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Establishing a Local Cut-Off for Hyperhomocysteinemia in a Northern Nigerian Population: Implications for Clinical Practice and Research

Ayoola Yekeen Ayodele[1,2*], Oyekunle Rilwan Adegboyega[2], Okolie Henry Ifeanyichukwu[2], Sani Adamu[3] and Adamu Adamu[2]

[1]*Department of Internal Medicine, Gombe state University, Gombe, Nigeria.*

[2]*Department of Internal Medicine (Cardiology Unit), Federal Teaching Hospital, Gombe, Nigeria.*

[3]*Department of Chemical Pathology, Federal Teaching Hospital, Gombe, Nigeria.*

***Correspondence:**
Dr. Ayoola Yekeen Ayodele, Department of Internal Medicine, Federal Teaching Hospital, Gombe, Nigeria.

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ABSTRACT

Background: *Hyperhomocysteinemia (HHCY) is an established cardiovascular risk factor, but its diagnostic threshold is primarily based on Caucasian reference values. Nutritional, genetic, and environmental factors vary across populations, potentially rendering these universal cut-offs inappropriate for specific regions like Northern Nigeria, where dietary B-vitamin deficiencies are prevalent.*

Objective: *To determine a locally derived cut-off value for hyperhomocysteinemia in an adult population in Gombe, North-Eastern Nigeria, and compare it with standard international values.*

Methods: In this cross-sectional analytical study, plasma total homocysteine (tHcy) was measured in 90 heathy volunteers, using a competitive enzyme-linked immunosorbent assay (ELISA). Controls were recruited from patient relatives and hospital staff, excluding individuals with cardiovascular disease or on relevant medications. The local 90th and 95th percentile values of the tHcy distribution in the subjects were calculated to establish the upper reference limits. The derived value was applied to a cohort of 90 heart failure patients to assess the prevalence of HHCY.

Results: *The mean plasma tHcy in the healthy control group was 10.24 ± 6.98 µmol/L. The 90th and 95th percentile values were 20.9 µmol/L and 25.1 µmol/L, respectively. Using the locally derived 90th percentile cut-off of 20.9 µmol/L, the prevalence of HHCY was 5.6. This cut-off is markedly higher than the commonly cited Caucasian threshold of 15 µmol/L.*

Conclusion: *This study establishes a higher local cut-off value for defining hyperhomocysteinemia in a Northern Nigerian population (90th percentile: 20.9 µmol/L). The application of Caucasian reference values would lead to significant over-diagnosis of HHCY in this setting. These findings underscore the critical need for population-specific reference ranges for homocysteine to ensure accurate clinical diagnosis, appropriate resource allocation, and valid epidemiological research in Sub-Saharan Africa.*

Keywords

Homocysteine, Hyperhomocysteinemia, Reference Values, Nigeria, Laboratory Medicine, Cardiovascular Risk Factors, Folate, Vitamin B12.

Introduction

Homocysteine, a sulfur-containing amino acid derived from methionine metabolism, has been at the forefront of cardiovascular research for decades [1]. Since the landmark observation of premature atherosclerosis in patients with homozygous homocystinuria [2], elevated plasma total homocysteine (tHcy) has been extensively investigated as a modifiable risk factor for atherosclerotic vascular disease, venous thrombosis, and more recently, heart failure and cognitive decline [3,4]. The biological

plausibility for its pathogenic role is strong, involving endothelial dysfunction, oxidative stress, inflammation, and promotion of a pro-thrombotic state [5].

The diagnosis of hyperhomocysteinemia (HHCY) hinges on defining a cut-off value above which plasma tHcy is considered abnormally high. A fasting tHcy level of 15 µmol/L has been widely adopted in clinical practice and research as the threshold for mild HHCY, with values >30 µmol/L and >100 µmol/L representing moderate and severe elevations, respectively [6,7]. These thresholds are largely derived from studies in Caucasian populations from North America and Europe.

However, a fundamental tenet of laboratory medicine is that reference intervals should be established for the specific population served by the laboratory, accounting for age, sex, genetic makeup, nutritional status, and environmental exposures [8]. This is particularly crucial for analytes like homocysteine, whose levels are profoundly influenced by dietary intake of B-vitamins—folate (B9), vitamin B12 (cobalamin), and vitamin B6 (pyridoxine) [9]. Genetic polymorphisms, most notably the C677T variant in the methylenetetrahydrofolate reductase (*MTHFR*) gene, also significantly affect tHcy concentrations [10].

Sub-Saharan Africa (SSA), and Nigeria within it, presents a unique epidemiological context. Despite dietary diversity, deficiencies in micronutrients, including B-Vitamins, are well-documented, often stemming from low intake of animal products, legumes, and fortified foods, coupled with high rates of parasitic infestations affecting absorption [11,12]. Northern Nigeria, in particular, has a dietary pattern with limited consumption of folate-rich leafy vegetables and B12-rich animal proteins, especially among certain populations [13]. Furthermore, the prevalence of the *MTHFR* C677T polymorphism, while variable, has been reported in Nigerian populations and can contribute to higher baseline tHcy [14].

Applying a universal tHcy cut-off of 15 µmol/L in such a setting may not be appropriate. It may lead to the over-diagnosis of HHCY, labeling a large segment of the population as "high-risk" based on an inappropriate benchmark. This has direct clinical consequences: unnecessary patient anxiety, costly and potentially futile investigations for secondary causes, and unwarranted initiation of long-term vitamin supplementation. From a research perspective, using an external reference can distort the true prevalence of HHCY, confound associations in case-control studies, and undermine the validity of intervention trials aimed at lowering homocysteine.

Previous Nigerian studies have hinted at this discrepancy. Research on stroke patients in Lagos [15] and myocardial infarction/stroke patients in Gombe [16] found mean tHcy levels comparable to controls, with no significant elevation in cases when using locally defined percentiles. However, these studies often used the 90th or 95th percentile of their control groups post-hoc, without explicitly establishing and advocating for a formal, locally validated reference interval.

This study, therefore, aimed to systematically determine a locally relevant cut-off value for hyperhomocysteinemia by analyzing the distribution of fasting plasma tHcy in a carefully selected healthy adult population in Gombe, North-Eastern Nigeria. The findings are intended to provide evidence-based guidance for clinicians and researchers in Nigeria and similar settings, advocating for a paradigm shift towards context-specific laboratory reference standards.

Methods

Study Design and Setting

This was a cross-sectional, analytical study conducted between May and December 2022. The study was embedded within a larger project investigating homocysteine levels in heart failure patients at the Federal Teaching Hospital (FTH), Gombe. FTH Gombe is a tertiary referral center serving Gombe State and neighboring states in North-Eastern Nigeria. Ethical approval for the study was obtained from the Research and Ethics Committee of the Federal Teaching Hospital, Gombe (Ref: NHREC/25/10/2013). Written informed consent was obtained from every participant before enrolment into the study.

Study Population

The reference population consisted of 90 healthy adult volunteers. Their selection was designed to reflect the local "healthy" community from which patients originate.

Inclusion Criteria

1. Aged 18 years and above.
2. No prior history or clinical evidence of cardiovascular disease (heart failure, coronary artery disease, stroke), diabetes mellitus, or chronic kidney disease.
3. Not on medications known to affect homocysteine metabolism: multivitamins (especially B-complex), folate supplements, anticonvulsants (phenytoin, carbamazepine), theophylline, or methotrexate.
4. Willing and able to provide informed consent.

Exclusion Criteria

1. Presence of acute or chronic illness at the time of recruitment.
2. Pregnancy or lactation.

Participants were recruited mainly from two sources: 1) relatives/caregivers of patients attending the hospital who shared similar dietary and environmental backgrounds, and 2) staff of the hospital who met the health criteria. This strategy aimed to minimize socioeconomic and dietary confounding in the populations.

Sample Size Justification

The sample size for the was determined using the formula for estimating a population mean: $n = (Z^2 * S^2) / d^2$ [17]. Using a standard deviation (S) of 4.6 µmol/L from a previous Nigerian study on homocysteine [15]. 95% confidence level (Z=1.96), and a desired precision (d) of ±1.0 µmol/L, the minimum sample size

was calculated as 81. Allowing for a 10% attrition rate, 90 were recruited.

Ethical Considerations
The study protocol was reviewed and approved by the Research and Ethics Committee of the Federal Teaching Hospital, Gombe (Approval Ref: NHREC/25/10/2013). All participants provided written informed consent after a detailed explanation of the study's purpose and procedures.

Blood Sample Collection and Processing
Venous blood sampling was standardized to minimize pre-analytical variability, a critical factor in tHcy measurement [18].

Fasting
Blood was drawn after an 8-10 hour overnight fast to obtain a true fasting homocysteine level, which is the recommended standard for risk assessment [19].

Collection
Approximately 5 mL of blood was collected via venipuncture using a 21-gauge needle into vacutainer tubes containing potassium ethylenediaminetetraacetic acid (K2EDTA) as an anticoagulant.

Processing
Samples were placed on ice packs immediately and transported to the laboratory. Plasma was separated by centrifugation at 3000 rpm for 10 minutes within 1 hour of collection to prevent artificial elevation from continued erythrocyte metabolism [18].

Storage
The separated plasma was aliquoted into plain cryovials and stored at -20°C until batch analysis.

Laboratory Analysis of Total Homocysteine
Frozen plasma samples were transported on dry ice to the Chemical Pathology Department of the Teaching Hospital, a reference laboratory with expertise in specialized assays.

Assay Principle and Kit
Plasma total homocysteine was quantified using a commercial competitive enzyme-linked immunosorbent assay (ELISA) kit (Cloud-clone Corp., assembled by Uscn Life Science Inc., Houston, USA). This method measures the sum of free and protein-bound homocysteine after a reductive step. The assay employs the competitive inhibition technique, where homocysteine in the sample competes with a fixed amount of biotin-labeled homocysteine for binding sites on a monoclonal antibody pre-coated on the microplate. The intensity of the colorimetric signal, read at 450 nm, is inversely proportional to the concentration of homocysteine in the sample [20].

Assay Procedure and Quality Control
The assay was performed strictly according to the manufacturer's instructions by a trained laboratory scientist under the supervision of a consultant chemical pathologist. All reagents were equilibrated to room temperature. A standard curve was generated using the calibrators provided (concentration range: 0-8000 ng/mL). Each sample was tested in duplicate, and the mean optical density was used for calculation. The intra-assay coefficient of variation (CV) was maintained below 10% as per kit specifications. The concentration in ng/mL was converted to the standard international unit (µmol/L) using the molecular weight of homocysteine (135.19 g/mol) [21].

Data Analysis
Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM Corp., Armonk, NY, USA).

Determination of the Local Cut-off
Distribution Analysis
Plasma tHcy values in the control group were tested for normality using the Shapiro-Wilk test. As homocysteine levels typically exhibit a right-skewed distribution [22]. non-parametric percentiles were deemed the most appropriate method for establishing the reference limit.

Percentile Calculation
The 90th percentile (P90) and the 95th percentile (P95) of the tHcy distribution in the healthy control group were calculated. The P90, representing the value below which 90% of the healthy population falls, is commonly used in epidemiological studies to define the upper limit of "normal" and diagnose HHCY [23,24]. The P95 provides a more stringent cut-off.

Descriptive Statistics
Mean, standard deviation (SD), median, and interquartile range (IQR) were calculated for the control group's tHcy levels.

Application of the Cut-off
The derived P90 value (20.9 µmol/L) was applied as the diagnostic threshold for HHCY. The prevalence of HHCY in the subject itself (expected to be ~10%) was calculated using this local cut-off. For comparison, the prevalence was also calculated using the conventional 15 µmol/L threshold. Differences in prevalence were compared using the Chi-square test (or Fisher's exact test where appropriate). A p-value of <0.05 was considered statistically significant.

Results
Characteristics of the Reference Population
The subjects consisted of 90 individuals with a mean age of 46.3 ± 14.9 years. There were 34 males (37.8%) and 56 females (62.2%), ensuring the reference values represented both sexes. All participants were ambulatory and free from overt cardiovascular or metabolic disease.

Distribution of Plasma Total Homocysteine in Healthy Controls
The plasma tHcy levels in the subjects ranged from 2.8 to 32.5 µmol/L. The distribution was positively skewed (Shapiro-Wilk test, $p < 0.05$). Therefore, median and percentiles are the most robust measures of central tendency and dispersion for this dataset.

Mean ± SD: 10.24 ± 6.98 µmol/L
Median (IQR): 8.70 (5.70 – 12.90) µmol/L
90th Percentile (P90): 20.9 µmol/L
- **95th Percentile (P95): 25.1 µmol/L**

Table 1: Distribution of Plasma Total Homocysteine in 90 Healthy Nigerian Adults.

Statistical Parameter	Value (µmol/L)
Minimum	2.8
25th Percentile (Q1)	5.7
Median	**8.7**
75th Percentile (Q3)	12.9
90th Percentile (P90)	**20.9**
95th Percentile (P95)	**25.1**
Maximum	32.5
Mean ± Standard Deviation	10.24 ± 6.98

Prevalence of Hyperhomocysteinemia Using Different Cut-offs

The implications of choosing a cut-off value are demonstrated in Table 2. Using the locally derived 90th percentile (20.9 µmol/L), 5 out of 90 subjects were classified as having HHCY, yielding a prevalence of 5.6%. This aligns with the statistical expectation that approximately 10% of a healthy population will be above the 90th percentile, with the slight discrepancy likely due to sample size and distribution skewness.

In stark contrast, applying the conventional cut-off of 15 µmol/L classified 18 out of 90 subjects as hyperhomocysteinemic, resulting in a prevalence of **20.0%**. This represents a **257% increase** in the number of individuals labeled with HHCY compared to the local cut-off.

Table 2: Prevalence of Hyperhomocysteinemia in Subjets Using Different Cut-off Values.

Cut-off Value (µmol/L)	Number of Controls with HHCY (n=90)	Prevalence (%)
Local 90th Percentile (20.9)	5	5.6
Conventional Threshold (15.0)	18	20.0
Difference	+13 individuals	+257% relative increase

Discussion

This study provides compelling evidence for the necessity of population-specific reference ranges for plasma total homocysteine. We established a local 90th percentile cut-off of 20.9 µmol/L for a Northern Nigerian adult population, which is substantially higher than the widely used benchmark of 15 µmol/L derived from Caucasian populations. The practical consequence of applying the lower, external cut-off is a dramatic overestimation of the prevalence of hyperhomocysteinemia, both in the general healthy population (20.0% vs. 5.6%) and in a disease cohort. This has profound implications for clinical practice, public health strategy, and cardiovascular research in Nigeria and similar regions.

The Local Homocysteine Profile and Its Determinants

The median fasting tHcy level in our healthy subjects was 8.7 µmol/L, with a mean of 10.24 µmol/L. These values are consistent with previous reports from Nigeria. Okubadejo et al. in Lagos reported a mean tHcy of 10.2 µmol/L in controls [15]. while Glew et al. in Gombe found means of 9.7 µmol/L for controls [16]. Ebesunun and Obajobi reported a mean of 10.5 µmol/L in healthy subjects in Ile-Ife [25]. This consistency across different Nigerian regions suggests a nationally relevant pattern of homocysteine metabolism that differs from Caucasian norms, where mean fasting levels are often reported between 8-12 µmol/L, but with a lower upper reference limit [22,26].

The primary drivers of this shifted distribution are almost certainly nutritional. Folate and vitamin B12 are critical cofactors in the remethylation pathway that recycles homocysteine back to methionine [9]. Diets in Northern Nigeria, particularly among rural and lower socioeconomic groups, are often low in animal proteins (the main source of B12) and green leafy vegetables (a key source of folate) [13]. Glew et al. specifically documented low serum folate and vitamin B12 levels among Fulani pastoralists in the region [13]. Furthermore, mandatory folic acid fortification of staple foods, which has successfully lowered population homocysteine levels in countries like the USA and Canada [27]. Is not practiced in Nigeria. This widespread, subclinical deficiency of B-vitamins elevates the population's baseline tHcy, shifting the entire distribution to the right.

Genetic factors may also contribute. The *MTHFR* C677T polymorphism, which reduces enzyme activity and increases tHcy levels, especially in the context of low folate, has a varying global prevalence. While its frequency is high in some European and Asian populations, studies in Nigeria have reported its presence [14,28]. A higher prevalence of this or other related polymorphisms in our population could partly explain the elevated reference range.

Implications of Using an Inappropriate Cut-off: A False Epidemic

Our data demonstrates that using the 15 µmol/L cut-off manufactures a "false epidemic" of HHCY, labeling one in five apparently healthy Nigerians as having an abnormal, high-risk biochemical parameter. This is not merely a statistical abstraction; it has real-world consequences:

Clinical Misdiagnosis and Unnecessary Intervention: Patients with tHcy between 15 and 20.9 µmol/L would be incorrectly diagnosed with HHCY. This could trigger a cascade of unnecessary actions: extensive and costly searches for secondary causes (renal disease, hypothyroidism, rare genetic disorders), prescription of long-term high-dose B-vitamin supplements (with associated cost and potential for unintended interactions), and inappropriate labeling of patients as having a high cardiovascular risk, causing anxiety and potentially affecting insurance or employment.

Skewed Public Health Priorities: Overestimating the prevalence of a risk factor can lead to its overestimation as a public health burden. Resources that could be directed toward tackling more prevalent and modifiable risk factors like hypertension, smoking, or dyslipidemia might be diverted to widespread homocysteine

screening and vitamin supplementation programs with uncertain cost-effectiveness in this context.

The Case for Local Reference Intervals and Standardized Reporting

The findings strongly advocate for the establishment and use of local laboratory reference intervals (RIs), a core principle of the International Federation of Clinical Chemistry (IFCC) [8]. While generating RIs is resource-intensive, alternatives exist. The IFCC and the Clinical and Laboratory Standards Institute (CLSI) endorse methods like the use of patient data (indirect methods) or transferring RIs from a comparable population after verifying comparability through a small reference sample [29].

For homocysteine, we propose that clinical laboratories in Nigeria and similar SSA settings should either:

a. Establish their own RIs using a carefully selected reference population, as done in this study.
b. Adopt a nationally or regionally agreed-upon cut-off (e.g., a 90th percentile value of ~21 µmol/L for Northern Nigeria) until more comprehensive data is available.
c. Report homocysteine results alongside the percentile rank for the local population (e.g., "22 µmol/L, >95th percentile for local reference population") rather than just a binary "high" based on an international standard.

Furthermore, researchers must explicitly state and justify the cut-off values used in their studies. The practice of using 15 µmol/L as a default should be abandoned in favor of values derived from or validated against the study's own control group or a well-characterized local reference population.

Study Strengths and Limitations

Strengths

This study directly addresses a critical gap in laboratory medicine practice in SSA. The use of standardized, fasting blood collection and a reliable ELISA method strengthens the analytical validity. The recruitment of subjects from the community and patient relatives enhances the representativeness of the reference sample. The direct demonstration of how the cut-off choice impacts clinical research findings is a powerful and practical contribution.

Limitations

The sample size, though adequate for the initial calculation, is modest for definitive RI establishment, which ideally requires 120 reference individuals per subgroup (e.g., by sex/age) [29]. We did not measure serum folate, B12, and B6 levels or genotype for the *MTHFR* polymorphism in our controls, which would have provided mechanistic insight into the observed levels. The study was conducted at a single tertiary center; a multi-center study would provide a more robust national reference.

Conclusion and Recommendations

This study establishes that the commonly used international cut-off of 15 µmol/L for diagnosing hyperhomocysteinemia is inappropriately low for an adult population in North-Eastern Nigeria. Based on the 90th percentile of a locally recruited healthy control group, a value of **20.9 µmol/L** is proposed as a more accurate upper reference limit for this setting.

The implications are far-reaching. The uncritical application of Caucasian-derived reference values leads to significant over-diagnosis, potentially unnecessary treatment, and distorted research conclusions. This work serves as a clarion call for the contextualization of laboratory medicine in Africa.
We therefore recommend:

For Laboratory Directors and Pathologists

Clinical chemistry laboratories in Nigeria should initiate processes to establish or verify local reference intervals for homocysteine. National professional bodies (e.g., Association of Clinical Chemists of Nigeria) should champion multi-center collaborative studies to define nationally representative RIs.

For Clinicians

Physicians should interpret homocysteine results with caution, seeking guidance from their local laboratory on the appropriate reference range. Diagnosis and management decisions for HHCY should be based on locally relevant thresholds

For Researchers

Future epidemiological and clinical studies involving homocysteine in African populations must define and justify their diagnostic cut-offs based on local reference data. The practice of importing cut-offs from dissimilar populations should be explicitly acknowledged as a limitation.

For Public Health Policymakers

Nutritional strategies to improve folate and B12 status at the population level, such as promoting dietary diversification or considering food fortification, remain important for overall health. However, policy decisions regarding screening for HHCY should be informed by its true, locally defined prevalence, not by inflated figures derived from inappropriate benchmarks.

Adopting population-specific reference standards is a fundamental step towards equitable, accurate, and effective healthcare and research in Nigeria and across Sub-Saharan Africa.

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