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HEMOGLOBIN GENOTYPE HAS NO INFLUENCE ON PAIN PERCEPTION OF UNDERGRADUATE STUDENTS IN ILORIN, NORTH-CENTRAL NIGERIA

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Abstract
Pain perception is influenced by various factors, including genetics, awareness, age, gender, and environmental factors (Hussain and Karim, 2019). This study aimed to determine if hemoglobin genotype variability affects human perception of pain. 208 participants aged 17-45 years from the University of Ilorin were recruited using purposive sampling. The participants were counselled and verbal consent was gotten from them before the procedure. Pain threshold and tolerance were assessed using the cold pressor test. 5mls of blood was drawn from antecubital vein and stored in Ethylenediamine tetraacetic acid (EDTA) bottles at 2º-6ºC until hemoglobin electrophoresis was carried out. Data were analyzed using SPSS version 28.0 and presented as mean ± SD, median IQR, frequency as well as percentages and p-value < 0.05 was considered statistically significant. Sociodemographic results showed that 2.4% of participants were older than 35 years, 97.6% are unmarried and 85.6% are Yoruba. Hemoglobin electrophoresis revealed that 64% of participants were HbAA, 28.8% HbAS, 4.8% HbSS, and 2.4% HbAC. No significant variation was found in pain threshold between genotypes, though participants with the HbAS genotype had the highest pain threshold and the lowest pain tolerance, this warrants further investigation in larger sample. Higher pain threshold and tolerance may be conferred by HbS and HbC characteristics.

Keywords: Hemoglobin Genotype; Pain Perception; Pain Threshold; Pain Tolerance.

Introduction

Pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage IASP (2020). Mild forms of pain could be ignored in humans, but moderate to severe pain usually affects the functionality of affected individuals. Globally, pain is one of the most common indications of hospital consultations, and it occurs as a result of the complex interplay of multiple factors with resultant discomfort and negative impact on well-being (Afolayan, 2015; James, 2013). The determinant of pain threshold and perception in humans could be linked to race, gender, ethnicity, as well as social interpretation of painful experiences, making research into this topic a challenging one (Young *et al*., 2012; James, 2013). Worldwide, it is estimated that 15 - 50% of people are confronted with pain at any given time with significant loss of productivity, increased healthcare utilization and hospital admissions (Cordell *et al*., 2002; Young *et al*., 2012).

Despite numerous studies to define the effects of environmental factors on pain sensitivity and perception, the role of these factors in the explanation of the pathophysiology remains minimal (Belfer, 2013). Increasing evidence suggests that genetic factors contribute significantly to individual differences in responses to both clinical and experimental pain. Twin studies have demonstrated that genetic influences play a significant etiological role in clinical pain states (Shankar *et al*., 2011; Norbury *et al*., 2015). However, Fillingim, (2008) reported that inter-individual variability in pain experience is mediated by interactions among numerous biopsychosocial factors, not solely genetic influences. Dispositional characteristics such as gender, race/ethnicity, personality, and age have been associated with pain responses, as have situational variables like mood states, stress, and transient biological factors (Green *et al*., 2004).

The hemoglobin genotype is also an important genetic variance in humans, as the differences across the classes (AA, AS, AC, SC, SS, and CC) are seen in some of their clinical characteristics. It is estimated that about 5% of the world's population have mutations in hemoglobin genes resulting in sickle cell trait (heterozygous) and anemia (homozygous). The hemoglobin S results from the replacement of glutamic acid by valine at position 6 of the beta chain of the hemoglobin molecule while hemoglobin C is due to the glutamic acid being replaced by lysine (Tossea *et al*., 2018).

Pain is a central concern in sickle cell disease (SCD), challenging both patients and clinicians due to its acute and chronic forms, particularly vaso-occlusive crises (VOC), the most common pain type in SCD (Sagi *et al*., 2021). VOC episodes, often recurrent and unpredictable, drive frequent emergency visits and reduce life expectancy. Besides general pain from causes like trauma or inflammation, individuals with SCD endure a distinct baseline pain, with 30% of adults reporting daily chronic pain independent of acute VOC (Sagi *et al*., 2021). The mechanisms behind chronic pain in SCD are complex, involving sensory hyperexcitability, systemic inflammation marked by interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF), and chemokine ligand-2 (CCL-2), along with neuroinflammation in the central and peripheral nervous systems (Gupta *et al*., 2018). Both animal and human studies reveal SCD-associated hyperalgesia and allodynia (Jacob *et al*., 2015).

While there is an extensive body of literature exploring pain experiences and management among sickle cell disease (SCD) patients (Smith *et al*., 2008; Ballas *et al*., 2012), research specifically examining pain threshold and tolerance across various hemoglobin genotypes remains limited. The majority of SCD studies tend to focus on the frequency, intensity, and management of acute and chronic pain crises associated with hemoglobin S (HbS), often overlooking the potential variability in pain perception across other genotypes (da Guarda *et al*., 2020). Only a few studies have investigated the comparative pain thresholds and tolerances in individuals with different hemoglobin variants, such as HbA, HbS, and HbC, which could provide insights into the underlying genetic and physiological mechanisms of pain beyond SCD contexts (Sagi *et al.*, 2021; Dampier *et al*., 2019).

This gap in the literature underscores a need for broader research that investigates whether specific genotypes confer differences in pain sensitivity or tolerance, which would not only deepen understanding of the genetic influences on pain, but also refine pain management strategies across patient populations (Kirkham *et al*., 2023). Our study addresses this gap by comparing pain thresholds and tolerance across hemoglobin genotypes, contributing to a foundational knowledge base that may inform future genetic and clinical investigations. This approach is intended to foster a more nuanced, genotype-informed perspective on pain management, challenging traditional assumptions about SCD patients' pain experiences and providing a framework for future research on genetic markers influencing pain perception.

Despite varying levels of sensitivity to pain among different genotypes, there is a major challenge of subjectivity, and pain is usually described as subjective in nature, therefore, the lack of ideal measurement of pain in humans has led to some studies using different methods of assessment with no one standing out (Stewart *et al*., 2021). The assessment of pain threshold and tolerance using a cold pressor test is a simple, affordable, and yet readily available method of comparing pain perception in the different hemoglobin genotype groups, especially in low-resource settings like ours. This study is aimed at assessing the pain threshold and tolerance amongst the different genotypes and determining whether the variability in blood genotype could affect the way humans perceive pain.

Methodology

Ethical Consideration

This study complied with ethical standards for human studies based on institutional and national guidelines. Ethical approval was obtained from the University of Ilorin Ethical Research Committee with approval number UERC/ASN/2019/1551, before the commencement of this study. Verbal informed consent was also obtained from each participant at recruitment into the study.

Study Area

This study was conducted at the College of Health Sciences, University of Ilorin, a tertiary educational institution located in the North-Central geo-political zone of Nigeria. The College of Health Sciences is made up of the following departments: Anatomy, Physiology, Medicine, Nursing, Physiotherapy, Radiography, and Medical Laboratory Sciences (MLS), with a total of 3,110 students.

Study Design

This was a cross-sectional study involving consenting students of the College of Health Sciences, University of Ilorin aged 17 – 45 years. Students who had surgery or major trauma in the last 3 months and those with ongoing vaso-occlusive crisis or had crisis in the last two weeks, chronic pain syndrome or any form of neuropathies, and recent use of opioid or opioid abuse were excluded from the study.

Sample size determination and sampling techniques

The minimum sample size for this study was calculated using Leslie Fischer's Formula (Araoye, 2004).

$$n = \frac{z^2pq}{d^2}$$

n = minimum sample size

z = standard normal deviation set at 1.96 for a 95% confidence interval

p = prevalence of SCD in Nigeria South – Eastern Community = 1.62% (Nwabuko *et al*., 2022)

q = 1 – p

d = desired level of precision (0.05)

Then, n = 208

To eliminate bias, the sample size was divided into proportions for each department in the College of Health Sciences, University of Ilorin based on the population of their students to give 18.3% (Anatomy), 17.4% (Physiology), 27.0% (Medicine), 24.4% (Nursing), 5.9% (Physiotherapy) and 7.0% (Medical Laboratory Sciences). Subsequently, 38, 36, 56, 51, 12, and 15 students were recruited from Anatomy, Physiology, Medicine, Nursing, Physiotherapy and Medical Laboratory Sciences respectively. Students who met the inclusion criteria were recruited consecutively using a purposive sampling technique.

Preparation of participants

After obtaining informed consent, each participant was allowed to sit comfortably on a cushioned chair in the testing facility at room temperature in a quiet environment. Instructions were explained to the participants clearly as pain threshold was defined as the point between "about to be painful" and "just became painful," while pain tolerance was defined as the point at which the subject can no longer withstand the pain. Data on socio-demographics, and medical and surgical history were obtained from the students. Interactions between study participants and investigators were limited to those necessary to collect data to avoid inconsistencies.

Sample collection and processing

Consenting students were counseled and had their blood samples collected for Hb electrophoresis. About 5mls of blood were withdrawn from the antecubital vein into the EDTA bottle and stored at a temperature of 2^0C to 6^0C in the physiology laboratory of the University of Ilorin until analysis. The blood samples were lysed with Tris-buffer solution (Hopax Fine Chemicals Ltd, Taiwan). The lysed blood was then placed in the cellulose acetate paper (Titan III$^@$ Cellulose Acetate), and then transferred into the electrophoretic tank (DY-300, Wincom Company Ltd., China). Hemoglobin genotypes were separated into different bands after 30-60 minutes at a voltage of 220v. The position, as well as the location of the bands, were then recorded and compared to the prepared control to confirm the genotype. The principle of electrophoresis was based on the movement of charged particles of hemoglobin in the alkaline buffer of pH between 8.2 – 8.6. Cellulose acetate paper was used in the study because it supports the rapid separation of the hemoglobin genotype with minimal preparation time.

Assessment of pain threshold and pain tolerance

Pain threshold and pain tolerance were assessed using the cold pressor test. The process of cold pressor test entailed the participants immersing their dominant hands in a bowl containing iced water (0^0C). The water was maintained at a constant temperature by repeatedly adding ice cubes. The subjects were encouraged to keep their hands in the water for as long as possible until the pain became intolerable when they could remove their hands. Pain threshold was determined to be the time when the subject started feeling pain, and notified the investigator by saying “in pain”, which is the point between “about to be painful” and “just became painful”. Pain tolerance was recorded as the entire duration of how long the participant could bear the pain. It is the point from when the participant dipped their hands into the water to when the hands were withdrawn from the water due to intolerable pain (Rahim-Williams *et al.*, 2007). The time taken for these to occur was recorded in seconds using a stopwatch. The process was repeated three times and the average time was recorded for analysis.

Data Analysis

Data analysis was performed using SPSS version 28.0 (IBM, Armonk, NY, USA), and data was presented in frequency and percentages. Descriptive statistics were presented in mean ± SD, median as well as IQR. One-way ANOVA was used to compare the difference of multiple means and p-value < 0.05 was considered statistically significant.

Results

The socio-demographic variable of participants

A total of 208 participants comprising 92 males and 116 females, were recruited for the study. The majority of the students were aged 17 – 25 years with only 2.4% older than 35 years. Also, the subjects were predominantly (97.6%) single and largely (85.6%) from the Yoruba tribe. Overall, 64.0% had AA genotype with SS accounting for 4.8% and AC and AS 2.4% and 28.8% respectively as shown in **Table I.**

Pain threshold and tolerance among various hemoglobin genotypes

Table II shows the pain threshold among various hemoglobin genotypes in all participants. There was no statistically significant difference in the pain threshold among different genotypes (F=1.109, p=0.347). Participants with the AS Genotype had the highest pain threshold (26.73 ± 46.70) while AA students had the least (18.44 ± 19.61).

Furthermore, there was no statistically significant difference in the pain tolerance among various hemoglobin genotypes (F=0.716, p=0.544), despite the AC and SS groups having the highest and lowest recorded tolerance respectively (774.20 ± 1582.08 and 207.00 ± 175.93). This is shown in **Table III.**

Table 1: Socio-demographic variables of participants

Variables	Frequency	Percentage (%)
Age		
17 – 25	162	77.9
26 – 30	29	13.9

31 – 35	12	5.8
36 – 45	5	2.4
Gender		
Male	92	44.2
Female	116	55.8
Religion		
Islam	118	56.7
Christianity	89	42.8
Traditional	1	0.5
Marital Status		
Single	203	97.6
Married	5	2.4
Ethnicity		
Yoruba	178	85.6
Hausa	2	1.0
Fulani	6	2.9
Igbo	10	4.8
Others	12	5.8
Hb Genotype		
AA	133	64.0
AC	5	2.4

AS	60	28.8
SS	10	4.8
SC	0	0

Table II: Pain threshold among the different hemoglobin genotypes

Genotype	**Pain threshold** **Median (IQR) (secs)**	**Mean ± SD (secs)**	**F**	***p*-value**
AA	14.00 (8.00 – 23.50)	18.44 ± 19.61	1.109	0.347
AC	15.00 (5.50 – 53.50)	26.60 ± 31.15		
AS	15.00 (10.00– 31.50)	26.73 ± 46.70		
SS	15.50 (10.50 – 29.25)	19.90 ± 12.99		

F: ANOVA (Analysis of Variance); SD: Standard deviation; IQR: Interquartile range

Table III: Pain tolerance among the different hemoglobin genotypes

Genotype	Pain tolerance Median (IQR) (secs)	Mean ± SD (secs)	F	*p*-value
AA	66.00 (31.00 – 482.50)	464.42 ± 804.65	0.716	0.544
AC	20.00 (16.00 – 1909.50)	774.20 ± 1582.08		
AS	130.00 (40.50 – 641.00)	541.38 ± 829.87		
SS	182.00 (123.50 – 235.50)	207.00 ± 175.93		

F: ANOVA (Analysis of Variance); SD: Standard deviation; IQR: Interquartile range

Discussion

Pain threshold and tolerance are dependent on myriad factors that vary for different genetic compositions in humans. Despite the established role of genetics in pain perception, our study found no significant correlation between hemoglobin genotypes and pain perception. We recruited 208 undergraduate students (116 females and 92 males) and determined their hemoglobin genotypes using hemoglobin electrophoresis. Pain threshold and tolerance were assessed using the cold pressor test, with the time recorded in seconds. Statistical analysis revealed no significant differences in pain perception (both threshold and tolerance) among the different hemoglobin genotypes.

The age range for this study was between 17 to 45 years and this is not surprising as this study was conducted among students of tertiary educational facility in North-Central Nigeria. The prevalence of SCD in the study was 4.8% (all SS, with no SC), and this was higher than the 1.62% recorded by Nwabuko *et al*. in South-Eastern Nigeria, further establishing the truth about regional variation in the prevalence of this disease (Nwabuko *et al*., 2015). In Nigeria, a prevalence of SCD of 1-3% has been reported with HB-SS being the predominant variants while HB-SC occurs sporadically, especially in the South-Western region of the country (Nwogoh *et al*., 2012, Nwabuko *et al*., 2015; Nubila *et al*., 2013).

The use of heat stimulus for pain threshold and tolerance assessment has generated concerns and interest as many SCD patients use heat as an analgesic method when in pain. In addition, many physicians have recommended heat therapy as a form of management for pain (Wethers *et al*., 2000). In alignment with previous studies, cold pressor test (CPT) was used in this study to assess pain tolerance and threshold. According to von Baeyer et al. (2005), CPT allows for greater control over the stimulus's location, intensity and duration. Cold temperature is a familiar stimulus that both young and old would likely encounter daily, and CPT uses this temperature making it easier for subjects to agree to the study and also co-operate. In addition, using the CPT frees the subjects from the influence of factors like anxiety and fatigue that may be present in clinical settings (von Baeyer *et al.,* 2005).

This study reported no significant ($p=0.347$) difference in pain threshold among different hemoglobin genotypes. Notably, HB-AA had a lower threshold (18.44 ± 19.61 secs) compared to HB-SS variant (19.90 ± 12.99 secs). This is in contrast to the findings by Brandow *et al*. (2013) in a cross-sectional study at Wisconsin Sickle Cell Centre where SCD patients had significantly lower heat and cold pain thresholds than the controls, these findings could be linked to altered central and peripheral neuronal mechanisms. However, the differences in the pain threshold from their study were observed on the tested hand, but not the foot where the pain perception was similar for various hemoglobin genotypes. The reason for the regional differences is not properly understood, hence, the need for further research in this area.

However, findings from this study were similar to the reports by O'leary *et al*., where they evaluated the pain threshold and sensory processing of 27 subjects, alongside 28 controls. They found no significant difference (p=0.58) in cold pain threshold among the study participants. Despite the similarity between our studies, O'Leary *et al*. conducted their study aged 10.3 – 18.3 years, and there were variations in their findings for different parts of the body, as well as for heat and cold pain thresholds (O'leary *et al*., 2014). Therefore, it is imperative that there is a need to further analyze the critical mechanisms of pain thresholds (heat and cold) on different parts of the body with the involvement of all age groups, because, Brandow *et al* had earlier reported the association between age and pain threshold between SCD patients and controls.

This study shows that there was no significant difference (p=0.54) in pain tolerance among the Hb variants, though the lowest pain tolerance was reported in the HbSS group (207.00 ± 175.93 seconds). This finding was similar to an American study where there was no reported difference in the pain threshold and tolerance of the participants (Khaleel *et al*., 2017). This further buttress the fact that there is no difference in pain tolerance of various Hb genotypes despite the variations in peripheral blood flow, chronic inflammation, and unpredictable neuronal functions (Khaleel *et al*., 2017). However, a conclusion could not be drawn from their study because, heat stimulus was used to assess pain tolerance in contrast to our study where we used the cold pressor test.

Interestingly, our result despite not being significant, showed a trend which suggests that the S and C carrier traits may confer higher pain threshold [AS (26.73 ± 46.70) < AC (26.60 ± 31.15) < SS (19.90 ± 12.99) < AA (18.44 ± 19.61)] and tolerance [AC (774.20 ± 1582.08) < AS (541.38 ± 829.87) < AA (464.42 ± 804.65) < SS (207.00 ± 175.93)] on the individuals. However, the disease state (SS) does not confer higher pain threshold and tolerance as seen in the lower values compared to other genotypes. Although, Simoni *et al*. (2017) reported that blood groups influence pain perception, up until the writing of this manuscript, our study is the first study to report this trend that blood genotype carrier traits S and C may influence pain perception. Further investigations are needed to confirm this result.

Conclusion

Our study concludes that while hemoglobin S and C traits may be associated with increased pain threshold and tolerance, this effect does not reach statistical significance. This finding challenges the common belief that individuals with sickle cell disease (SCD) generally exhibit lower pain thresholds and tolerance compared to those with other hemoglobin variants. It also aligns with broader research suggesting that, while genetic and biological factors do influence pain perception, hemoglobin genotype alone is not a primary determinant.

Recommendations

Consequently, we recommend that clinical practices consider this perspective to avoid unwarranted assumptions or stigmatization of SCD patients regarding pain tolerance, as they may experience pain similarly to individuals with other hemoglobin genotypes. Future studies could further investigate other genetic markers and their potential roles in pain perception, given the complex, multifactorial nature of pain experience.

Conflict of interests

The authors declare no competing interest.

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