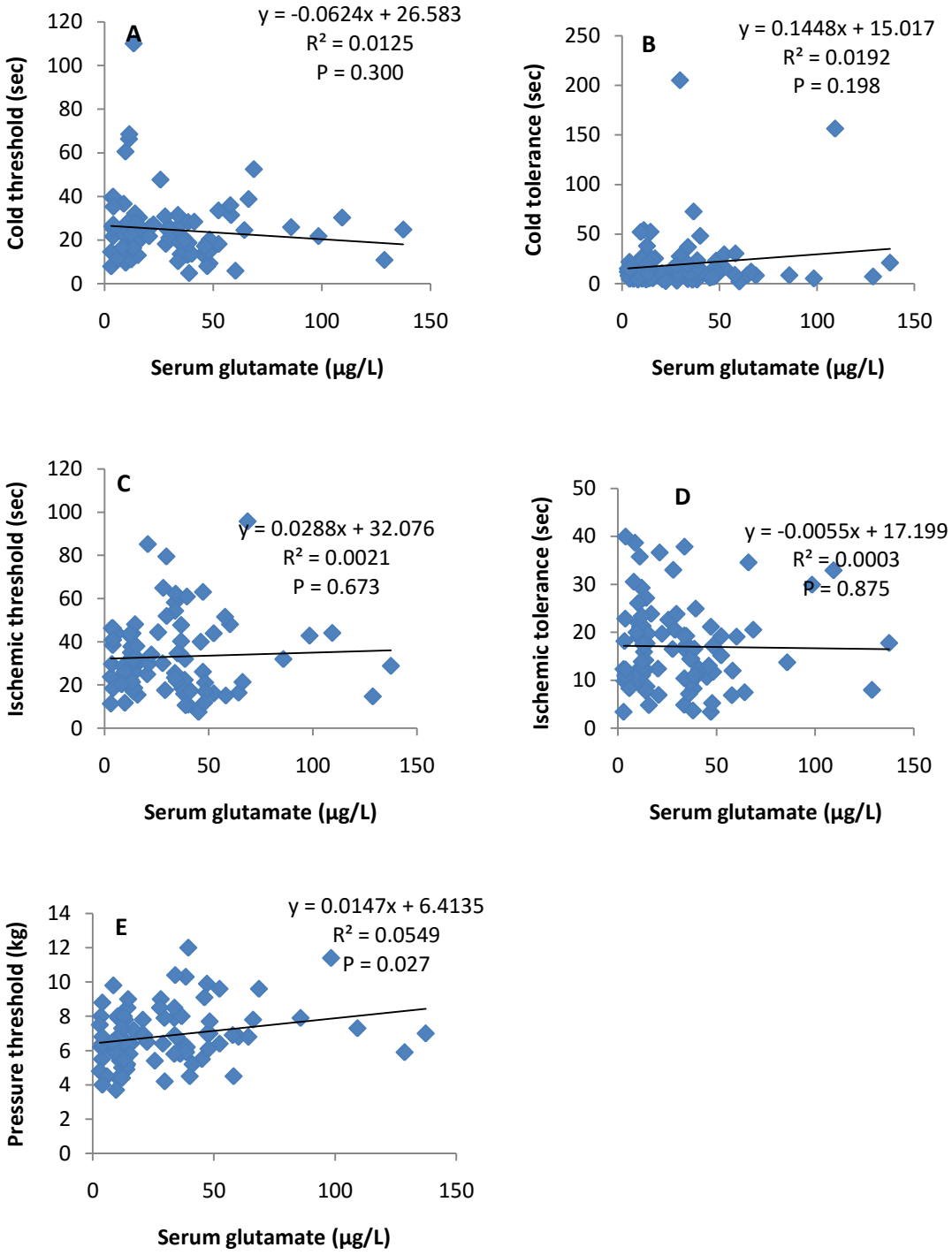


Figure 4.14: Scatter diagram showing relationships between serum glutamate concentration and cold pressor pain threshold (a), cold pressor pain tolerance (b), ischaemic pain threshold (c), ischaemic pain tolerance (d) and pressure pain threshold (e) among healthy Nigerian adult population in Zaria. Unit of parameters indicated in parenthesis

4.20 Relationship between Serum Serotonin and Experimental Pain Response

Pearson's correlation analysis of serum serotonin concentration and experimental pain responses in a Nigerian adult population showed that serum serotonin concentration is insignificantly correlated with cold pressor pain threshold ($R^2 = 0.0074$; $p = 0.479$). There is a negative but insignificant correlation between serum serotonin concentration and ischaemic pain threshold ($R^2 = 0.0052$; $p = 0.545$), but a negative and statistically significant correlation with ischaemic pain tolerance ($R^2 = 0.1107$; $p = 0.007$). Correlation between serum serotonin concentration and pressure pain threshold is found to be negative but statistically insignificant ($R^2 = 0.0259$; $p = 0.162$) (Figure 4.15).

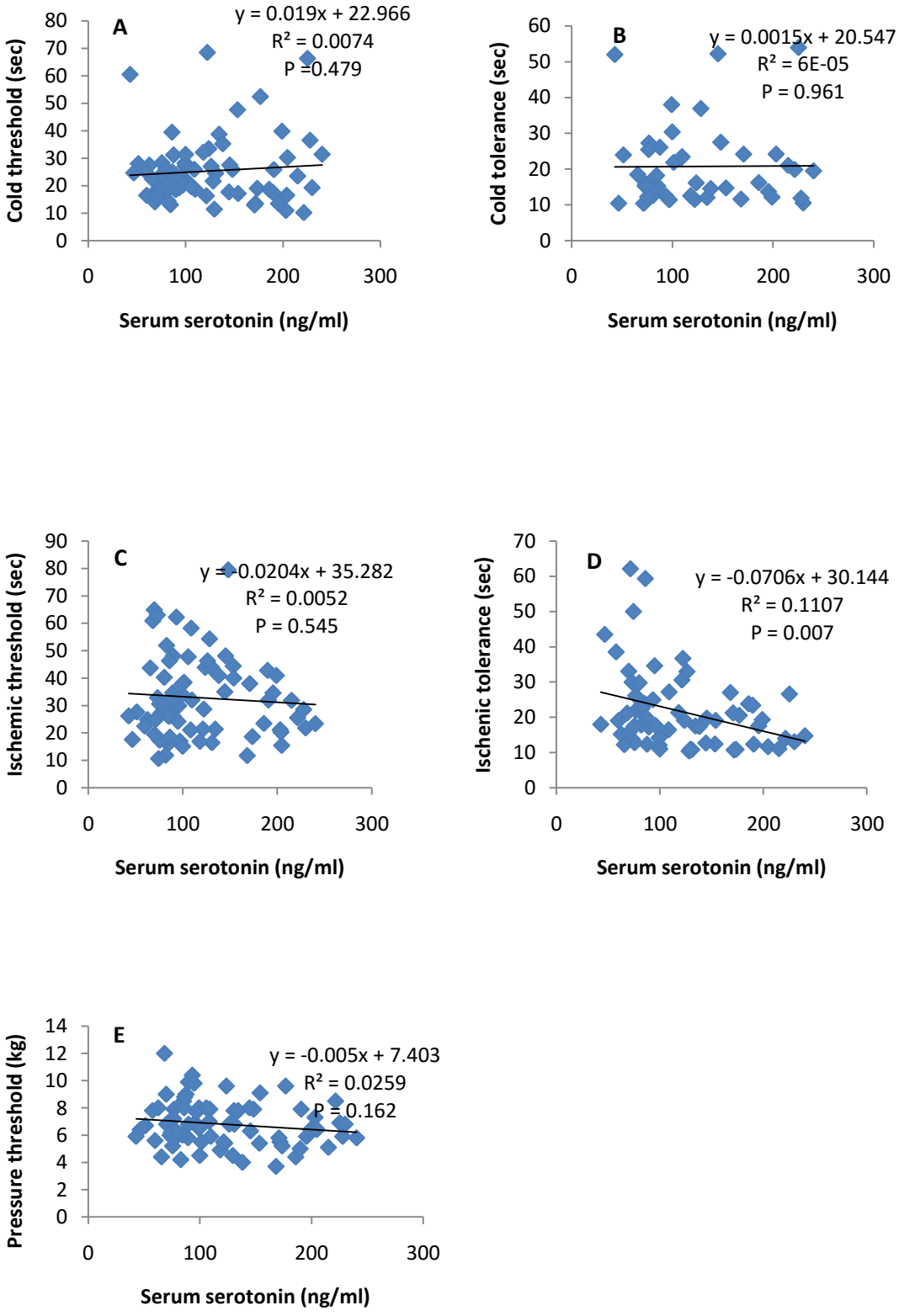


Figure 4.15: Scatter diagram showing relationships between serum serotonin concentration and cold pressor pain threshold (a), cold pressor pain tolerance (b), ischaemic pain threshold (c), ischaemic pain tolerance (d) and pressure pain threshold (e) among healthy Nigerian adult population in Zaria

4.21 Correlation between Serum Glutamate and Serotonin Concentrations with Experimental Pain in Males and Females

There was a significant positive correlation between serum glutamate concentration and cold pressor pain tolerance in males ($r = 0.4571$, $p < 0.01$), but not in females ($r = 0.1146$, $p > 0.05$). Serum serotonin was also significantly and positively correlated with cold pressor pain tolerance in males ($r = 0.5499$, $p = 0.0001$), but not in females ($r = 0.1410$, $p > 0.05$). On the other hand, a significant positive correlation between serum glutamate and pressure pain threshold was recorded in females ($r = 0.4377$, $p < 0.01$), but not in males ($r = 0.1502$, $p > 0.05$) (Figure 4.16).

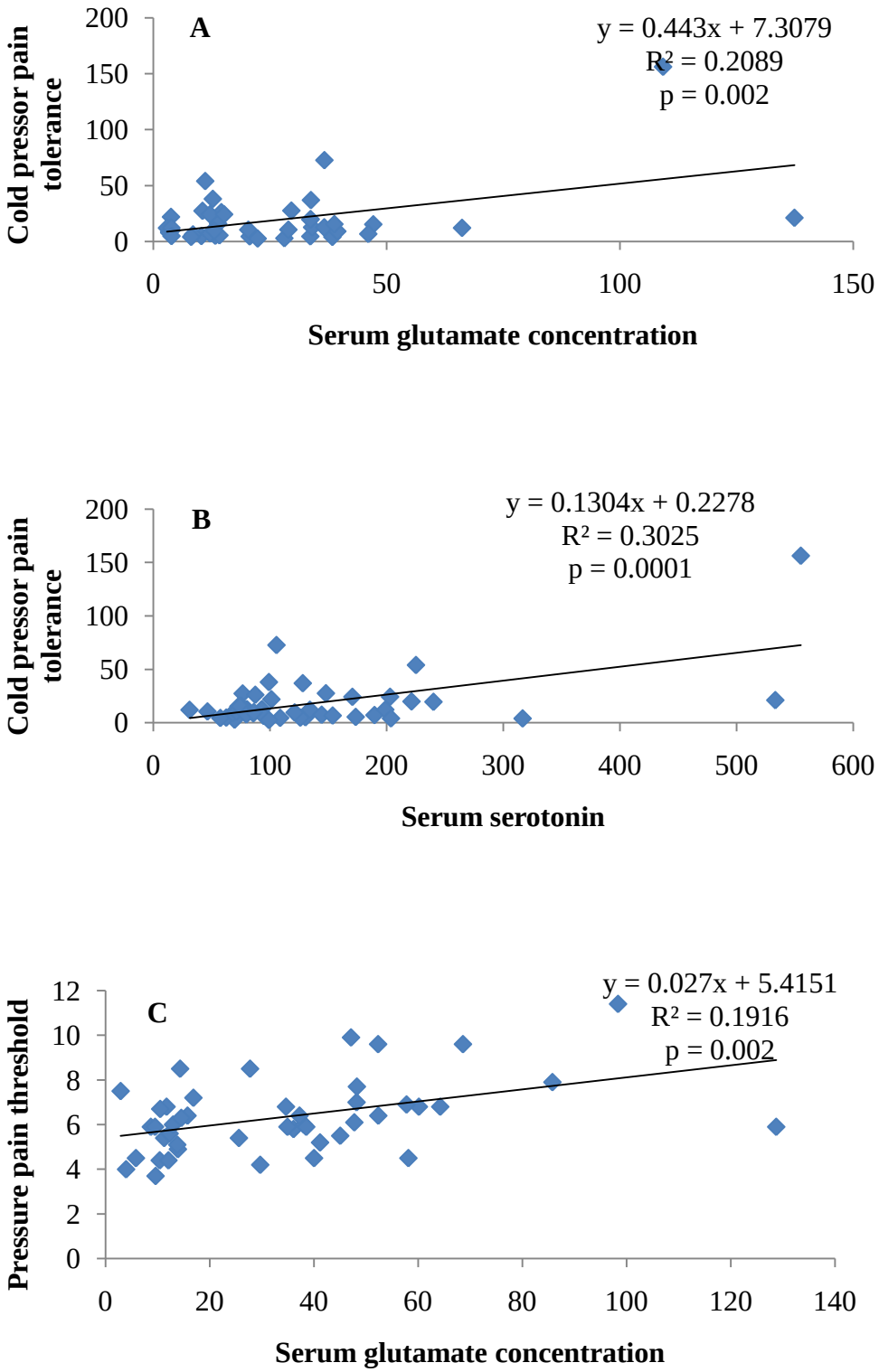


Figure 4.16: Scatter diagram showing relationships between serum serotonin and glutamate concentrations with experimental pain outcomes in males (a, b) and females (c) among healthy Nigerian adult population in Zaria

4.22 Sex Differences in Serum Concentration of Na^+, K^+ and Cl^-

The result of sex differences in serum electrolytes showed that the male subjects have lower serum sodium concentration (143.65 ± 52.95) than the females (148.83 ± 48.71), though the difference is not statistically significant [p = 0.527; t(155) = -0.634]. Difference in serum potassium concentration also shows no statistical significance [p = 0.099; t(155) = -1.660], but male participants have lower concentration (5.09 ± 2.08) compared to the females (5.77 ± 3.09). Analysis of serum chloride concentration shows that male participants have slightly lower serum chloride (93.42 ± 11.89) than females (94.10 ± 10.13), but the difference is not statistically significant [p = 0.706; t(155) = -0.378] (Figure 4.17).

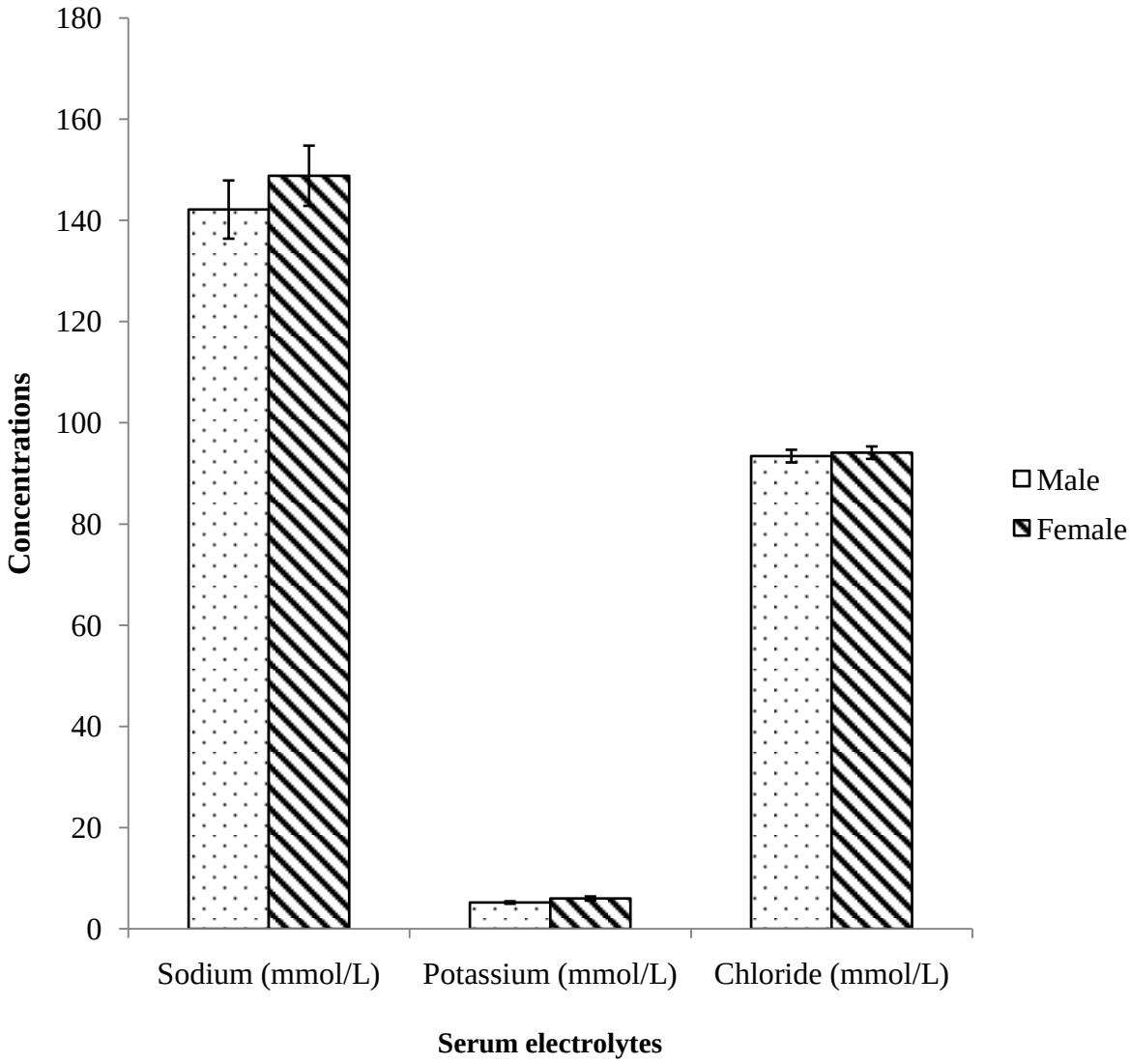


Figure 4.17: Sex differences in serum concentration of Na, K and Cl among healthy adult population in Zaria. Unit of parameters indicated in parenthesis

No statistically significant differences between males and females (independent samples *t*-test)

4.23 Age Differences in Serum Concentration of Na^+, K^+ and Cl^-

The result of age differences in serum concentration of sodium, potassium and chloride showed that serum sodium concentration is 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 is a decrease with increase in age, as participants in the youngest age group (20 – 30 years) have the highest concentration (6.03 ± 2.91), and those in the oldest age group (above 50 years) have 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) (Figure 4.18).

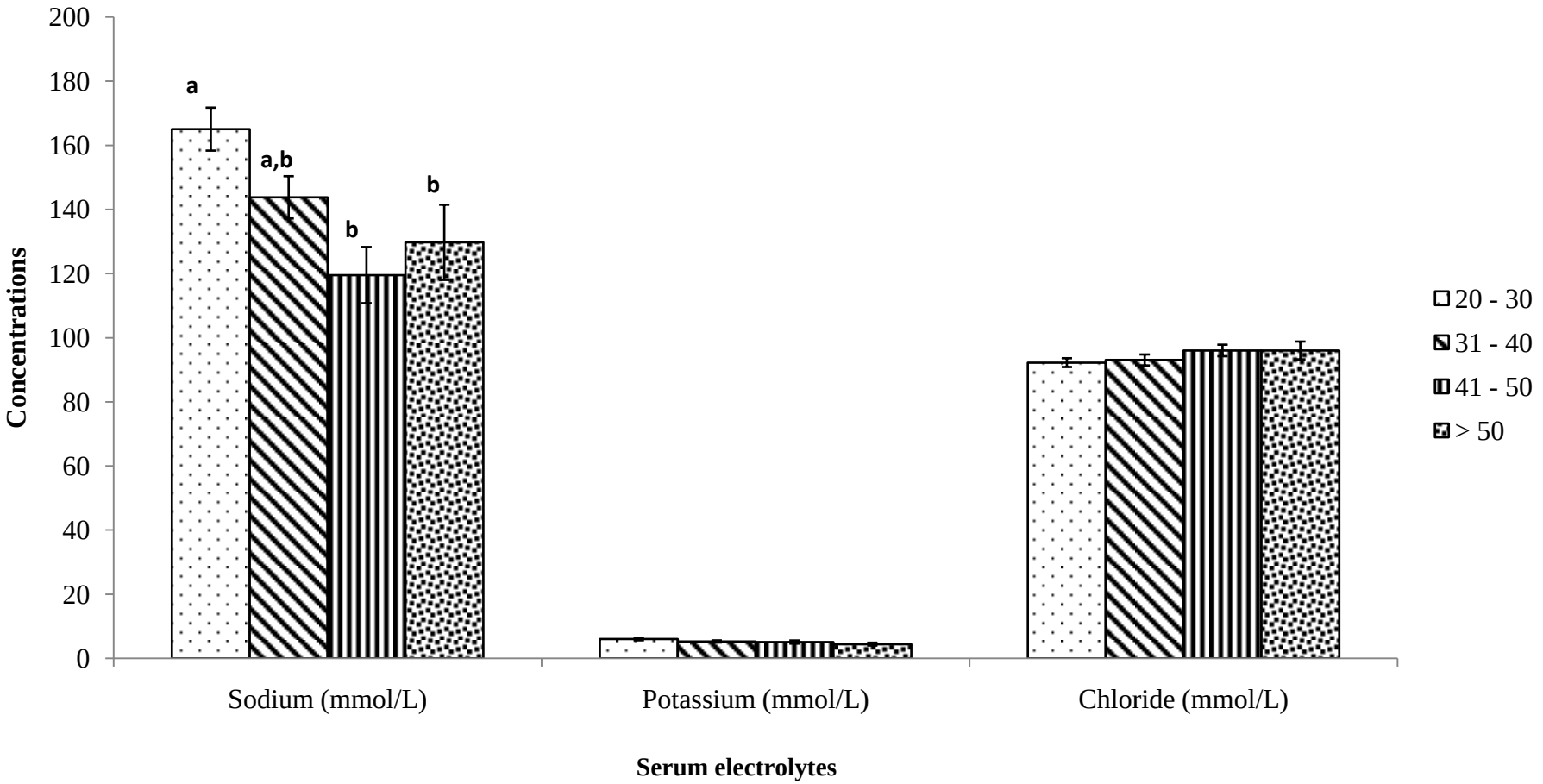


Figure 4.18: Age differences in serum concentration of Na, K and Cl among healthy Nigerian adults in Zaria

Mean difference of bars with different superscript letters[a,b,] is statistically significant ($p < 0.05$) between the age groups for the corresponding variable (ANOVA). Unit of parameters indicated in parenthesis

4.24 Ethnic Differences in Serum Concentration of Na^+, K^+ and Cl^-

Result of ethnic differences in serum concentration of sodium, potassium and chloride showed that the Hausas have statistically significant lower serum sodium (135.21 ± 47.37) when compared to 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 have significantly higher serum sodium compared to Fulanis (123.22 ± 51.15) (p = 0.001), and the difference between the Fulanis and subjects in the others tribe group is 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 have the highest concentration (6.26 ± 3.25), while those in the others group have 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 shows that subjects in the Fulani group have the highest (95.92 ± 9.94), and the Yorubas have the lowest (89.40 ± 13.14) (Figure 4.19).

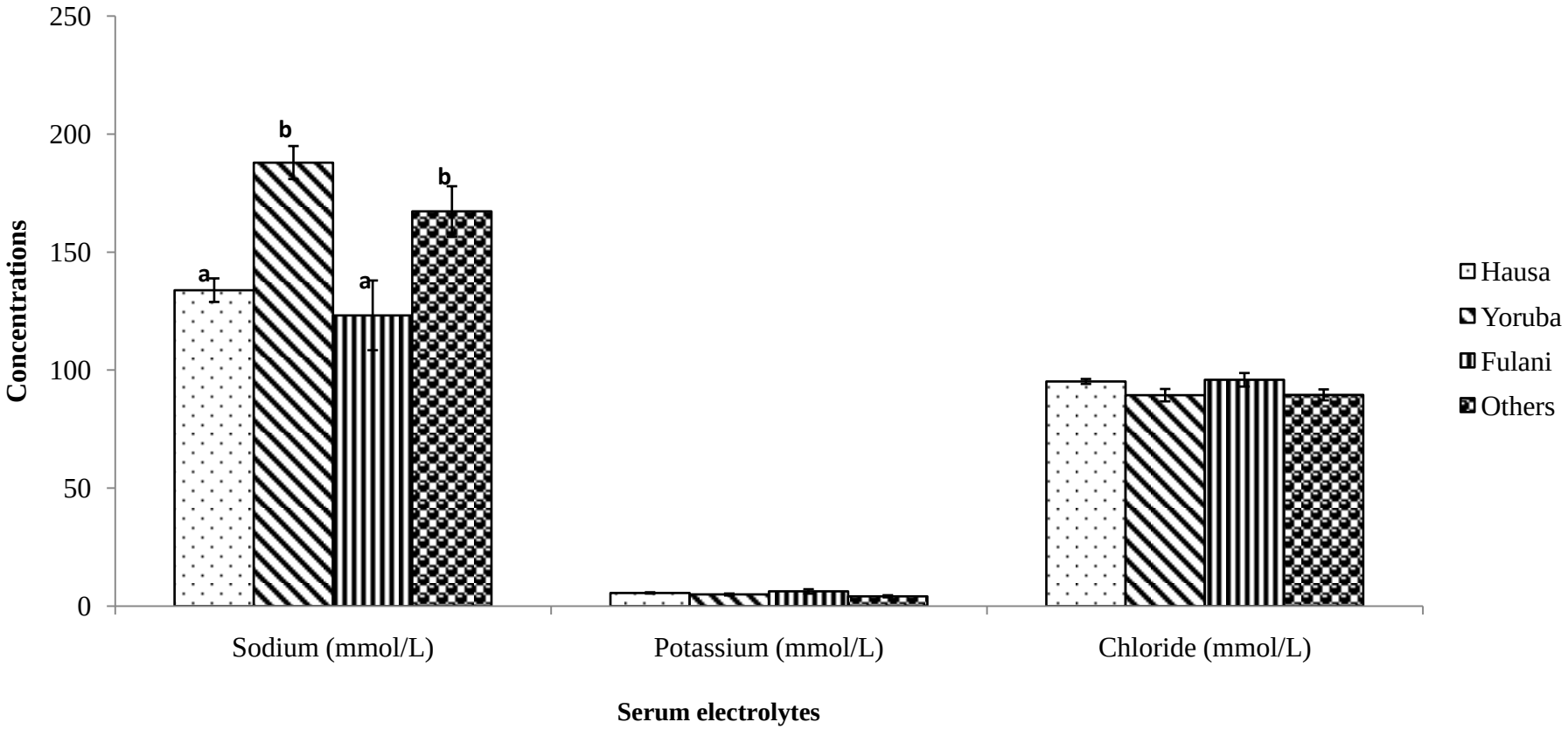


Figure 4.19: Ethnic differences in serum concentration of Na, K and Cl in a healthy adult Nigerian population in Zaria

Mean difference of bars with different superscript letters[a,b,] is statistically significant ($p < 0.05$) between the groups for the corresponding variable (ANOVA)**.** Unit of parameters indicated in parenthesis

4.25 Sex Differences in Serum Concentration of MDA, GSH and SOD

Male subjects were found to have slightly higher serum MDA concentration (23.68 ± 1.53) than the females (23.16 ± 1.31), though the difference is not statistically significant [p = 0.762; t(155) = 0.259]. Difference in serum GSH concentration does not also statistically vary [p = 0.391; t(155) = 0.865], but the males have higher GSH concentration (30.69 ± 0.68) than the females (29.79 ± 0.78). Analysis of serum SOD concentration shows that male participants have lower serum SOD (13.51 ± 1.00) than the females (14.07 ± 0.98), but the difference is also not statistically significant [p = 0.740; t(155) = -0.402] (Figure 4.20).

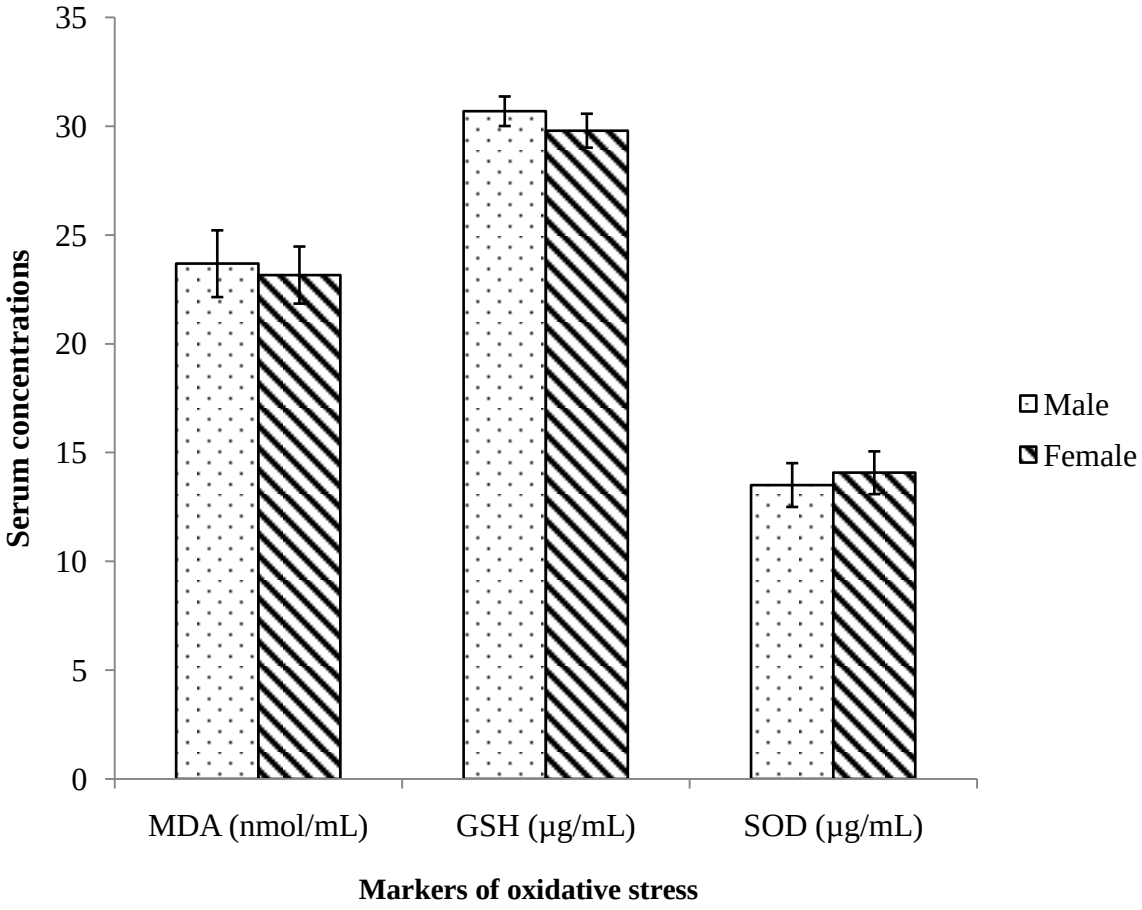


Figure 4.20: Sex differences in serum concentration of MDA, GSH and SOD among healthy Nigerian adult population in Zaria

No statistically significant difference between males and females for all the parameters (independent samples *t*-test, $p > 0.05$). Unit of parameters indicated in parenthesis

4.26 Age Differences in Serum Concentration of MDA, GSH and SOD

The result of age differences in serum concentration of MDA, GSH and SOD showed that serum MDA concentration is 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 shows no statistically significant variation among the studied subjects (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 have the highest (31.50 ± 0.85). Analysis of serum SOD concentration also shows no statistically significant age variation among the studied participants (p = 0.117; F = 2.017), but the result shows that there is 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) (Figure 4.21).

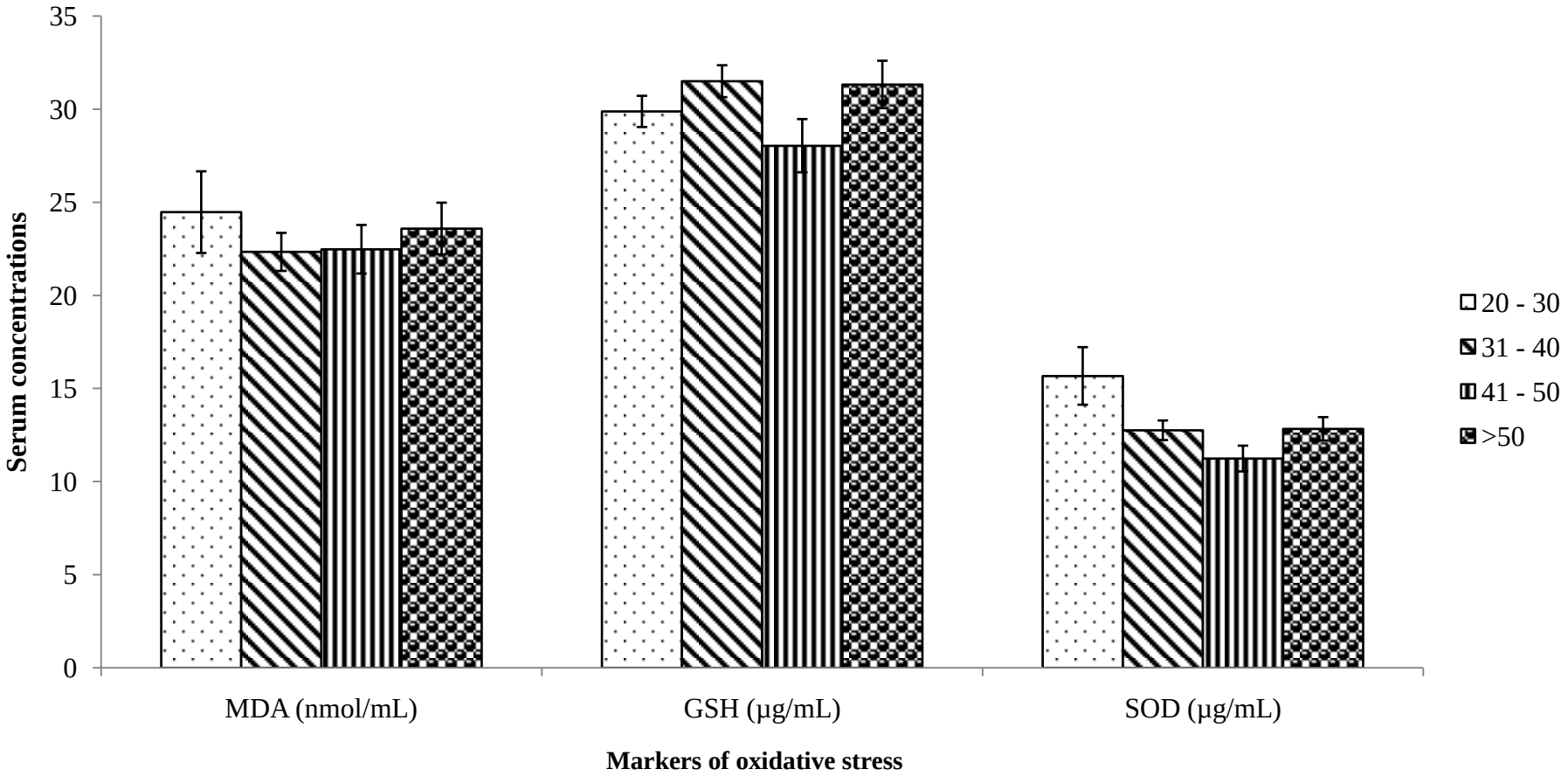


Figure 4.21: Age differences in serum concentration of MDA, GSH and SOD in a healthy Nigerian adult population in Zaria

No statistically significant difference between the different age groups for all the parameters (ANOVA, $p > 0.05$). Unit of parameters indicated in parenthesis

4.27 Ethnic Differences in Serum Concentration of MDA, GSH and SOD

Result of ethnic differences in serum concentration of MDA, GSH and SOD showed that subjects that belong to the Yoruba ethnic group have the highest serum MDA concentration (26.25 ± 3.49), while those in the Others group have 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 subjects (p = 0.792; F = 0.346), though the Fulanis have the lowest GSH concentration (28.74 ± 2.13), while the Hausas have the highest (30.58 ± 0.77). Serum SOD concentration analysis shows no statistically significant ethnic differences among the studied population (p = 0.136; F = 1.894), but the result shows that subjects in the Fulani group have the least serum SOD concentration (10.71 ± 0.76), while the Yorubas have the highest (16.30 ± 2.44) (Figure 4.22).

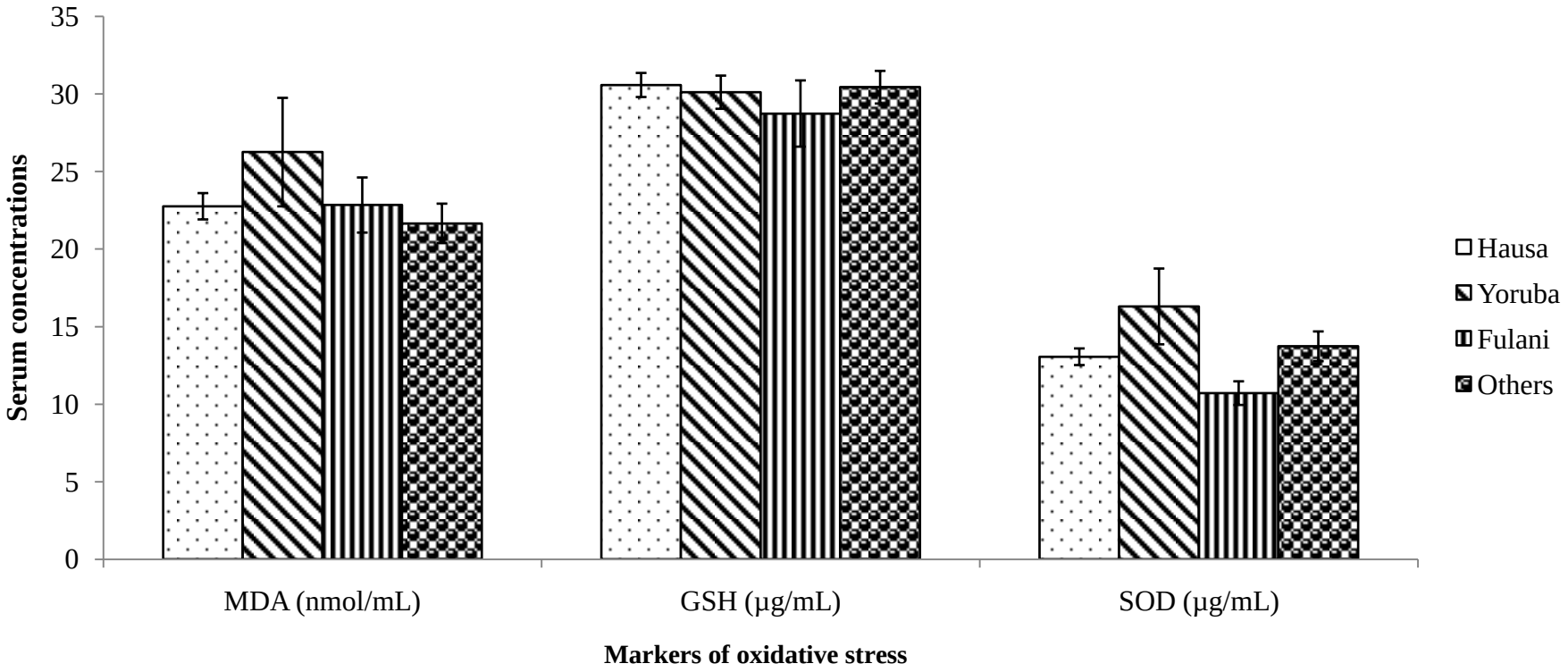


Figure 4.22: Ethnic differences in serum concentration of MDA, GSH and SOD in a healthy Nigerian adult population in Zaria

No statistically significant difference between the different groups for all the parameters (ANOVA, $p > 0.05$). Unit of parameters indicated in parenthesis

CHAPTER FIVE

5.0 DISCUSSIONS AND LIMITATIONS

5.1 Discussion

The present study was carried out to investigate the influence of sex, age and ethnicity on experimental pain perception in an adult Nigerian population in Zaria. The experimental protocols and blood sample collections were performed during the same season in all subjects to minimise seasonal variations. All subjects were duly explained the aim as well as implication of the study, and gave written consent. It was hypothesized that sex, age and ethnicity will influence experimental pain outcome among adult Nigerians. Furthermore, the hypothesis also stated that correlation between pain perception and serum concentrations of glutamate and serotonin would provide support for more comprehensive research to determine possible mechanisms underlying the inter-individual variability in experimental pain perception. Subjects with potential confounding variables (like inflammatory diseases and drug use) were excluded from the study.

Results of this study indicate that sex, age and ethnicity influence the outcome of some experimental pain models among the studied population. Over time, many researchers have investigated inter-individual variability in pain responses using different experimental protocols. Studies have shown that females are more sensitive to experimental pain than males (Silva *et al.,* 2016). Physiological, socio-cultural and psychological variables are believed to be the factors that contribute to sex differences in pain behaviour. Due to the complex interplay among the multiple factors involved in the manifestation of persistent pain across several domains, discriminating which factors are most important for the purpose of treatment was very challenging. Pain

expression and, hence, the importance of specific factors commonly varies across individuals, and also in one individual over time (Ferrell *et al.,* 2002).

Result of the present study showed that males have higher cold pressor pain threshold and tolerance than females, though the differences were not significant. This is in agreement with the finding of Ogedengbe *et al.* (2015). Nikolov *et al.* (2014) and Umar *et al.* (2017) reported higher cold pressor threshold in males, but no difference in cold pressor tolerance, while Edwards *et al.* (2004) and Silva *et al.* (2016) concluded that males have significantly higher cold pressor pain threshold and tolerance than females. Mitchell *et al.* (2004) reported that gender differences were found in the response to cold pressor pain, with men tolerating the stimulus for significantly longer than women. Logan and Gedney (2004), who assessed stability by using fore-head cold pressor applications, reported no differences in pain report between men and women. In another study on a Libyan population by Tashani *et al.* (2010), cold pressor pain threshold was found to be significantly higher in men, but there were no significant differences between the sexes in pain tolerance, intensity, or unpleasantness. A study by de Nazare *et al.* (2014) reported significantly lower cold pressor pain threshold in males than females.

Effect of sex on pressure pain response in the present study showed that male participants had a significantly higher pressure pain threshold when compared to the females. This agrees with the findings of previous studies that obtained similar results, when pressure algometry was applied in other body locations. Garcia *et al.* (2007) and Rui *et al.* (2015) reported that women showed a lower pressure pain threshold than men. Hogeweg *et al.* (1992) also reported a significantly higher pressure pain threshold

in males than females. In addition, Chesterton *et al.* (2003) showed that healthy females exhibited significantly lower mean pressure pain threshold in the first dorsal interosseous muscle than males, which was maintained for 14 repeated measures within a 1 hour period. A study by Lee *et al.* (2008) reported that pressure pain threshold in males was higher than in females at different muscles of both sides. On the other hand, the result of the present study disagree with the finding of Lemming *et al.* (2015), who described that analysis of the temporal aspects of pain sensitivity did not show any significant differences related to sex. Age and sex have been shown to play little role in the outcome of pressure algometry test (Antonaci *et al.,* 1992). Though pressure algometry has been used for evaluation of pain, determination of therapeutic effects, and follow-up surveys of treatments in many musculo-skeletal diseases, it may be influenced by factors such as inter-observer reproducibility, sex, and dominant and non-dominant sides (Park *et al.,* 2011).

Sex differences in pressure algometry, as reported in this study, may be related to hormonal influences as well as some gender differences in specific pain modality (Lemming *et al.,* 2015). This difference has clear implications for the use of different gender subjects in laboratory-based experimental designs utilising pressure algometry. The difference may be due to the fact that men tend to respond to pain more slowly than women because of the traditional and social recognition that men must endure pain. Thicker muscles and subcutaneous tissues of men in view of anatomical characteristics (Park *et al.,* 2011), may contribute to the difference observed in this study, suggesting that men may be less susceptible to pain than women due to anatomical and physiological characteristics. Kusumadevi *et al.* (2011) reported a moderately significant higher pain scores in women than the men in the age group of

21-30 years, suggesting that the increased sensitivity to pain in women appear to be greatest in middle age.

Result of ischaemic pain test in the present study showed that male participants had significantly higher pain threshold when compared to the females. On the other hand, pain tolerance for ischaemic pain does not significantly vary, though male participants had slightly higher tolerance than the females. This finding agrees with that of Umar *et al.* (2017), which also showed higher ischaemic pain threshold in males than in females. Bragdon *et al.* (2002) and Edwards *et al.* (2004) reported no sex difference in ischaemic pain threshold and tolerance, while Fillingim and Maixner (1996) and Bulls *et al.* (2015) reported that women demonstrated shorter time to ischeamic pain tolerance than men. The lower ischaemic pain sensitivity in the males than the females in this study may be linked to higher blood pressure among the male participants, as systolic, diastolic, and mean arterial pressures have been demonstrated to significantly correlate with ischaemic pain responses in males, but not in females; with higher blood pressure being associated with lower pain sensitivity (Fillingim and Maixner, 1996). Bragdon *et al.* (2002) reported that higher systolic blood pressure was correlated with higher heat pain threshold and tolerance in men only, while oestradiol level was found to be strongly associated with higher ischaemic pain tolerance in women. Baroreceptor-mediated blood pressure-related analgesic mechanism appears to predominate in men, while an endogenous opioid mechanism predominates in women (Bragdon *et al.*, 2002).

Sex differences in the perception of painful stimuli have been of interest to scientists for some time, and the consensus is that males show greater tolerance than females.

Sex differences in experimental pain may depend on the stimuli used and the nature of the chosen end point (Binder *et al.,* 2000), and the magnitude of such differences may be affected by variations in the methodology used to induce the pain, such as the stimulus type, anatomic location, stimulus duration and characteristics, as well as stimulus application (i.e. whether the stimulus is self-applied) (Paller *et al.,* 2009).

Sex differences in responses to experimental pain have been investigated using a wide variety of stimulus modalities, including mechanical (blunt pressure and punctate mechanical stimuli), electrical, thermal (heat and cold), ischaemic, and chemical stimuli (e.g. capsaicin, hypertonic saline). Pain responses have been assessed by a number of different outcome measures, including behavioural indices of threshold and tolerance, and self-report measures of pain intensity and unpleasantness (Bartley and Fillingim, 2013). Cold stress, for example, stimulates free nerve endings to regulate temperature by sending afferent signals to central nervous system. Activated receptors induce reflex vasoconstriction via stimulation of myelinated Aδ fibres related to pain sensation, and non-myelinated C fibres that are responsible for perception of nociceptive stimuli (Silva *et al.,* 2016).

The lack of statistical significance for the cold pressor pain in the present result may be attributed to the fact that the subjects were properly oriented to control for psycho-social influences on their pain perception. The participants were oriented to report their actual and not psychological pain, thus, bringing the females' pain responses similar to those of the males. Males are more motivated to suppress pain expressions due to socio-cultural and psychological influences of the male sexual role, while the female sexual role encourages pain expression and produces lower motivation to suppress

pain. Sex of the experimenter may also be a factor in the outcome of the present study, as the entire experiment in all the subjects was carried out by the same male experimenter. Men and women have been reported to show lower pain sensitivity, when they were processed by biological male personnel than by biological female personnel before the cold pressor pain test. Meanwhile, women who interacted with a transgendered researcher reported higher pain sensitivity than women processed by biological male or female researchers (Vigil *et al.*, 2014).

This study failed did not assess the menstrual and pregnancy status of the female participants, as well as their blood sex hormonal level, which may modulate their pain response due to hormonal influence. Pain sensation have been shown to increase during the luteal phase of menstrual cycle, and high serum progesterone and oestradiol concentrations correspond to lower values for pain intensity (Silva *et al.*, 2016). Withdrawal latency was reported to be significantly reduced during the luteal phase, compared with the follicular phase. The follicular phase is characterised by high oestrogen levels and women were reported to be less sensitive to pain at a time when circulating oestrogen levels are high, as oestrogen has been shown to play a role in the regulation of opiate expression (Nikolov *et al.*, 2014).

Another compounding factor may be possible contraception practice of the female volunteers, which was also not evaluated in this study. In a study that evaluated sex differences in response to cold pressor pain in normally menstruating women, women maintained on oral contraceptives, and men, pain threshold was found to be higher in normally menstruating women compared with women maintained on oral contraceptives and men (Kowalczyk *et al.*, 2006). The influence of sex hormones on

pain-related variability in humans has been associated with the distribution of sex hormones and their receptors in areas of the peripheral and central nervous systems associated with nociceptive transmission. Although oestradiol and progesterone's effects on pain sensitivity are relatively complex (both exert pro-nociceptive and anti-nociceptive effects on pain), testosterone, on the other hand, appears to be more anti-nociceptive, given the association between decreased androgen concentrations and chronic pain (Bartley and Fillingim, 2013). Chemical/hormonal contraceptives influence pain threshold and tolerance in females, as significantly lower ischaemic pain threshold has been linked to oral contraceptive use in healthy women (Bragdon *et al.,* 2002). Stening *et al.* (2007) reported that pain responses vary across the menstrual cycle, and that the differences are related to differences in hormone levels. Gonadal hormones have been suggested to play complex roles in pain sensation, which may be either pro-nociceptive or anti-nociceptive, depending on the hormone profile (Nikolov *et al.,* 2014). Pain perception may be influenced not by absolute levels of sex hormones, but rather their dynamic changes, and it is possible that it is the reduced progesterone concentrations, from a high level, rather than the progesterone concentration itself that triggers the increased pain sensations during late luteal phase (Stening *et al.,* 2007).

Sex steroid-dependent, gestation-associated anti-nociception has also been demonstrated in experimental animals, as well as pregnancy-induced analgesia in humans (Stening *et al.,* 2007), although a study by Binder *et al.* (2000) demonstrated that there are no gender-based differences in the severity or the time course of adjuvant-induced inflammation nor in the magnitude of the hyperalgesia in the ankle joints of CFA induced arthritic rats.

It has been suggested that sex chromosomes and steroidal actions on development lead to differences in pain physiology between men and women. Animal and human studies also suggested that sex-specific hormones (e.g. oestrogen) influence sex-specific responses to pain. Further investigations have identified neurobiological differences between men and women in brain regions related to pain sensitivity, such as the periaqueductal gray and the strength of its connectivity with the amygdala, caudate and the putamen nuclei (Bulls *et al.*, 2015).

Sex differences in the sensation of pain may vary significantly across studies because of the different methods used as well as the body area being stimulated. Other factors that can influence the results are the psychological characteristics of each participant, such as levels of anxiety, depression, or previous painful experiences of each subject (de Nazari *et al.*, 2014).

Age-related anatomical and functional changes in the somato-sensory system have been documented in both human and animal studies, ranging from reduction of myelinated and unmyelinated peripheral nerve fibres, as well as signs of damage, including Wallerian degeneration. Number and size of sensory neurones in dorsal root ganglia (DRG) also decreases with advancing age after a mid-adult peak (Yezierski, 2012).

Result of age differences on cold pressor pain did not show statistically significant variation in the present study, but pressure pain threshold was found to be significantly lower among the 41 – 50 age group. Ischaemic pain threshold was found to be

significantly higher among the oldest age group (>50), though ischaemic pain tolerance was not significantly different. The effect of age on pain sensation may dependent on the type of pain induced. Study has shown that whereas pressure pain threshold decreases with age, heat pain threshold remain unchanged (Lautenbacher *et al.,* 2005). Umar *et al.* (2017) reported increase in cold pressor pain tolerance with advancing age among young adult population, but no difference in ischaemic pain response. Earlier onset of ischaemic pain and greater pain severity was reported among elderly subjects by Rittger *et al.* (2011). In a meta-analysis of studies on pain threshold and tolerance by Lautenbacher *et al.* (2017), pain threshold was reported to increase with age, especially using heat stimulus, while pain tolerance did not show substantial age-related changes.

In a study by Wandner *et al.* (2012) that assess a respondent's view of the typical young adult, middle-aged adult, and older adult with respect to pain sensitivity and willingness to report pain using the age expectation of pain questionnaire, participants indicated that typical older adult is more sensitive to pain than typical younger adult. Frailty may be implicated in the influence of age on pain perception, as pain and frailty are both prevalent and have severe health impacts among older adults. Pain and depressive symptoms were reported to be positively associated with physical frailty, and depressive symptoms were associated with pain. The positive association of pain with frailty is persistent and partially mediated by depression, and co-morbid depression and pain have an additive interaction on physical frailty (Tian *et al.,* 2018). Another finding suggests that with increased age, there is a small increase in pain threshold (Walton *et al.*, 2011), indicating that pain threshold increases as people age.

Effect of ageing on pain depends on pathophysiologic, pharmacokinetic, and pharmacodynamic changes in the elderly as well as psychosocial factors (Belfer, 2013). Mecklenburg *et al.* (2017) reported that, as in human volunteers, thresholds of baseline thermal and mechanical nociception in naive aged mice are higher than in naive adult mice, but postoperative thermal and mechanical hypersensitivity has almost the same magnitude in aged and adult mice. Age possibly alters basal nociception by modifying neuronal circuitries within pain pathways. A systematic review found that pre-existing pain, anxiety, age, and type of surgery are the most significant predictive factors for the intensity of postoperative pain (Ip *et al.*, 2009).

Animal studies examining the effects of age on pain sensitivity have resulted in conflicting observations that include increases, decreases or no change in cutaneous sensibilities with advancing age. Altered expression of neurotransmitters and receptors has been observed in the spinal cords of old animals. Immunohistochemical studies reveal decreased labelling of calcitonin gene related peptide (CGRP), substance P, nitric oxide, and somatostatin in the dorsal horn of aged rats. Evidence of a progressive age-related loss of serotonergic and noradrenergic terminals in the dorsal horn also suggests the potential for changes in descending modulatory pathways. Decreased numbers of opiate receptors and the decreased efficacy of opiate-mediated antinociception may also contribute to age-related changes in the processing and perception of nociceptive information. Molecular alterations in gene expression for trophic factors, neuropeptides, cell adhesion molecules, ion channels or genes related to mitochondrial function and calcium handling are also additional contributing factors to age related changes in pain perception (Yezierski, 2012). These age-related changes

may be best conceptualised as a reduced capacity in the functional reserve of the pain system, at both ends of the intensity spectrum (Gibson and Farrell, 2004).

A study using latency of responses to paw lick and tail flick in rats described a decrease in thermal and electric shock sensitivity with increasing age that correlated with a decrease in the number of opiate receptors in the frontal poles, striatum and hippocampus. Young animals were reported to have significantly shorter thermal response latencies than older animals, which correlated with a decrease in the expression of Nav1.8 sodium channels, thus providing a mechanistic explanation for the decrease in thermal sensitivity. Observations also reveal a decrease in expression of the ion channel TRPV1 (Yezierski, 2012).

A correlation analysis identifies an increase in the time of pain perception with increasing age, as reports found that advanced age results in loss of pain perception. It is believed that the degree of conservation of the peripheral nervous system is lower in older people, which predisposes damages in the afferents related to pain perception. Age in males plays a relevant role in modulating both pain threshold and tolerance, whereas women's age is not correlated (de Nazare *et al.,* 2014).

Effect of ethnicity on cold pressor pain shows no statistically significant variation in the present study, though participants belonging to the Fulani ethnic group were found to have the highest pain threshold, but the lowest pain tolerance. Yorubas had the lowest pain threshold, while participants belonging to others ethnic group had the highest pain tolerance. Perception of pressure pain showed statistically significant variation by tribe in the present study, with Yorubas having significantly higher

pressure pain threshold compared to Hausas, though the difference was not statistically significant when compared with the Fulanis and the others. Ischaemic pain threshold, on the other hand, showed no significant ethnic variation, but Fulani ethnic group showed significantly higher ischaemic pain tolerance than participants in all the other groups.

Experimental pain studies in the USA indicated that African–Americans report greater sensitivity (i.e. lower threshold) and reduced pain tolerance to a variety of pain tests when compared with non-Hispanic whites, including thermal pain, cold pressor pain, ischaemic pain and electrical stimulation (Campbell and Edwards, 2012). Ahn *et al.* (2017) also reported that Asian-Americans with knee osteoarthritis had significantly greater experimental pain sensitivity than non-Hispanic Whites. It has been reported that while African-Americans did not differ from whites on threshold measures of heat pain, ischaemic pain, and cold pressor pain, they exhibited significantly lower tolerances for each of the stimulus modalities (Campbell *et al.*, 2005; Cruz-Almeida *et al.*, 2013). Edwards *et al.* (2001) reported that African Americans reported greater severity of pain and more pain-related disability, as well as lower ischaemic pain tolerance than whites. The lack of significant differences in some of the pain modalities used in this study may be attributed to unequal sample size between the ethnic groups.

Differences in a variety of socio-cultural factors, ranging from family traditions and religious beliefs influence disparities in pain. Ethnicity may have a major influence on the meaning of pain and how it is appraised and responded to emotionally and behaviourally (Campbell and Edwards, 2012). Racial/ethnic differences in experimental pain sensitivity were reported to be more pronounced with supra-

threshold than with threshold stimuli (Kim *et al.*, 2017a). Statistically significant ethnic difference in post-operative pain score and morphine usage has been reported, which persisted after controlling for age, body mass index and duration of operation (Tan *et al.*, 2008). While pain tolerance and supra-threshold ratings of pain unpleasantness may primarily reflect the affective-motivational dimension of pain, pain threshold and supra-threshold ratings of pain intensity may be more strongly associated with sensory-discriminative aspects of the experience. It has been suggested that ethnic differences in pain responses may be most apparent for the affective-motivational dimension of pain (Campbell *et al.*, 2005).

In addition to socio-cultural factors, ethnic disparities in pain are also associated with biological and psychological influences. Ethnic group differences in allele frequencies for polymorphisms of pain-related genes (e.g. COMT and OPRM1) may contribute to ethnic differences in pain responses, as the COMT and OPRM1 genes have been associated with pain-induced mu-opioid receptor binding, experimental pain sensitivity, and risk for developing chronic pain (Ahn *et al.*, 2017).

Lack of significant difference in cardiovascular parameters (SBP and DBP) among the ethnic groups may attribute to lack of significant differences in their perception of cold pressor and ischaemic pain threshold, as the pain task is a potent cardiovascular challenge and is associated with enhanced adrenaline release and cardiovascular reactivity. Differences in adrenergic and vascular responses to stress could potentially contribute to ethnic differences in clinical and experimental pain (Edwards *et al.*, 2001), though Sheffield *et al.* (2000) reported that resting blood pressure did not account for the race differences in pain, but rather it is possible that it is not blood pressure, but risk for hypertension, that is related to pain perception.

Result of the present study showed that BMI effect on cold pressor pain threshold, pressure pain and ischaemic pain threshold showed no statistically significant variation. However, overweight subjects had significantly higher cold pressor pain tolerance compared to the underweight and obese. Ischaemic pain tolerance was found to be significantly higher in subjects with normal BMI, when compared to the obese subjects. Pain threshold was reported to be higher in subjects with BMI > 25 using the cold pressor test, though pain tolerance showed no significant differences between groups by BMI (Petkova *et al.*, 2014). Individuals with a high BMI report higher pain intensity, compared with individuals with normal BMI (Astita *et al.*, 2015). Kivrak *et al.* (2016) found no correlation between BMI and pain perception, while report by Khimich (1997) showed that patients with fatness have higher pain sensitivity threshold than people of other categories. A comparative study reported that Libyan women had higher pain sensitivity response to cold pressor pain than Libyan men, but both sexes had lower pain responses when compared to their Western counterparts, and height and anxiety may have contributed to the differences between the sexes (Tashani *et al.*, 2010). Height, but not weight or BMI, was reported to be correlated with pain threshold in women, but not in men (Tashani *et al.*, 2010). Various disorders, including obesity, are associated with an increased risk of aggravation of pain, as overweight negatively affects the musculo-skeletal system disorders. Obesity is known to be one of the risk factors in some nociceptive pain conditions (e.g., osteoarthritis and post-laparotomy pain) (Hozumi *et al.*, 2016).

Pressure pain threshold measured at a site with small accumulations of subcutaneous fat (thenar eminence) was reported to be lower for the obese subjects compared with overweight and normal subjects (higher BMI predicted lower pressure pain threshold),

suggesting that obesity increases sensitivity to noxious pressure stimuli at body sites which are low in subcutaneous fat (Tashani *et al.,* 2017). Price *et al.* (2013) reported that obese subjects had higher thresholds for painful thermal stimuli on the abdomen, and decreases in sensitivity correlated with measures of adiposity.

Significant correlation between BMI and pressure pain threshold has been reported in male (Lee *et al.,* 2008). Icagasioglu *et al.* (2015) found no statistically significant correlation between obesity and pain threshold. The thickness of the subcutaneous tissues in obese people has been proposed to account for the reduction in amplitude of sensory/mixed nerve responses, when nerve conduction studies are performed using surface recording and percutaneous stimulation (Miscio *et al.,* 2005). Electrophysiological examinations also demonstrated that the motor and sensory action potentials are significantly impaired in obese subjects, and obesity has been shown to elevate experimental heat pain threshold. Obesity and depression deteriorate reciprocally, and depression is known as one of the major risk factors for aggravating pain (Hozumi *et al.,* 2016). Susceptibility to pain in obese individuals may be affected by increases in the concentrations of pro-inflammatory cytokines in the blood, as evidence suggest that obese individuals have elevated circulation levels of pro-inflammatory cytokines, such as TNF-α, IL-6, and C-reactive protein (Astita *et al.,* 2015).

Using the hot plate analgesymeter, mice that had leptin injection were found to have lower pain threshold than the control group, and leptin was proposed to have influence on pain threshold by affecting the secretion of neuroendocrine factors associated with

nociception such as beta-endorphin and alpha-melanocyte stimulating hormone (Kutlu *et al.,* 2003).

Manual asymmetry is important in every sensory and motor function, and superior performance has been linked to several factors, including the processing characteristics of the left hemisphere/right-hand system and task complexity (Ozcan *et al.,* 2004). Result of the present study showed that right handed participants reported a significantly higher cold pressor pain threshold than the left handed ones, but the cold pressor pain tolerance did not statistically vary between the groups. Pressure and ischaemic pain responses, on the other hand, did not significantly differ between the groups. Pressure pain was assessed in the dominant hand, while cold pressor and ischaemic pain were assessed in the non-dominant hand. This result is in agreement with a study of inter-group comparisons between right-handed and left-handed subjects that revealed no significant differences between the dominant hands of right-handed and left-handed subjects with respect to pressure pain threshold (Ozcan *et al.,* 2004), but disagree with the study of Pud *et al.* (2009), who reported no significant cold pressor pain difference in right and left handed individuals. Report by Murray and Safferstone (1970) shows that pain threshold and tolerance were greater for the right hand than for the left, regardless of the hand preference of the subjects. Experimental pain thresholds are reported to be lower when applied to the left hand than the right hand in right handed healthy volunteers (Wasan *et al.,* 2010). The right cerebral hemisphere is believed to play a unique role for pain sensitivity. In an assessment of pressure pain and cerebral laterality in right handed subjects, pain threshold was found to be lower on left hand than the right hand (Pauli *et al.,* 1999).

The lack of significant difference in the pressure and ischaemic pain tasks may be due to masking effort of the left handers, as left-handed people living in a world designed for right-handers, because left-handed persons use the right (non-dominant) hand for many tasks that would naturally be done by the dominant hand of right-handers. Perception has been presumed to be altered at locations where action is more likely to occur (Le Bigot and Grosjean, 2012).

In an examination of prefrontal responses to pain stimulation among newborns during their first acute pain experience after birth, Ozawa *et al.* (2011) showed that right-hand stimulation induced a greater prefrontal activation than the left-hand stimulation, suggesting that hand laterality affects pain perception even in neonates. In another examination of ten right-handed subjects using radiant heat stimuli, findings showed a dominant role of the left frontal operculum and adjacent dorsal insula (the cortical projection target of nociceptive pathways from the spinal cord to the posterior thalamic nuclei) in the early sensory-discriminative dimensions of pain processing, irrespective of stimulation side (Schlereth *et al.*, 2003).

Effect of Hb genotype on experimental pain tests in the present study showed that there is no statistically significant variation in cold pressor pain threshold between subjects with AA and AS genotypes. On the other hand, AA genotyped subjects have significantly lower cold pressor pain tolerance than the AS subjects. The study also showed that ischaemic pain threshold significantly vary by Hb genotype, with AA subjects having lower ischaemic pain threshold than their AS counterparts. Ischaemic pain tolerance, however, did not significantly vary by Hb genotype. Pressure pain perception does not show statistically significant variation between the groups, though

AA genotyped subjects have lower pressure pain threshold than the AS group. Only AA and AS genotyped subjects volunteered for the study, and it shows that AS genotype is associated with increase in pain threshold and tolerance.

Pain perception is a complex process that is influenced by a variety of environmental and genetic factor (Diatchenko *et al.*, 2005), and pain in sickle cell disease (genotype SS) is associated with colder temperatures and touch, suggesting hypersensitivity to tactile stimuli (Brandow *et al.*, 2013). Research in pain genetic suggests that variability in gene loci contributes to individual experiences in pain sensitivity, and that even in multi-factorial pain syndromes, there is substantial heritability (Adegbola, 2011). Evidence suggests that some phenotypic features, such as eye or hair colour, predict pain. For example, findings indicated that dark pigmentation phenotype is more sensitive in response to cold pressor pain (Holmgaard *et al.*, 2017).

Result of the present study showed that cold pressor pain threshold shows no statistically significant variation by blood group, but cold pressor pain tolerance, on the other hand, was significantly lower among the O^- group subjects when compared to O^+ and A^+ groups. A study that used the cold pressor test reported no significance in pain threshold and pain tolerance among the A, B, AB or O blood group and between Rh positives and negatives of any of the groups (Shankar *et al.*, 2011).

Participants with O^+ blood group and those with A^+ had significantly higher ischaemic pain threshold than those with AB^+ group. Result of ischaemic pain tolerance showed that subjects with O^- blood group had significantly lower tolerance compared to all the other groups. For the pressure pain response, participants belonging to the AB^+ blood

group had the highest pressure pain threshold, while those in the A^+ group had the lowest, though the mean differences did not significantly vary between all the groups. A study by Simoni *et al.* (2017) reported that participants with B blood group displayed the lowest mechanical pain sensitivity, while participants with blood type AB exhibited the strongest conditioned pain modulation effect.

Studies have demonstrated genetic influences as important aetiological contributor towards clinical pain states, as blood groups may be differentially associated with various diseases. A significant disease associations described for non-O (subjects of group A, B. or AB) versus O subjects is susceptibility to arterial and venous thromboembolism. Evidence also supports that blood group O provides a selective advantage against severe malaria, and is associated with peptic ulceration. Blood group A has also been associated with gastric cancer (Shankar *et al.*, 2011).

Blood groups have become targets in epidemiological investigations as they are well known genetically determined traits with clear polymorphic expression in populations. The ABO antibodies can also be considered as part of the innate immune system against some bacterial pathogens and enveloped viruses that carry ABO-active antigens. Several genetic, developmental, and clinical conditions can affect ABO blood typing, with implications for epidemiology studies (Cooling, 2015).

Result of pain catastrophizing in the present study showed that median difference in pain catastrophizing by sex is statistically significant, with male participants having less pain catastrophizing score than females (i.e. females catastrophize more than males). Age and ethnicity, on the other hand, did not significantly influence pain

catastrophizing outcomes. Pain catastrophizing, in this study, was determined using the visual analogue scale (VAS), which is a widely used evaluation tool in the scientific and clinical universe (Silva *et al.,* 2016). The findings of the present study are in agreement with those of Edwards *et al.* (2004) and Umar *et al.* (2017) that also reported higher pain catastrophizing score in females than in males. Study indicated that sex differences in complaints of painful day-to-day symptoms are accounted for by the significant differences between men and women in reports of catastrophizing (Edwards *et al.,* 2004). Pain catastrophization was found to have a positive correlation with postoperative pain. It has been suggested that thought intrusion may be interpreted as signals of coping failure, thereby increasing the threat value of pain stimulus, nevertheless, catastrophizers may have come to expect that their pain experience will be high regardless of variations in thought intrusions (Ip *et al.,* 2009). It was found that pain catastrophizing was significantly associated with cold pressor pain threshold outcomes (Trost *et al.,* 2015), as also evident in the present study. High levels of catastrophizing are associated with heightened pain intensity, psychological distress, and disability (Trost *et al.,* 2015). Thorn *et al.* (2004) suggested that sex difference in pain catastrophizing is partially accounted for by the dispositional tendency to describe oneself as emotionally vulnerable. Pain catastrophizing may be situational as well as dispositional (sex, age, race). Women were reported to catastrophize more than men in response to osteoarthritis pain (Keefe *et al.,* 2000), and it has been suggested that female may possess a lower diffuse noxious inhibitory control neural circuitry which predisposes them to pain catastrophizing (Leung, 2012).

The result of the present study showed no significant effect of age on pain catastrophizing, which is in agreement with Lynch *et al.* (2007) that found no sex or

age differences in pain catastrophizing, but disagree with Ruscheweyh *et al.* (2011) that reported that pain catastrophizing change during life span, with a higher engagement of emotional versus sensory pain processing in younger compared with older adults. Significant associations has been found between catastrophizing and both pain intensity and psychological adjustment to samples of youths with chronic pain and disabilities (Engel *et al.*, 2012).

Lack of significant ethnic differences in pain catastrophizing in the present study may be due to close genetic association of the studied population, as pain catastrophizing has been associated with specific genotypes. Ethnic differences in pain catastrophizing has been demonstrated in a study by Hsieh *et al.* (2010), where *t*-test analysis revealed that Chinese subjects reported greater catastrophizing than European Canadian. Catechol-o-methyltransferase (COMT) diplotype (high versus low activity) has been shown to modulate pain ratings and pain catastrophizing in shoulder pain, where low COMT activity is associated with higher pain ratings and higher tendency for pain catastrophizing. Val(158)met single nucleotide polymorphism in the COMT gene has also been reported to mediate pain catastrophizing and maladaptive pain coping in fibromyalgia. Higher level of pain catastrophizing has been associated with enhanced serum interleukin-6 (IL-6) activity (Leung, 2012).

Pain tolerance and supra-threshold ratings of pain unpleasantness reflect affective-motivational aspects of pain, whereas pain threshold and supra-threshold ratings of pain intensity reflect the sensory-discriminative aspects of pain. Sensory-discriminative aspects of pain are those that describe the location, intensity, and duration of painful stimuli, while affective-motivational aspects of pain describe how pain is qualitatively

experienced. Thus, ethnic differences in pain responses may be most apparent for the affective-motivational dimension of pain because it is more influenced by psychosocial factors than the sensory-discriminative aspects of pain (Hsieh *et al.,* 2010).

Another compounding factor for lack of significant effect of ethnicity on pain catastrophizing in the present study may be poor assessment measure, resulting from being asked to characterize pain using an unfamiliar descriptive context. People from different cultures conceptualize and describe pain using different cognitive frameworks. For example, when asked to rate their pain on a linear numeric scale, some Native American patients tended to choose a "favorite or sacred" number instead of the number that correctly indicated their level of pain (Narayan, 2010).

Result of sex differences in blood glutamate concentration in the present study showed that male subjects have significantly lower serum glutamate concentration compared to the female subjects. This result disagree with the finding of Saito *et al.* (2016) and Zlotnik *et al.* (2010), who reported that blood glutamate concentration was significantly higher in men than in women. Stover and Kempski, (2005) also reported a more sustained increase in blood glutamate in male than in female patients following isoflurane anaesthesia. The lower glutamate concentration may explain the observed higher experimental pain thresholds 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 (Osikowicz *et al.,* 2013). The positive and significant correlation recorded between serum glutamate and pressure pain threshold in females and not in males is in agreement with the observed lower pressure pain threshold and higher serum glutamate obtained in females than

males in the present study. 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 (Chiu *et al.,* 2009). GOT and GPT are two enzymes that are able to metabolize blood glutamate, thus, facilitating the lowering of extracellular levels of brain glutamate. Pitkanen *et al.* (2003) reported higher concentrations of essential amino acids and branched chain amino acids in men than in women, but women had higher aspartate, glycine, serine and taurine than men.

Another factor may be non-pregnant status of the female participants since pregnant women were excluded from the study. Pregnancy is associated with increase level of placental hormones, and blood glutamate level has been reported to be inversely correlated with levels of plasma oestrogen and progesterone, and it is suggested that oestrogen plays a significant role in the autoregulation of blood and brain glutamate levels. An increase in glutamate would increase the secretion of GnRH, which in turn would stimulate the secretion of oestrogen. This oestrogen then would down-regulate the elevated glutamate levels in the plasma, thus decreasing glutamate levels in the brain (Zlotnik *et al.,* 2010). Study has found that testosterone and oestradiol cause a decrease glutamate decarboxylase (GAD) mRNA expression in males, but increase in females (Bosma *et al.,* 2001).

Serum glutamate concentration in the present study was found to decrease with age, as participants in the 20 – 30 age group have the highest serum glutamate concentration, while those in the above 50 age group have the lowest, though the differences were not statistically significant. This agrees with a previous study by Koochekpour *et al.* (2012)

that reported no significant differences in serum glutamate levels among different age groups of normal subject. Pitkanen *et al.* (2003) reported a decrease with age of total blood protein, water and sodium. Another study by Saito *et al.* (2016) found a significantly higher level of glutamate in old than in young subject.

Result of the present study showed that mean serum glutamate concentration is highest among participants belonging to the other ethnic groups, and lowest among the Hausa ethnic group, though the differences were also not statistically significant. This is in agreement with the report of Song *et al.* (2014) that showed that differences in peripheral glutamate level in schizophrenia is independent of age, sex, medication, and ethnicity. Ethnic difference in serum glutamate concentration has been demonstrated by Koochekpour *et al.* (2012), with African-Americans having higher serum glutamate than Caucasian-Americans. South Asian and African Surinamese had been reported to have higher plasma amino acid (including glutamic acid) than the Dutch (van Valkengoed *et al.,* 2017). A previous study has shown that administration of amitriptyline (serotonin reuptake inhibitor) was followed by significant reduction of serum glutamate levels and numeric pain rating scale score in chronic tension type headache patients (Surbakti *et al.,* 2017a). Positive correlation between serum glutamate and headache intensity has also been reported (Surbakti *et al.,* 2017b). Patients with multiple sclerosis were also reported to have statistically significant elevated serum glutamate (Al Gawwam and Sharquie, 2017).

Glutamate is regarded as an important pain mediator in peripheral tissue, at the DRG and in the brain (Wozniak *et al.,* 2012), and low blood glutamate levels has been shown to strongly correlate with the brain's extracellular glutamate concentrations

(Zlotnik *et al.,* 2010). Glutamate, as the major excitatory neurotransmitter in the central nervous system, has been implicated in pain pathophysiology. It act via ionotropic receptors, (including NMDA, AMPA and kinate receptors) as well as metabotropic glutamate receptors (mGluR). Kainate receptors function as mediators and modulators of synaptic transmission and plasticity by regulating presynaptic neurotransmitter release and postsynaptic currents. 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 and to inhibit cortical spreading depression in the mammalian cortex (Andreou *et al.,* 2015).

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 is in agreement with some previous findings (Mann *et al.,* 1992; Hranilovic *et al.,* 2007; Hodge *et al.,* 2012). A study by Langeslag *et al.* (2012) reported no significant sex difference in serum serotonin concentration in women and men that were not in love, though there was a significantly higher serum serotonin level in women that where in love than in men that were in love. Nishizawa *et al.* (1997) reported higher brain serotonin concentrations in males, while Ortiz *et al.* (1988) and Weiss *et al.* (2005) reported significantly lower blood serotonin in males than in females, with female heritability also having larger additive component.

Result of the present study showed that serum serotonin concentration was positively correlated with cold pressor pain tolerance in males only. This may explain the higher cold pressor pain tolerance observed in males than females. This is in agreement

finding of Martin *et al.* (2017) that showed a significant reduction in threshold and tolerance level of heat thermode during acute tryptophan depletion (ATD) condition in healthy subjects, indicating positive correlation between serotonin level and heat pain. A study by Farhanchi *et al.* (2018) reported an inverse association between serum serotonin and postoperative pain. Research showing role of serotonin in pain processing has had conflicting outcomes and may depend on type of serotonin receptor that has been activated. Activation of 5-HT1A, 5-HT1B, 5-HT1D and 5-HT7 serotonin receptor subtypes is associated with anti-nociception, whilst activation of 5- HT2A and 5-HT3 is associated with pro-nociception (Ossipov *et al.,* 2014). Manipulation of serotonin level via ATD has been shown to improve pain tolerance in healthy volunteers, where as other findings reported no effect of ATD to outcome of cold pressor pain. Morphine-induced analgesia was also reported to be abolished by ATD, thus, indicating the complex involvement of serotonin in pain processing (Martin *et al.,* 2017). There have been mixed results reported from the use of selective serotonin reuptake inhibitors (SSRIs), which increase serotonin within the brain, as choice of pain trestment.

Serotonin synthesis is controlled by sex hormones. Testosterone and estrogen have been shown to be positively correlated with serum serotonin. Rate of serotonin synthesis is higher in normal males than in normal females (Farhanchi *et al.,* 2018). On the other hand, serotonin transporter protein may be reduced in synaptic junctions by the sex hormones and may result in enhancement of synaptic transmission. Thus, the pain perception can be expected to be diferent between genders and postmenopausal age (Farhanchi *et al.,* 2018). Serotonin, as a neurotransmitter, has been proposed to play various roles in pain modulation and signaling mechanisms in both peripheral and

central nervous systems. In the peripheral nervous system, serotonin and some inflammatory mediators such as prostaglandins, bradykinin, histamine, potassium ion, and substance P may be responsible for sensitizing the peripheral neurons to infammatory pain, while it may have various effects in stimulating or inhibiting pain signal transmission in the CNS (Farhanchi *et al.,* 2018).

The serotonergic (5-hydroxytryptamine, 5-HT) system is implicated in the control of numerous behavioral and physiological functions, and modulate mood, emotions, sleep, appetite, and cognitive functions. Impairment of the central serotonergic neurotransmission has been associated with the etiology of various psychiatric disorders, addictions, and altered behaviors such as extreme violence, hostility, aggression, suicidal behavior, and impulsivity. The 5-HT transporter (5-HTT) regulates the entire 5-HT system and its receptors via modulation of extra-cellular fluid 5-HT concentration (Noskova *et al.,* 2008). The serotonin transporter (5-HTT) terminates serotonergic neurotransmission by sodium-dependent uptake of serotonin into the presynaptic neuron (Kunugi *et al.,* 1997). Whole blood 5-HT levels have been reported to be higher in women compared to men, a difference that appears to be genetically determined. Men synthesize serotonin significantly faster than women. 5-HT functions to coordinate complex sensory and motor patterns during a variety of behavioural states and is implicated in the pathology of mood disorders, sleep and eating disorders, and schizophrenia. Sex differences in 5-HT function may underlie the known sex difference (women > men) in the prevalence of depression and may impact pharmacological treatments that target 5-HT neurotransmission (Cosgrove *et al.,* 2007).

Availability of diencephalon 5-HT transporter (which functions to regulate 5-HT signalling by modulating synaptic 5-HT levels, and is the principal target of many efficacious antidepressants) was found to be lower in depressed women in an age-dependent manner but not in depressed men (Staley *et al.,* 2006). Study demonstrated higher 5-HT transporter availability in females vs. males (Staley *et al.,* 2001).

The lack of significant difference in serum serotonin concentration in this study may be due to inability to consider the reproductive status of the studied population, as well as their exposure to exogenous steroids. Study by Wang *et al.* (2014) reported significantly higher serum serotonin concentration in post-menopausal women compared to pre-menopausal and young adult females, while Modder *et al.* (2010) reported significantly lower serum serotonin concentration in post-menopausal women not on hormone replacement therapy than in pre-menopausal women and post-menopausal women on hormone replacement therapy. Women using oral contraceptives were reported to have significantly higher blood serotonin than those on injectable contraceptives and control (Faryal *et al.,* 2016). Another study has shown that oestradiol-17, in its positive feed-back mode for stimulating gonadotrophin release, increases significantly the amount of mRNA for the 5-hydroxytryptamine 2A receptor (5-HT2AR) and the serotonin transporter (SERT) in the dorsal raphe nucleus (DRN) of acutely ovariectomized female rats, which was accompanied by a significant increase in the density of 5-HT2AR binding sites in cerebral cortex and other higher brain centres, and of SERT sites in the basolateral amygdala and lateral septum. Positron emission tomography scan has also shown that long-term oestradiol treatment increases the density of 5-HT2AR in the forebrain of women (Sumner *et al.,* 2007).

Associations between blood serotonin and anthropometric measures differed among males (significant negative correlations with total body fat) and females (no significant correlations with body fat). Both lean mass (positive correlation) and fat mass (negative correlation) are significantly correlated with serotonin in males, but the correlations were not significant in females (Hodge *et al.,* 2012). Report shows a significant negative correlation between serum serotonin concentration with BMI and also with femur neck trabecular volumetric bone mineral density, while there is a positive correlation between serum serotonin and serum calcium and phosphorous in pre-menopausal women only (Modder *et al.,* 2010). Diastolic blood pressure was reported to correlate significantly with blood 5HT in older individuals (50–65 years) (Ortiz *et al.,* 1988).

Age was found not to significantly affect serum serotonin concentration in the present study, though it was found to be lowest among the youngest subjects (20 – 30 age group), and highest among subjects in the 41 – 50 age group. This is in agreement with some previous studies (Morgan *et al.,* 1987; Mann *et al.,* 1992; Hranilovic *et al.,* 2007; Taborskaya *et al.,* 2016), but in disagreement with the study of Kumar *et al.,* (1998) that reported that concentration of 5-HT in platelets was significantly higher in older women (≥ 61 years) than in younger women (< 60 years), while concentration of 5-HT in plasma of older women was significantly lower than that of the younger women. Serotonin was also reported to show a decreased binding capacity with age in frontal cortex and hippocampus (Marcusson *et al.,* 1984). In a study that investigated the effect of ageing and reduction in ovarian function on blood serotonin level, it was observed that amenorrbeic, natural postmenopausal and ovariectomised women had lower blood serotonin levels than regularly menstruating women, but the blood

serotonin level increase after oestrogen replacement in postmenopausal women to values similar to those observed in regularly menstruating women. Blood serotonin level was inversely correlated with age in ovariectomised women, and a direct relationship was also observed between serum oestradiol level and blood serotonin level (Gonzales and Carrillo, 1993). Tolstenkov *et al.* (2017) reported a decline in serotonin level with age, while Jonassen *et al.* (2012) reported no statistically significant differences between the 5-HT transporter gene-linked polymorphic region (5-HTTLPR) genotypes (the polymorphic/promoter region in the serotonin transporter gene SLC6A4) with age. Neuronal level of serotonin was reported to decrease with age (Yin *et al.,* 2014). Central serotonin transporter (SERT) availability was reported to show a significant inverse correlation with age in living human subjects, declining by 29.5% over the age range 18 to 88 years (van Dyck *et al.,* 2000).

The effect of age on serotonin level may be associated with age related changes in feeding frequency and type of food, which is not common in the studied population. Feeding a diet restricted in the amino acid tryptophan (the precursor of serotonin) from weaning to two years of age markedly reduces serotonin levels in all brain regions in rats, while returning the animals to a normal diet produces a moderate recovery of the brain serotonin level (Timiras *et al.,* 1984).

Mean serum serotonin concentration was found to be highest among the participants belonging to others ethnic groups, and lowest among the Yoruba ethnic group, but the difference was also not statistically significant. Significant ethnic differences in genotype and allele frequencies of the 5-HTTLPR has been demonstrated in healthy individuals from four European populations living in Croatia and the Russian

Federation, including Croats, Bashkirs, Tatars and Russians (Noskova *et al.,* 2008), between Caucasian and Japanese populations (Kunugi *et al.,* 1997), between African-Americans and European-Americans (Odgerel *et al.,* 2013), as well as between Russians and Tuvinians (from Kyzyl, The Republic of Tuva) (Savostyanov *et al.,* 2015).

The high level of uncertainty in serotonin result may be due to the fact that serotonin circulates in very low concentrations in the plasma, with most of it present in platelets (Lee *et al.,* 2014), though Mann *et al*. (1992) reported no significant difference in blood and platelet serotonin concentrations. Another reason may be differences in food intake, homogeneity of the participants, or the season. Though frequency of food intake has been shown to be un-associated with serotonin concentrations, there are reports of seasonal 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 (Mann *et al.,* 1992).

Data from the present study show that serum electrolytes concentrations did not statistically vary by sex, though the male subjects were found to have lower serum sodium, potassium and chloride concentrations than the females. Result of age differences showed that serum sodium concentration is significantly higher among subjects in the younger age group of 21 – 30 years than those in the 41 – 50 years group and those in the above 50 years group. Difference in serum potassium and chloride concentrations, on the other hand, showed no statistically significant variation by age in the present study. 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. A previous study by Levine *et al.* (1961) reported no significant sex or age differences in serum sodium and potassium 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).

Biologically, sodium and potassium play a significant role in maintenance of homeostasis by participating in various physiological activities such as neuromuscular irritability, nerve conduction, inhibition of free radical formation, etc. Altered levels of these elements may induce series of abnormal events such as slow movement, postural abnormality, impaired balance, membrane damage and peripheral vascular resistance (Dutta *et al.,* 2015). 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. The Kv4.2 potassium channel modulates pain plasticity and related inflammation in spinal cord dorsal horn neurons. The Kv7 (KCNQ or M channels) family is widely expressed in the nervous system, including nociceptors, and M channel blocker injection in the hind paw of rats induced moderate pain, while injection of M channel enhancer was also reported to produce an analgesic effect. Reduced Kv7 function was also involved in inflammatory pain. Diclofenac which is popularly used to treat pain and inflammation was demonstrated to activate Kv7.2/Kv7.3 channels (Kianifard and Chopra, 2018).

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 a 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 the elderly subjects have higher blood pressure, 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 elderly 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 lack of significant difference in serum potassium may be due to the strict exclusion criteria used in the study, as subjects on any form of medication were excluded in the analysis. The elderly are commonly known to be prescribed many medications that interfere with urinary excretion of electrolytes/potassium (Schlanger *et al.,* 2010). Significantly raised plasma sodium concentrations in hypertensive blacks than in Caucasians has been observed, and suggested to be due to increase in a sodium transport inhibitor, a depressed sodium efflux rate constant as well as a reduction in the activity of oubain-sensitive component of the Na/K ATPase pump (Iyalomhe *et al.*, 2008).

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. A study by Kim *et al.* (2017b) 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). 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.

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, such as 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. Reduced glutathione is 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 represents 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, as GSH is synthesized from glutamate, cysteine, and glycine, catalyzed sequentially by two cytosolic enzymes, γ-glutamylcysteine synthetase and GSH synthetase. Glutathione is the predominant low-molecular-weight thiol in animal cells, mostly present in the cytosol. With the exception of bile acid, which may contain up to 10 mmol/L, extracellular concentrations of GSH are relatively low (e.g., 2–20 mol/L in plasma).

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 (Marcourakis *et al.*, 2008) 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. Lipoperoxidation 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 agens. Reactive oxygen species play an important role in allodynia and hyperalgesia mainly through central sensitization, as well as in the pathogenesis of rheumatoid arthritis (Sczallasi *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).

5.2 Limitations of the Study

i. Though the same examiner performed the experimental pain test throughout the study, inability to assess experimenter characteristics such as ethnicity and sex in this study may have contributed to the observed results, as influence of experimenter on the present pain outcome cannot be ruled out.

ii. Inability to assess psychological status, and lack of data on psychiatric diagnoses were also limitations in this study, as there was a possibility that psychological and psychiatric status may influence inter-individual variability in pain response.

iii. All the participants were recruited from a homogenous urban population, thus, limiting the degree to which these findings can be applied to the general population.

iv. Inability to assess menstrual and pregnancy status, as well as contraceptive use by the female participants.

v. Another limitation is the lack of data on dietary habit as well as herbal medicine utilization.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Result of this study showed that sex did not significantly influence the outcome of cold pressor pain responses, though the male volunteers had slightly higher values than the females. Pressure pain, on the other hand, was found to have a significant variation by sex, with the male participants having a higher pressure pain threshold than the females. Ischaemic pain threshold was significantly higher in males than in females, while ischaemic pain tolerance was found to be statistically similar.

Age differences in perception cold pressor pain showed no statistical variation among the studied population. Effect of age on pressure pain perception showed a somewhat age dependent decrease, though the difference does not statistically vary between all the age groups studied. The oldest age group (above 50 years) were found to have significantly higher ischaemic pain threshold when compared to the 20 to 30 and 41 – 50 age groups. Ischaemic pain tolerance, on the other hand, showed no statistically significant age variation among the studied participants.

Ethnicity was found to have no statistically significant effect on the threshold and tolerance to cold pressor pain in this study, but pressure pain threshold was found to be significantly higher among the Yorubas when compared to the Hausas. Ethnic differences on ischaemic pain threshold showed no statistically significant variation among the studied subjects, but ischaemic pain tolerance showed statistically significant variation, with the Fulani tribal group having higher tolerance than participants classified as Others ethnic group.

When effect of BMI on cold pressor pain test was analyzed among the participants, obese subjects were found to have the highest pain threshold, while underweight subjects had lowest, though the differences were not statistically significant across all the BMI groups. Cold pressor pain tolerance was also found not to be significantly influenced by BMI. The BMI was also found to not significantly affect the outcome of pressure pain test in this study. Effect of BMI on ischaemic pain threshold was found to be statistically similar among the studied groups, but result of ischaemic pain tolerance showed that subjects with normal BMI had a statistically significant higher ischaemic pain tolerance than their obese counterparts.

A highly statistically significant difference was observed on the effect of handedness on cold pressor pain threshold, with right handed participants having a higher threshold than their left handed counterparts. Effect of handedness on cold pressor pain tolerance, on the other hand, does not show statistically significant variation. Differences on pressure pain threshold between right and left handed participants showed no statistically significant variation among the studied population. Responses of the participants to ischaemic pain test also showed no significant effect of handedness.

Haemoglobin genotype was observed to significantly influence the outcome of cold pressor pain tolerance in this study, but not the cold pressor threshold. Subjects with the AA genotype were found to have significantly lower cold pressor pain tolerance than their AS counterparts. The AA genotyped subjects also have a significantly lower ischaemic pain threshold than the AS ones. Ischaemic pain tolerance, however, does

not show statistically significant variation by Hb genotype. Effect of Hb genotype on pressure pain threshold does not also show statistically significant variation between the groups.

When data was analyzed with blood group as a factor, mean cold pressor pain threshold showed no significant variation, but the cold pressor pain tolerance was significantly lower among the O^- group subjects when compared to O^+ and A^+ groups. Subjects with O^+ blood group and those with A^+ had significantly higher ischaemic pain threshold than those with AB^+ group, but there was no statistically significant variation in ischaemic pain tolerance. For the pressure pain response, participants belonging to the AB^+ blood group had the highest pressure pain threshold, while those in the A^+ group had the lowest, though the mean differences was not also statistically significant.

Result of pain catastrophizing shows that median difference in pain catastrophizing by sex is statistically significant, with male subjects having less pain catastrophizing score than the females. Age and ethnicity do not significantly affect pain catastrophizing score in this study. Male subjects were found to have significantly lower serum glutamate concentration than females, but serum serotonin concentration showed no statistically significant sex variation. Serum glutamate and serotonin were found to have no statistically significant variation by age and ethnicity. Serum concentrations of sodium, potassium and chloride do not statistically vary by sex, but effect of age showed a statistically significant higher serum sodium concentration among participants in the 20 – 30 age group than those in the 40 – 50 and above 50 groups.

Serum sodium, but not potassium and chloride showed ethnic variation, with Hausa and Fulani having lower than the other groups.

6.2 Conclusion

Perception and response to experimentally induced pain, as well as pain catastrophizing among adult Nigerian subjects showed variation by sex, age and ethnicity, depending on the model used. Other factors such as BMI, blood group, Hb genotype and handedness were also found to influence the outcomes of some experimental pain models. Variations in serum concentrations of glutamate and serotonin were also investigated in this study, and were found to correlate with some of the experimental pain outcomes. Serum concentration of sodium, but not potassium and chloride, was also found to be influenced by sex, age and ethnicity in this study. Serum concentrations of MDA, GSH and SOD did not statistically vary among the studied population.

6.3 Recommendations

Based on the findings of this study, the following recommendations were made:

i. Clinicians should explore the possibility of individualised pain therapy based on sex, age and ethnicity.
ii. It is essential to use combination of experimental pain modalities when studying inter-individual variability in human pain responses.
iii. Experimental pain models can be used in clinical settings to deduce possible clinical pain outcome
iv. Other studies can extrapolate serum glutamate concentration from pressure pain threshold using the formula:

$y = 0.0147x + 6.4135$

y = pressure pain threshold and

x = serum glutamate concentration

v. The BMI, Hb genotype, blood group and handedness of patients should be put into consideration during pain treatments.

vi. Further studies should be carried out using other experimental pain models, as well as more chemical and genetic analysis, to ascertain the possible mechanism of inter-individual variability in pain outcomes.

6.4 Contributions to Knowledge

Despite encountered challenges and limitations, this study has been able to make the following contributions to knowledge:

i. Cold pressor pain threshold and tolerance are statistically similar between males and females among the studied population [$t(157) = 0.11$; $p = 0.93$]. Pressure pain threshold is significantly higher in males than females [$t(159) = 4.09$; $p = 0.00$]. Ischaemic pain threshold is also significantly higher in males than females [$t(159) = 3.67$; $p = 0.00$].

ii. There is no statistically significant effect of age on cold pressor pain threshold [$F(3,153) = 1.93$; $p = 0.13$] and tolerance [$F(3,153) = 1.22$; $p = 0.31$]. Pressure pain threshold is significantly lower among participants in the 41 – 50 age group [$F(3,155) = 2.22$; $p = 0.04$]. Members that are above 50 years have significantly higher ischaemic pain threshold than those of 20 – 30 years ($p = 0.03$) and 41 – 50 years ($p = 0.02$).

iii. Ethnicity does not affect the outcome of cold pressor pain threshold ($p = 0.34$) and tolerance ($p = 0.41$) among the studied population. Members of the Yoruba ethnic group have higher pressure pain threshold than the Hausas [$F(3,154) =$

4.14; p = 0.01]. Ischaemic pain threshold show no ethnic variation [F(3,154) = 0.86; p = 0.46], but ischaemic pain tolerance is higher among the Fulanis than the Others [F(3,154) =2.39; p =0.03].

iv. Body mass index (BMI) has no effect on cold pressor pain threshold [F(3,155)=0.51; p=0.68], but overweight subjects have significantly higher cold pressor pain tolerance than underweight and obese subjects [F(3,155)=3.69; p=0.03]. Pressure pain threshold is also not influenced by BMI [F(3,157)=0.38; p=0.77], but ischaemic pain tolerance in higher in subjects with normal BMI than those that are obese [F(3,157) = 1.77; p = 0.01].

v. Right handed individuals have a higher cold pressor pain threshold than left handed ones [t(108)=3.63; p=0.00], but handedness does not have effect on cold pressor pain tolerance [t(108)=1.42; p=0.17] and pressure pain threshold [t(108)=-1.28; p=0.24].

vi. Subjects with AA Hb genotype have lower cold pressor pain tolerance [t(156)=-1.31; p=0.03] and ischaemic pain threshold [t(156) =-0.78; p = 0.04] than those with AS, but ischaemic pain tolerance [t(156) =1.89; p = 0.06] and pressure pain threshold [t(156)=-1.72; p=0.09] are not affected by Hb genotype.

vii. Subjects with O^- blood group have lower cold pressor pain tolerance than those with O^+ [F(4,150) = 2.59; p = 0.03] and A^+ [F(4,150) = 2.59; p = 0.02]. Ischaemic pain threshold is lower in AB+ subjects than in O^+ (p = 0.03) and A^+ (p = 0.04).

viii. Pain catastrophizing using the visual analogue scale was lower in males than in females (p = 0.04), but age and ethnicity have no effect on outcome of the visual analogue pain catastrophizing scale.

ix. Male subjects have lower serum glutamate concentration than females (p = 0.03), but serum serotonin concentration have no sex variation (p = 0.30). Age and ethnicity do not influence serum glutamate (p = 0.13; p = 0.10) and serotonin (p = 0.57; p = 0.19) concentrations.

x. Serum glutamate concentration have a strong positive correlation with pressure pain threshold (R^2 = 0.0549; p = 0.03).

xi. Serum glutamate concentration can be extrapolated from pressure pain threshold using the relationship: y = 0.0147x + 6.4135, where

y = pressure pain threshold and

x = serum glutamate

xii. Serum concentration of sodium [t(155)=-0.63; p=0.53], potassium [t(155)=-1.66; p = 0.09] and chloride [t(155)=-0.38; p = 0.71] are not influenced by sex, but serum sodium concentration is higher among younger subjects (20 – 30 years) than older subjects (41 – 50 years, p = 0.00; above 50 years, p = 0.02). Hausas and Fulanis have lower serum sodium concentration than the Yorubas and others.

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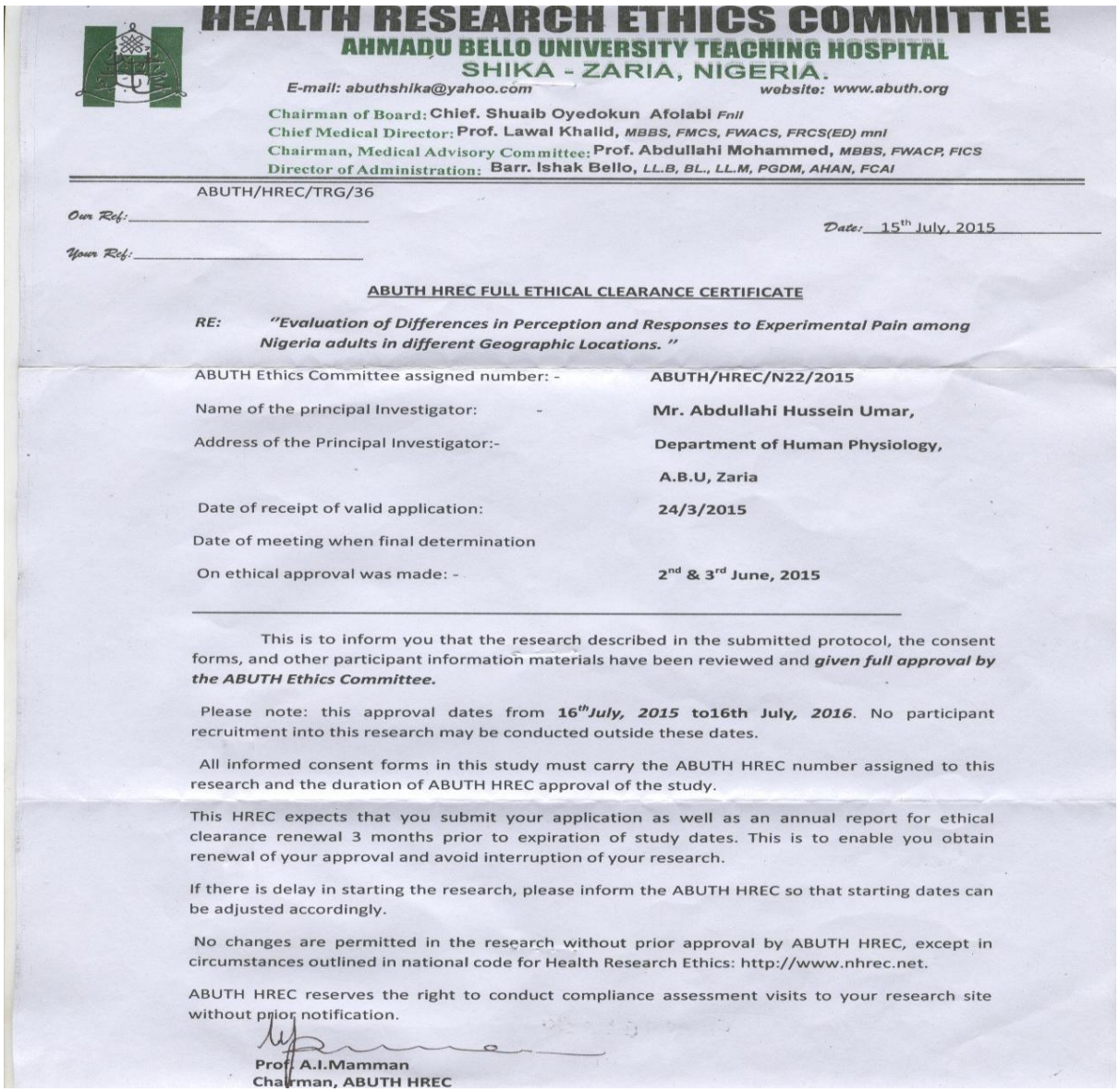
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APPENDICES

Appendix I

Copy of ethical approval obtained from the Ahmadu Bello University Teaching Hospital Health Research Ethics committee



HEALTH RESEARCH ETHICS COMMITTEE

AHMADU BELLO UNIVERSITY TEACHING HOSPITAL

SHIKA - ZARIA, NIGERIA.

E-mail: abuthshika@yahoo.com website: www.abuth.org

Chairman of Board: Chief. Shuaib Oyedokun Afolabi *Fnil*
Chief Medical Director: Prof. Lawal Khalid, *MBBS, FMCS, FWACS, FRCS(ED) mnl*
Chairman, Medical Advisory Committee: Prof. Abdullahi Mohammed, *MBBS, FWACP, FICS*
Director of Administration: Barr. Ishak Bello, *LL.B, BL., LL.M, PGDM, AHAN, FCAI*

ABUTH/HREC/TRG/36

Our Ref:

Your Ref:

Date: 15th July, 2015

ABUTH HREC FULL ETHICAL CLEARANCE CERTIFICATE

RE: ***"Evaluation of Differences in Perception and Responses to Experimental Pain among Nigeria adults in different Geographic Locations."***

ABUTH Ethics Committee assigned number: -	**ABUTH/HREC/N22/2015**
Name of the principal Investigator: -	**Mr. Abdullahi Hussein Umar,**
Address of the Principal Investigator:-	**Department of Human Physiology,**
	A.B.U, Zaria
Date of receipt of valid application:	**24/3/2015**
Date of meeting when final determination	
On ethical approval was made: -	**2nd & 3rd June, 2015**

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and ***given full approval by the ABUTH Ethics Committee.***

Please note: this approval dates from **16th *July, 2015* to16th July, *2016***. No participant recruitment into this research may be conducted outside these dates.

All informed consent forms in this study must carry the ABUTH HREC number assigned to this research and the duration of ABUTH HREC approval of the study.

This HREC expects that you submit your application as well as an annual report for ethical clearance renewal 3 months prior to expiration of study dates. This is to enable you obtain renewal of your approval and avoid interruption of your research.

If there is delay in starting the research, please inform the ABUTH HREC so that starting dates can be adjusted accordingly.

No changes are permitted in the research without prior approval by ABUTH HREC, except in circumstances outlined in national code for Health Research Ethics: http://www.nhrec.net.

ABUTH HREC reserves the right to conduct compliance assessment visits to your research site without prior notification.

Prof. A.I.Mamman
Chairman, ABUTH HREC

Appendix II

Copy of subject's consent form

INFLUENCE OF AGE, SEX AND ETHNICITY ON PERCEPTION AND RESPONSE TO EXPERIMENTALLY INDUCED PAIN IN AN ADULT POPULATION OF ZARIA, NIGERIA

Subject consent form

I …….. have read the information provided here in and it has been clearly explained to my satisfaction. I voluntarily accept to participate in this study and understand that I have the right to withdraw from the study at any time, without compromising the quality of the research.

( ) Yes ( ) No

…… ……

Signature/thumb print of respondent Date

……

Name of subject's legal guardian

…… …….

Signature/thumb print of subject's legal guardian Date

Address:……

……

…….

GSM/Telephone Number……..

Appendix III

Copy of self administered questionnaire used during the study

INFLUENCE OF AGE, SEX AND ETHNICITY ON PERCEPTION AND RESPONSE TO EXPERIMENTALLY INDUCED PAIN IN AN ADULT POPULATION OF ZARIA, NIGERIA

QUESTIONNAIRE

1. Age: ( ) 20 to 30 ( ) 31 to 40 ( ) 41 to 50 ( ) > 50
2. sex: ( ) male ( ) female
3. State of origin:…….
4. LGA:……
5. Tribe:……
6. Place of residence:……
7. Marital status: ( ) single ( ) married ( ) divorced ( ) widowed
8. Religion:……..
9. Education: ( ) No ( ) Primary ( ) Secondary ( ) Tertiary
10. Occupation:…….
11. Skin colour:…….
12. Handedness: ( ) Left handed ( ) Right handed
13. Physical disability:……
14. Average monthly income: ( ) low ( ) average ( ) high
15. Genotype:…….
16. Blood group:…….
17. Weight (Kg):…….

18. Height (m):……

19. Cigarette smoking: ( ) Yes ( ) No. If yes, for how long?.....

20. Alcohol drinking: ( ) Yes ( ) No. If yes, for how long?.....

21. Daily Exercise: ( ) No ( ) mild ( ) moderate ( ) severe

22. Are you engaged in any sport activity? ( ) Yes ( ) No.

23. If yes (21), kind of sport:…….

24. How often do you travel out of your place of residence:

    ( ) Never

    ( ) Very often (at least once in a year)

    ( ) Often (at least once in three years)

    ( ) Less often (once in five years)

25. Have you travel out of your place of residence in the last five years? () Yes( ) No

26. If yes (24), please state places visited:.....……

27. Are you suffering from any of the following medical conditions?

    a. Hypertension

    b. Diabetes

    c. Arthritis (joint pain)

    d. Liver disease

    e. Kidney disease

    f. Others (specify)……..

28. Are you currently (or in the last four weeks) under any medication? Yes ( ) No ( )

29. If yes (27), please state drug(s) being taken …….....

.....