Research Article

Population-Specific Reference Intervals for Whole-Blood GSH/GSSG Ratio in Healthy Emirati Adults According to CLSI and ISO 15189 Guidelines

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Article Info

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Keywords

Glutathione, GSSG, Reference interval, UHPLC, Oxidative stress, Emirati population, CLSI C28-A3, ISO 15189

Abstract

Background: The glutathione redox system, expressed as the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG), is a key indicator of cellular oxidative status. Manufacturer-provided reference intervals are typically derived from non-local populations and may not be applicable across different ethnic and geographic groups.

Objective: To characterize the distribution of whole-blood GSH/GSSG ratio in healthy Emirati adults using ultra-high-performance liquid chromatography (UHPLC) and to establish population-specific reference intervals in accordance with CLSI C28-A3 and ISO 15189 guidelines.

Methods: In this cross-sectional study, whole-blood samples from 120 apparently healthy Emirati adults were analyzed using a commercial UHPLC assay. The GSH/GSSG ratio was calculated for each participant. Due to non-normal distribution, reference intervals were derived using a nonparametric approach. Both the conventional 95% reference interval (2.5th–97.5th percentiles) and the central 90% interval (5th–95th percentiles) were calculated. Bootstrap resampling (20,000 iterations) was used to estimate 95% confidence intervals for the central 90% limits.

Results: The GSH/GSSG ratio demonstrated a right-skewed distribution with a median (IQR) of 4.76 (3.58–6.77). Most participants (89.2%) had values below the manufacturer-reported reference interval (10–15), with only 5.0% within this range. The nonparametric 95% reference interval was 1.66–28.40. The central 90% interval was 2.53–17.20, with bootstrap 95% confidence intervals of 2.21–2.97 (lower) and 14.92–19.73 (upper).

Conclusion: Manufacturer-provided reference intervals do not reflect the distribution observed in healthy Emirati adults. Population-specific reference intervals are therefore necessary. Reporting both 95% and 90% intervals, supported by bootstrap estimates, enhances clinical interpretation for both diagnostic and screening applications.

Introduction

Oxidative stress, defined as an imbalance between the production of reactive oxygen species and antioxidant defense capacity, plays a central role in the pathophysiology of numerous chronic and age-related diseases, including cardiovascular disease, diabetes, neurodegeneration, and cancer [1,2]. Maintenance of cellular redox homeostasis is therefore essential for normal physiological function, and reliable biomarkers of redox status are increasingly important in both clinical and research settings.

Among endogenous antioxidant systems, the glutathione redox couple, consisting of reduced glutathione (GSH) and oxidized glutathione (GSSG), represents a primary regulator of intracellular redox balance [3]. The GSH/GSSG ratio is widely recognized as a sensitive and integrative indicator of oxidative stress, reflecting both antioxidant capacity and oxidative burden [4,5]. Unlike absolute concentrations of GSH or GSSG alone, the ratio provides insight into redox dynamics and has been associated with metabolic, inflammatory, and degenerative conditions [6,7].

Despite its recognized biological relevance, clinical interpretation of the GSH/GSSG ratio remains challenging, as many assays rely on manufacturer-derived or externally established reference intervals that may not be fully representative of the populations in which they are applied [8]. Increasing evidence suggests that redox biomarkers, including glutathione-related parameters, exhibit significant inter-population variability, influenced by ethnicity, diet, lifestyle, environmental exposures, and genetic background [9,10]. Application of non-local reference intervals may therefore lead to systematic misclassification of healthy individuals.

International laboratory standards explicitly recognize this limitation. The Clinical and Laboratory Standards Institute (CLSI) C28-A3 guideline emphasizes the need for population-specific reference interval verification or establishment, particularly when demographic or ethnic differences are expected [11]. Similarly, ISO 15189 requires clinical laboratories to ensure that reference intervals are appropriate for the population served and to justify their clinical applicability [12]. However, population-specific reference intervals for redox biomarkers, including the GSH/GSSG ratio, remain scarce, particularly in Middle Eastern populations.

To date, no published study has established whole-blood GSH/GSSG reference intervals for healthy Emirati adults using modern chromatographic techniques. Given the unique genetic, dietary, and environmental characteristics of the Emirati population, extrapolation from reference intervals derived in Western or East Asian cohorts may be inappropriate. Furthermore, advances in ultra-high-performance liquid chromatography (UHPLC) offer improved analytical specificity and sensitivity for glutathione measurement compared with older spectrophotometric or enzymatic methods [13].

The present study was therefore designed to address this knowledge gap by characterizing the distribution of whole-blood GSH/GSSG ratios in a cohort of apparently healthy Emirati adults and establishing population-specific reference intervals in accordance with CLSI C28-A3 and ISO 15189 guidelines. In addition to the conventional 95% reference interval, a central 90% interval was derived to support sensitivity-oriented screening and early redox imbalance detection, providing laboratories with greater interpretive flexibility.

Materials and Methods

Study design and participants

This cross-sectional study included 120 apparently healthy Emirati adults. Participants were recruited from the local community and reported no known acute or chronic illness at the time of sampling. As the study involved exclusively anonymized retrospective data, formal Institutional Review Board approval and ethical committee registration were not required in accordance with institutional policy and applicable regulations, and the requirement for informed consent was waived. The study was conducted in accordance with institutional data protection standards and the principles of the Declaration of Helsinki.

Sample collection and UHPLC analysis

Whole-blood samples were collected by venipuncture into anticoagulant-containing tubes and processed according to the manufacturer's instructions to preserve glutathione redox status. Reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations were measured using the Chromsystems "Glutathione in Whole Blood" HPLC reagent kit (Order No. 66000; Chromsystems Instruments & Chemicals GmbH, Gräfelfing, Germany), according to the manufacturer's instructions provided in the assay manual (Instruction Manual IM 66000 Glutathione EN 06/2016 R3). For pre-analytical handling, fresh whole-blood samples were processed immediately after collection. Stored frozen samples were thawed immediately prior to analysis and processed without delay together with calibrators and quality controls. Sample preparation was performed under chilled and light-protected conditions to minimize ex vivo oxidation of glutathione and alterations in the GSH/GSSG ratio. Repeated freeze–thaw cycles were avoided. The GSH/GSSG ratio was calculated for each participant.

Statistical analysis

Statistical analyses were performed using SPSS Statistics (IBM Corp., Armonk, NY, USA). Descriptive statistics were reported as mean (SD) and median (IQR). Distributional characteristics were assessed using histograms, boxplots, empirical cumulative distribution functions, and quantile–quantile plots. Due to non-normality, reference intervals were derived using a nonparametric percentile-based approach in accordance with CLSI C28-A3 guidelines.

Both a 95% reference interval (2.5th–97.5th percentiles)

and a central 90% interval (5th–95th percentiles) were calculated to support diagnostic and screening-oriented clinical interpretation, consistent with ISO 15189 laboratory practice. Observed values were compared with the manufacturer-provided reference interval (10–15). Bootstrap resampling (20,000 iterations) was used to estimate 95% confidence intervals for the central 90% reference limits. Outliers were identified using Tukey's criterion (Q3 + 1.5×IQR) and retained in the primary analysis.

Results

1. Study population and data completeness

Whole-blood GSH/GSSG ratio measurements were available for all 120 apparently healthy Emirati adults, and no missing or invalid data points were identified. All samples were included in the primary analysis.

2. Distributional characteristics of the GSH/GSSG ratio

The GSH/GSSG ratio demonstrated a right-skewed, non-normal distribution, with most values clustered at the lower end and a small proportion of high-value observations. The median (IQR) ratio was 4.76 (3.58–6.77), while the mean (SD) was 6.45 (5.51). Observed values ranged from 1.44 to 30.23, indicating substantial inter-individual variability.

Normality assessment using quantile–quantile plots confirmed deviation from Gaussian distribution on the raw scale, with partial improvement following logarithmic transformation (Figures 1 and 2).

Table 1 summarizes the descriptive statistics for the GSH/GSSG ratio in the study population.

Table 1: Distribution characteristics and descriptive statistical summary of whole-blood GSH/GSSG ratio values in apparently healthy Emirati adults (n = 120).

Parameter	Value
Mean (SD)	6.45 (5.51)
Median (IQR)	4.76 (3.58–6.77)
Minimum – Maximum	1.44 – 30.23
Skewness	2.89
Excess kurtosis	8.80

Figure 1: Normal Q-Q plot of raw GSH/GSSG ratio values. Quantile-quantile plot showing substantial deviation from normality, particularly in the upper tail.

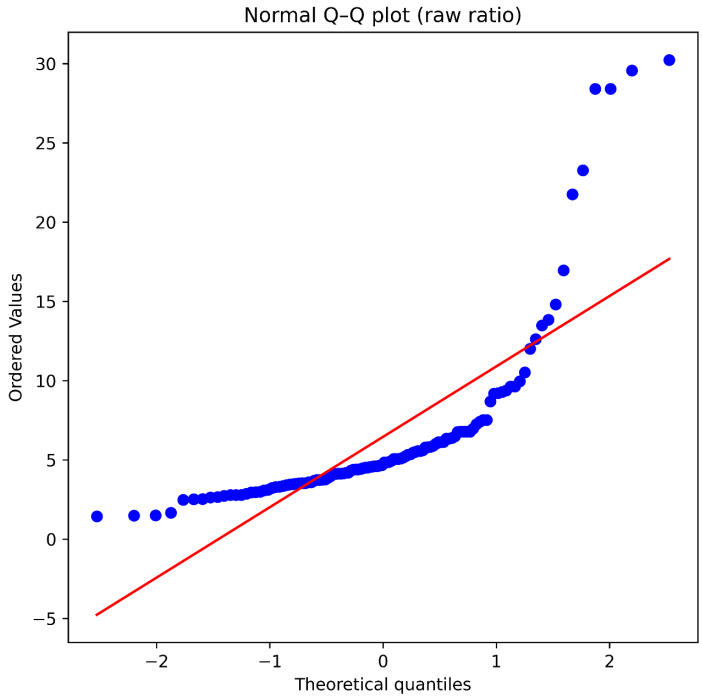
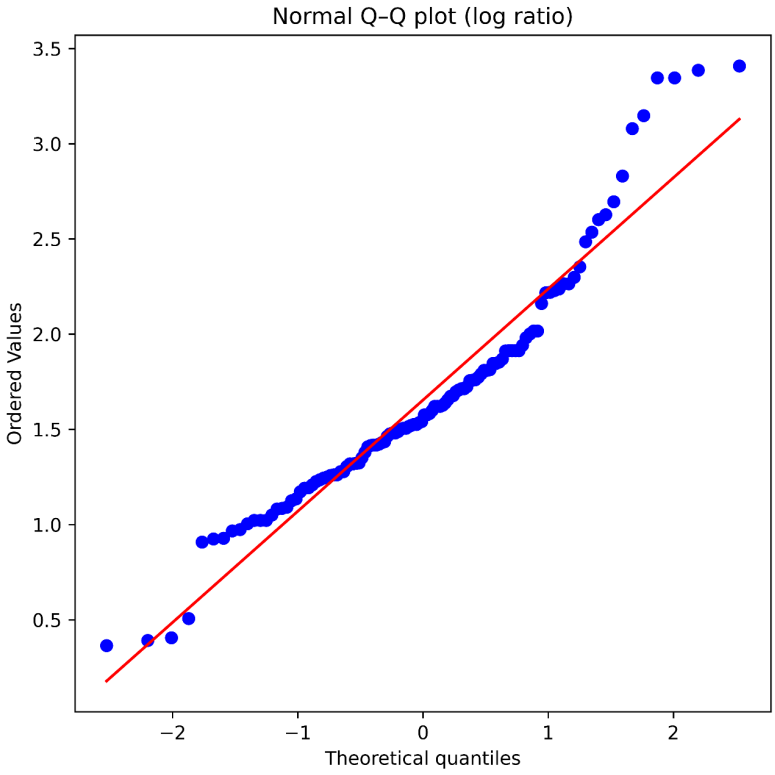
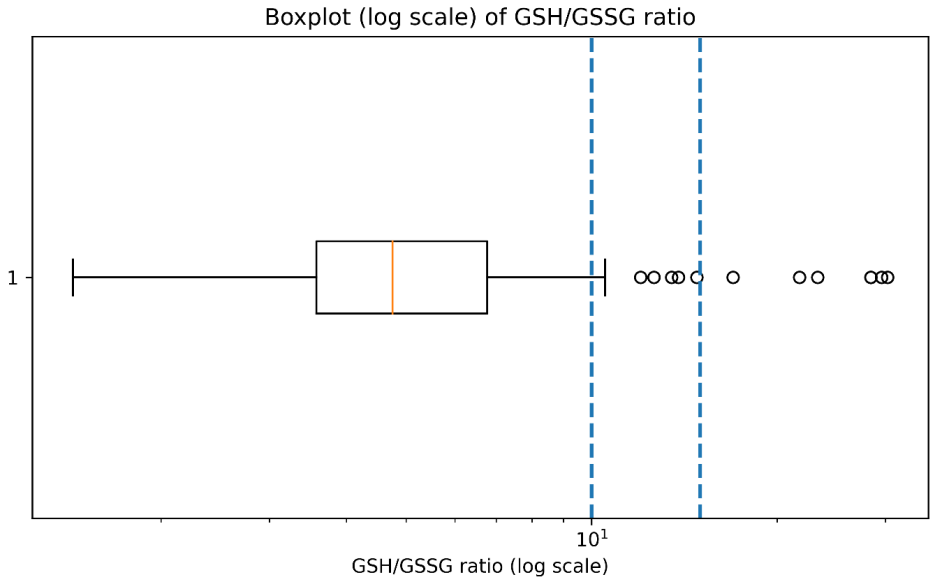


Figure 2: Normal Q-Q plot of log-transformed GSH/GSSG ratio values. Log transformation partially improves normality, but residual deviations persist, supporting the use of nonparametric methods.



The distribution and presence of high-value observations are further illustrated using a logarithmic-scale boxplot (Figure 3), which highlights right-skewness and inter-individual variability across the observed range.

Figure 3: Log-scale boxplot of whole-blood GSH/GSSG ratio in healthy Emirati adults. Horizontal boxplot displayed on a logarithmic scale illustrating the median, interquartile range, and high-value outliers of the GSH/GSSG ratio (n = 120). The log transformation highlights right-skewness and inter-individual variability in redox status.



3. Comparison with manufacturer-provided reference interval

The manufacturer-reported reference interval for the GSH/GSSG ratio (10–15) showed poor concordance with the observed distribution. A large majority of participants (89.2%) had values below the vendor's lower limit, while only 5.0% fell within the stated reference interval and 5.8% exceeded the upper limit.

This discrepancy is visually evident in the histogram and boxplot, where the vendor reference band lies above the central tendency of the population distribution (Figures 4 and 5). The categorical comparison with the manufacturer's reference interval is presented in Table 2.

Figure 4: Distribution of whole-blood GSH/GSSG ratio in healthy Emirati adults. Histogram with kernel density overlay showing the distribution of GSH/GSSG ratio values (n = 120). The shaded band represents the manufacturer-provided reference interval (10–15). Dashed vertical lines indicate the 2.5th and 97.5th percentiles derived from the study population.

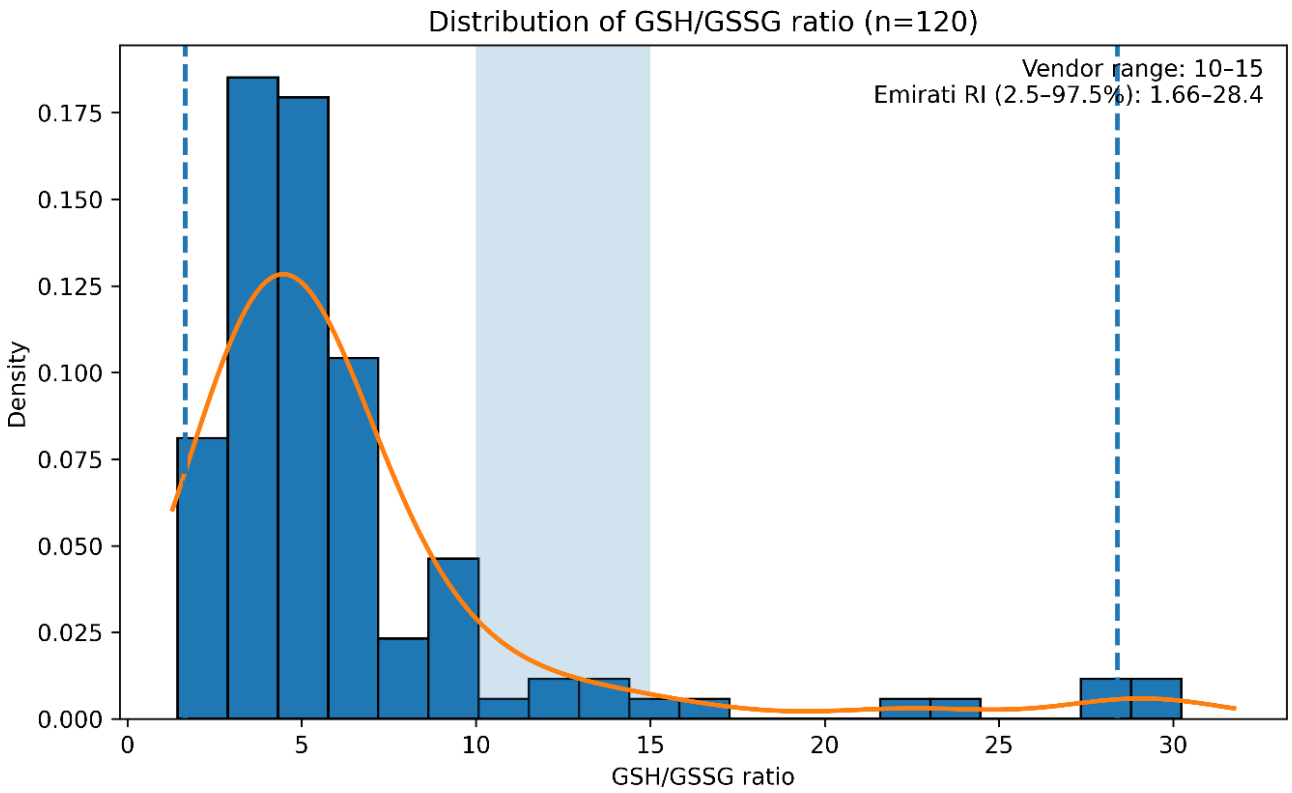


Figure 5: Boxplot of GSH/GSSG ratio relative to the manufacturer reference interval. Horizontal boxplot illustrating median, interquartile range, and outliers. The shaded region denotes the vendor reference interval (10–15), highlighting the predominance of values below this range.

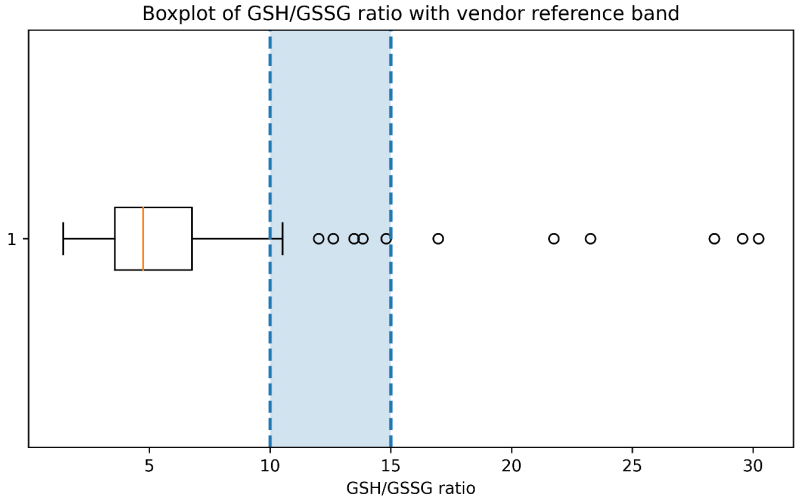
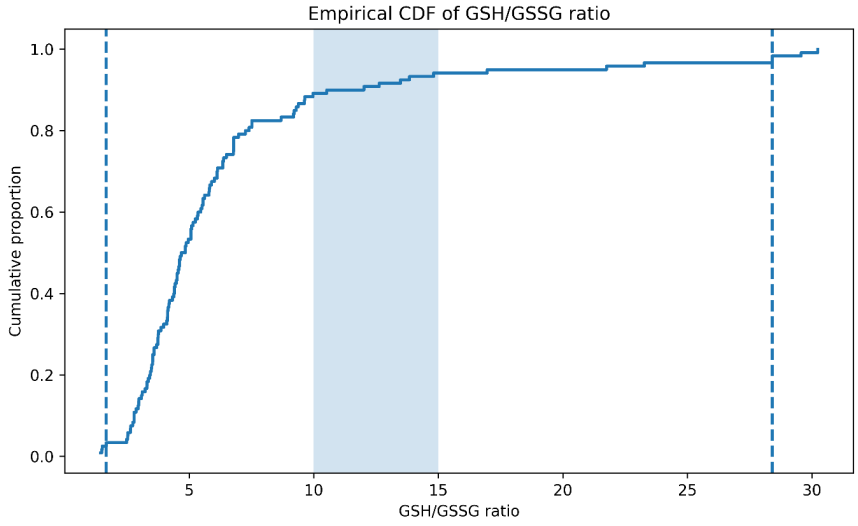


Table 2: Classification of whole-blood GSH/GSSG ratio values in healthy Emirati adults relative to the manufacturer-provided reference interval (10–15).

Classification relative to vendor range	Number (%)
Below 10	107 (89.2%)
Within 10–15	6 (5.0%)
Above 15	7 (5.8%)

The empirical cumulative distribution function (ECDF) further demonstrates this discordance by showing that approximately 90% of healthy individuals have GSH/GSSG ratio values below the manufacturer's lower cutoff of 10 (Figure 6).

Figure 6: Empirical cumulative distribution function (ECDF) of whole-blood GSH/GSSG ratio. The ECDF depicts the cumulative proportion of individuals across the observed range of GSH/GSSG ratio values. The curve demonstrates that the majority of healthy participants fall below the manufacturer-provided reference interval (10-15), supporting the derivation of population-specific reference intervals.

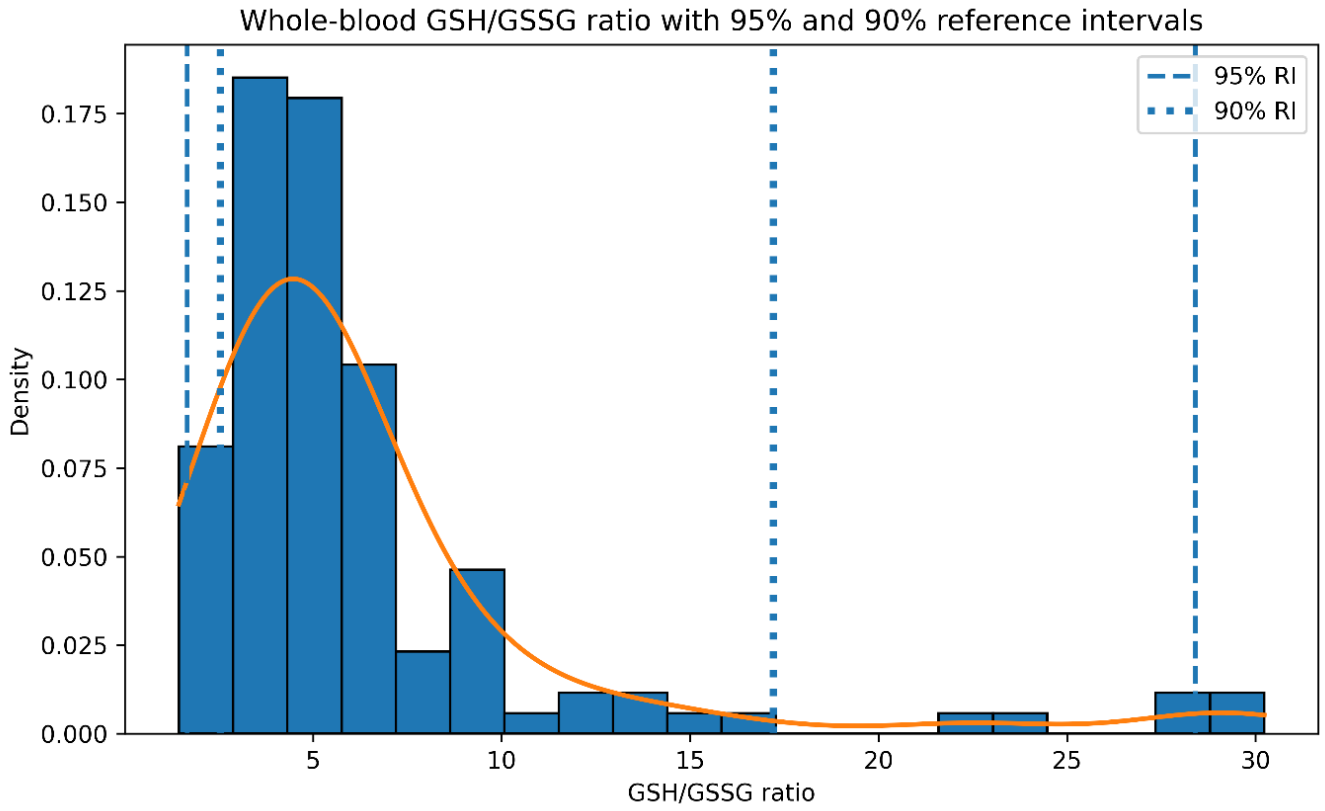


4. Population-specific reference interval estimation
Using a nonparametric percentile-based approach consistent with CLSI C28-A3 recommendations, a population-specific 95% reference interval (2.5th–97.5th percentiles) for the whole-blood GSH/GSSG ratio was derived as 1.66-28.40.
To support sensitivity-oriented screening and early redox imbalance detection, a central 90% reference interval (5th–95th percentiles) was also calculated, yielding limits of 2.53–17.20. The relationship between the observed distribution and both reference intervals is illustrated in the dual-interval distribution plot (Figure 7), where dashed lines represent the 95% reference interval and dotted lines indicate the central 90% interval. Both reference intervals are summarized in Table 3.

Table 3: Population-specific nonparametric diagnostic and screening reference intervals for whole-blood GSH/GSSG ratio in healthy Emirati adults derived according to CLSI C28-A3 guidelines.

Interval type	Percentiles	Lower limit	Upper limit	Intended clinical use
Reference interval (95%)	2.5th–97.5th	1.66	28.40	Diagnostic specificity
Central interval (90%)	5th–95th	2.53	17.20	Screening / early detection

Figure 7: Distribution of whole-blood GSH/GSSG ratio showing diagnostic and screening reference intervals. Histogram with kernel density overlay illustrating the distribution of GSH/GSSG ratio values in healthy Emirati adults (n = 120). Dashed vertical lines indicate the 95% reference interval (2.5th–97.5th percentiles), while dotted lines denote the central 90% interval (5th–95th percentiles). The dual-interval visualization highlights the distinction between diagnostic specificity and sensitivity-oriented screening thresholds.



5. Bootstrap confidence intervals for central 90% limits
Bootstrap resampling (20,000 iterations) was performed to assess the precision of the central 90% reference limits. The 95% confidence interval for the lower 5th percentile was 2.21–2.97, while the 95% confidence interval for the upper 95th percentile was 14.92–19.73, indicating acceptable robustness of the screening-oriented limits despite the skewed distribution. Bootstrap-derived confidence intervals are reported in Table 4 and contextualized visually in Figure 5.

Table 4: Bootstrap-derived 95% confidence intervals for the lower and upper limits of the central 90% whole-blood GSH/GSSG reference interval.

Reference limit	Point estimate	Bootstrap 95% CI
Lower limit (5th percentile)	2.53	2.21 – 2.97
Upper limit (95th percentile)	17.20	14.92 – 19.73

6. Outlier assessment and sensitivity analysis

Twelve participants were identified as high-value outliers based on Tukey's criterion (values > Q3 + 1.5×IQR). Exclusion of these individuals resulted in a narrower upper reference limit; however, the central tendency of the distribution and the observed discordance with the manufacturer-provided reference interval remained unchanged.

Accordingly, all participants were retained in the primary analysis, and both reference intervals were reported to reflect real-world biological variability in a healthy population.

Discussion

In this cross-sectional study of 120 apparently healthy Emirati adults, we established population-specific reference intervals for the whole-blood GSH/GSSG ratio using a validated UHPLC-based assay. The principal findings were: (i) the GSH/GSSG ratio exhibited a right-skewed, non-Gaussian distribution; (ii) the majority of participants had ratio values substantially lower than manufacturer-provided reference intervals; and (iii) the derived nonparametric 95% and central 90% reference intervals differed markedly from vendor-supplied limits derived from non-local populations.

The observed distribution of GSH/GSSG ratios in this cohort is consistent with prior reports indicating substantial biological variability in redox biomarkers among healthy individuals [9,10]. The predominance of lower ratio values, with a smaller subset of high-value observations, resulted in a right-skewed distribution, supporting the use of nonparametric methods for reference interval estimation as recommended by CLSI C28-A3.

A key finding of this study was the poor concordance between observed values and the manufacturer-provided reference interval. Nearly 90% of participants had GSH/GSSG ratios below the vendor's lower cutoff, indicating that application of the manufacturer's range would classify the majority of healthy Emirati adults as biochemically abnormal. Similar discrepancies have been reported in other populations when reference intervals derived from external cohorts are applied without local validation [8]. These findings underscore the limitations of universal reference intervals for redox biomarkers and reinforce the need for population-specific validation.

Comparison with previous population-based studies further highlights inter-population variability. Studies conducted in European and East Asian cohorts have reported higher median or mean GSH/GSSG ratios in whole blood or erythrocytes, although direct comparison is complicated by differences in analytical platforms, sample matrices, and reporting conventions [13,6]. Ethnic background, dietary antioxidant intake, environmental exposures, and lifestyle factors such as physical activity and smoking prevalence may all contribute to these differences [10,1]. Additionally, analytical methodology plays a critical role; UHPLC-based techniques generally provide greater specificity than older enzymatic or colorimetric assays, which may overestimate GSH or underestimate GSSG [13].

Several biological and methodological factors may explain the lower GSH/GSSG ratios observed in the present cohort. Diets characteristic of the region, differences in micronutrient intake, and environmental stressors such as heat exposure may influence baseline redox balance [2]. From an analytical perspective, whole-blood measurement captures both intracellular and extracellular glutathione pools, potentially yielding different distributions compared with erythrocyte-only or plasma-based assays [5].

An important strength of this study is the dual reporting of reference intervals. While the 95% reference interval remains the standard for diagnostic specificity, the central 90% interval may offer enhanced sensitivity for screening applications and early detection of redox imbalance. This approach is consistent with ISO 15189 guidance, which emphasizes clinically meaningful interpretation over rigid adherence to a single cutoff. The use of bootstrap-based confidence intervals further strengthens the robustness and transparency of the derived limits. From a clinical laboratory perspective, adoption of these population-specific intervals may improve the interpretation of GSH/GSSG results in Emirati adults by reducing false classification of healthy individuals as having abnormal redox status when non-local manufacturer-provided reference intervals are used. These intervals may also support more appropriate reporting, clinical decision-making, and longitudinal monitoring of oxidative stress–related conditions.

For laboratories operating under ISO 15189 standards, locally derived or locally verified reference intervals may provide stronger evidence that reported interpretive limits are appropriate for the population served. However, laboratories adopting these intervals should ensure that similar analytical methods, sample type, and pre-analytical handling procedures are used before implementation.

To our knowledge, this study represents the first establishment

of population-specific whole-blood GSH/GSSG reference intervals in healthy Emirati adults using UHPLC. The findings have direct implications for clinical laboratories and researchers using redox biomarkers in Middle Eastern populations. Adoption of locally derived reference intervals may reduce misclassification, improve clinical interpretation, and support more accurate assessment of oxidative stress–related conditions.

Several limitations should be acknowledged. The study cohort, while sufficient for nonparametric reference interval estimation, was limited to apparently healthy adults and did not include stratification by age, sex, or lifestyle factors. Future studies in larger cohorts should explore demographic stratification and longitudinal variability. Nonetheless, the present findings provide a necessary foundation for laboratory implementation and further research.

In conclusion, the GSH/GSSG ratio in healthy Emirati adults differs substantially from manufacturer-provided reference intervals derived from non-local populations. Establishment of population-specific reference intervals, supported by robust analytical methods and international guidelines, is essential for accurate clinical interpretation of redox biomarkers.

Conclusion

In conclusion, this study established population-specific reference intervals for the whole-blood GSH/GSSG ratio in apparently healthy Emirati adults using a UHPLC-based analytical approach. The observed distribution of GSH/GSSG ratios differed substantially from manufacturer-provided reference intervals derived from non-local populations, with the majority of healthy individuals exhibiting lower ratio values. These findings demonstrate that unverified application of external reference intervals may result in systematic misclassification when interpreting redox biomarkers.

By deriving both the conventional 95% reference interval and a central 90% interval in accordance with CLSI C28-A3 and ISO 15189 recommendations, this study provides laboratories with flexible, clinically meaningful tools for diagnostic and screening-oriented interpretation of redox status. The inclusion of bootstrap-based confidence intervals further strengthens the robustness and transparency of the proposed limits.

As the first study to report whole-blood GSH/GSSG reference intervals in healthy Emirati adults, this work addresses an important knowledge gap and highlights the necessity of population-specific validation for redox biomarkers. Future studies in larger and more diverse cohorts are warranted to explore demographic stratification, longitudinal variability, and clinical applicability in disease settings.

Acknowledgements

None.

Research Ethical

This study was conducted as a retrospective analysis of fully deidentified data obtained from routine laboratory records. In accordance with institutional policy and applicable regulations, research involving exclusively anonymized data does not require formal Institutional Review Board (IRB) approval or ethical committee registration, and the requirement for informed consent was therefore waived.

The study was conducted in compliance with institutional data protection standards and in accordance with the principles of the Declaration of Helsinki.

Author contributions

The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest

The authors do not have any conflict of interest.

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Data availability statement

The data generated and analyzed in the presented study are available from the corresponding author on request.

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