

Anwar Borai*, Kiyoshi Ichihara, Waleed Tamimi, Abdulaziz Masaud and Samia Sobki

Establishment of reference intervals for free light chains and immunoglobulins in Saudi population

<https://doi.org/10.1515/cclm-2023-0774>

Received July 22, 2023; accepted September 21, 2023;

published online October 4, 2023

Abstract

Objectives: Testing of serum-free light chains kappa (κ) and lambda (λ), along with ratio (FLCR) is essential for the diagnosis and management of monoclonal gammopathies. Accurate clinical diagnosis depends upon appropriate local population reference intervals (RIs). This study examined the Saudi population for serum-free light chains and other immunoglobulins to establish RIs and to explore variations in the test results by using the International Federation for Clinical Chemistry and Laboratory Medicine's global protocol for harmonized implementation of RI study.

Methods: A total of 180 healthy Saudi adults were recruited. All serum samples were assayed using the Freelite reagents from the Binding Site. The variation in reference values attributable to sex, age, BMI, and region was calculated by ANOVA as a standard deviation ratio (SDR). The RIs for the FLCR were derived by the parametric method and validated by using samples from patients with hypo- and hypergammaglobulinemia.

Results: The new RIs for free κ and FLCR were shifted to a higher side from the manufacturer-adapted RIs. Based on the SDR cutoff value (>0.4), between-sex partition RIs were not required for all analytes except IgM. Validation using patients with hypo- or hypergammaglobulinemia and without multiple myeloma, was all within the new RI. BMI,

smoking, and exercise were not relevant sources of variation for any analyte.

Conclusions: Locally derived RIs for free light chains and immunoglobulins analytes specific for Saudis were established after careful consideration of various factors. These RIs were more reliable than those provided as guidance by the manufacturer, or from other countries, for appropriate classification and prediction of disease progression for Saudi patients.

Keywords: free kappa; free lambda; kappa/lambda ratio; gammopathy; reference interval; Saudi Arabia

Introduction

Multiple myeloma (MM) is considered the most serious and prevalent subtype of plasma cell dyscrasias. Patients with MM are characterized by the abnormal proliferation of B-cell malignancy of plasma cells, which secrete monoclonal immunoglobulins in the bone marrow.

The common techniques, serum protein electrophoresis (SPE) and immunofixation (IFE), are used to monitor cell proliferation and staging of the disease [1]. Sometimes the levels of free light chains (FLCs) are too low to be detected by urine IFE. Nevertheless, the same patients continued to have abnormal serum levels of FLCs, which suggests that serum FLCs are more sensitive in such patients [2]. However, in some diseases, such as systemic immunoglobulin light-chain disease (amyloidosis), the diagnosis cannot be entirely relied upon a single test but requires a combination of IFE and FLCs tests [3].

The measurement of FLCs and their ratio may be applied on patients with clinical benign monoclonal gammopathies, as in monoclonal gammopathy of undetermined significance (MGUS), to symptomatic patients, as in multiple myeloma (MM) which ends up with bone destruction and renal damage or amyloid light-chain (AL) amyloidosis. FLCs measurement on blood samples obtained from such patients can be used for monitoring disease progression and patient management. Therefore, it is important to determine the reference interval (RIs) of FLC in healthy individuals.

The incidence of MM in the Saudi population is less than in other parts of the world. In 2014, MM and lymphomas

***Corresponding author: Anwar Borai**, PhD, King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences (KSAU-HS), King Abdulaziz Medical City, Ministry of National Guard, Riyadh, Saudi Arabia, E-mail: boraiaa@nghi.med.sa. <https://orcid.org/0000-0001-6504-9559>

Kiyoshi Ichihara, Faculty of Health Sciences, Yamaguchi University Graduate School of Medicine, Ube, Japan. <https://orcid.org/0000-0002-6039-4537>

Waleed Tamimi and Abdulaziz Masaud, King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences (KSAU-HS), King Abdulaziz Medical City, Ministry of National Guard, Riyadh, Saudi Arabia

Samia Sobki, Central Military Laboratory & Blood Bank, Prince Sultan Military Medical City, Riyadh, Saudi Arabia

accounted for 9.6–11 % of cancer-related deaths in Saudi Arabia [4] and MM represents about 1 % of total cancer cases with an incidence of 1 % in males and 0.7 % in females [5]. One of the reported claims for low reported cases of MM in Saudis compared to other populations was due to the unavailability of serum FLCs testing in many hospitals [6]. Since 2020, testing for FLC has become more commonly available, especially in tertiary hospitals around the Kingdom.

RIs are used as guidance for the clinical management of patients when any clinical decision needs to be taken by the physicians. Therefore, it is important to derive a reliable RI for FLCs in the Saudi population.

In 2002, Katzmann estimated the RIs for free kappa (κ), free lambda (λ), and the free light chains kappa/lambda ratio (FLCR) [7], and they were adopted by the manufacturer (Binding Site Inc) in the kit inserts of Freelite® reagents as guidance only. The kit inserts indicate that the RIs may vary with age, sex, sample type, diet, and population; and that each laboratory should verify or, if necessary, determine its own RI, however most of the clinical laboratories are still using those RIs reported by Katzmann. Thus, in such laboratories, FLCR above or below Katzmann limits indicates the presence of monoclonal gammopathy (MG), as the FLCR is used as the monoclonal proliferation index and the current International Myeloma Working Group (IMWG) guidelines have been modified accordingly [8].

Therefore, establishing RIs for FLC that represent the local (Saudi) population is of paramount importance to implement the IMWG optimal screening algorithm for MG by using the technique of FLC in addition to SPE and IFE [8]. However, a recently published article discussed how the results of FLCs can be different from one manufacturer to another and how inappropriate RIs may contribute to a high false-positive diagnosis rate. In addition to this, the study addressed the importance of establishing FLCs RIs using local patient populations in collaboration with clinicians [9].

Indeed, discrepancies in RIs have been reported between different populations e.g., South Africa [10], China [11], and Spain [12]. Previously we have established RIs for clinical chemistry, immunoassays and complete blood count parameters in Saudi population [13–15]. Therefore, in this study, we continued our investigation by establishing RIs for the FLCs and other immunoglobulins (IgG, IgM and IgA) using the internationally harmonized protocol for conducting the global reference value (RV) study, which was issued by the International Federation for Clinical Chemistry (IFCC), Committee on Reference Intervals and Decision Limits (C-RIDL) [16]. This is the first study conducted that aimed to determine RIs for FLCs and immunoglobulins in the Saudi population.

Materials and methods

Recruited subjects

A total of 180 healthy subjects were eventually enrolled from three central regions in Saudi Arabia. Subjects were distributed according to regions: 60 from the west, 50 from the center, and 70 from the east region. The recruitment process followed the IFCC/C-RIDL protocol [16, 17]. Subjects were selected from different professions, genders, and ages. The study was approved by the Research Ethics Committee, King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences (KSAU-HS), King Abdulaziz Medical City, Ministry of National Guard, Saudi Arabia (NRJ21J/154/06). Each subject was asked to fill out a questionnaire about lifestyles, food, and drink habits, medications in use, past medical history, etc. Each subject signed a written informed consent. All recruited subjects were investigated for common metabolic diseases, hematological disorders, and recent infections by collecting separate blood samples.

Exclusion criteria included evidence of existing, pre-existing and/or suspected unidentified MG or plasma cell proliferative disease, diabetes, recent infection, inflammation, renal dysfunction, liver disease, pregnancy, anemia, or any hematological disorder. Subjects were not screened for malaria, intestinal parasites, human immunodeficiency virus, and other infectious diseases, as these incidences are low in Saudi Arabia. Nine subjects were initially excluded because of the presence of diabetes. The final number of males and females was 81 and 99, respectively (45 % male and 55 % female), with a total BMI of $26.9 \pm 6.4 \text{ kg/m}^2$ (mean \pm SD) and age of 41.4 ± 14.2 years. All subjects were healthy Saudi citizens of age ≥ 18 years and were recruited according to the harmonized protocol of IFCC/C-RIDL for the scheme of recruitment, sampling, specimen handling and statistical methods for data analysis.

Blood collection and handling

Recruited subjects were asked to fast for 10 h the night before sample collection. Venipuncture was set between 7 and 10 a.m. the following morning. Blood collection was carried out in the venesection area of the Pathology Department of each hospital in each of the three regions. Blood samples using a plain tube were taken for the measurement of immunoglobulins (IgG, IgM, IgA), free kappa (κ), and free lambda (λ). Additional plain and EDTA blood samples tubes were collected for a health screening of each subject.

Healthy status of each subject was investigated in each participating laboratory by the measurement of fasting glucose, renal function (creatinine, urea, and electrolytes), liver function (alanine aminotransferase, lactate dehydrogenase, aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, total bilirubin, albumin, and total protein), lipid profile (cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein), bone profile (calcium and phosphorus), C-reactive protein and complete blood count analysis.

Measurements

All biochemical and hematological samples were collected separately and processed immediately after blood collection in

each participating laboratory. Blood samples for FLCs and immunoglobulins were separated, aliquoted in cryotubes, and frozen at -80°C until analysis. Samples from Jeddah and Hassa regions were transported on dry ice to Riyadh (central region) laboratory for FLCs and immunoglobulins analysis. All laboratories in Riyadh, Jeddah, and Hassa are accredited by the College of American Pathologists.

All samples were assayed for IgG, IgM, IgA, free κ and free λ using Freelite[®] and anti-IgG, IgA, and IgM reagents (The Binding Site Group Ltd, Birmingham, UK) in an Optilite[®] analyzer. The principle of measurement was based on turbidimetry. All samples were processed using the same lot numbers. The low and high levels of QC materials were provided as part of the Optilite Freelite (The Binding Site) kits. The daily QC results, between and within-day reproducibility of the assays were within the acceptable limits. The analytical ranges were IgG (1.5–36.7 g/L), IgA (0.1–8.6 g/L), IgM (0.2–7.7 g/L), free κ (2.6–140.3 mg/L) and free λ (4.1–155.4 mg/L). Biochemical and hematological parameters were performed in each region using Architect and Alinity automated systems (Abbott Diagnostics).

Statistical analyses

Partitioning criteria: The method of data partitioning by age or sex was adopted from Ichihara, 2008 [18]. The variability between subgroups in standard deviations classified by gender or age was expressed as the standard deviation ratio (SDR). The standard deviations (SD) between males and females (SD_{sex}), between-age subgroups (SD_{age}), and net between-individual SD (SD_{indiv}) were calculated using two-level nested ANOVA, and the SDR for each factor was calculated as the ratio of SD/SD_{indiv}: i.e., between-sex (SDR_{sex}), and for between-age (SDR_{age}). The SDR threshold of 0.40 was considered a set value for partitioning reference values (RVs) [18]. In applying the ANOVA, the RVs were divided into five age groups (≤ 30 , >30 –40, >40 –50, >50 –60, >60 years). Whenever the RV was highly skewed, the RV was first log-transformed, and then the SD was inverse-transformed using the transformed scale [18].

To define the RV for each analyte, multiple regression analysis (MRA) [19] was performed on all measured parameters using possible sources of variation such as in sex, age, BMI, smoking, and blood pressure. The level of cigarette smoking was categorized as follows: none, ≤ 20 , and >20 cigarettes/day. The importance of the variable was considered significant when its standardized partial regression coefficient (rp), which corresponds to the partial correlation coefficient, >0.2 in its absolute value.

Derivation of RI: The parametric method using two-parameter Box-Cox formula based on the Gaussian transformation of RV was used to calculate RI [17, 19]. The bootstrap method was used to calculate confidence intervals (CI) for lower and upper limits (LL and UL). After secondary exclusion, the final dataset was randomly resampled, allowing multiplicate sampling until the data size equaled the size of the source dataset. The resampled dataset was used to calculate the RI. We repeated this resampling and recalculation of RI 50 times and predicted the CIs for LL and UL from LLs and ULs of iteratively calculated RI.

Results

Source of variation and correlation among analytes

Table 1 shows standardized partial regression coefficients (rp) for different sources of variations in free κ , free λ , FLCR and immunoglobulins (IgG, IgM, IgA) analyzed by MRA. A $|rp|$ value of ≥ 0.20 was considered a practically significant “effect size” for the rp. An age-dependent increase in RV was observed in free κ (rp=0.387), free λ (rp=0.266), FLCR (rp=0.260), and IgA (rp=0.238), whereas sex-dependency of IgM was highly significant (rp=0.450). The effect of sex and age on RVs of different immunoglobulins, free κ , free λ , and FLCR are shown in Figure 1. As an additional finding, a smoking habit was related to a decrease in IgG (–0.229) while no associations were shown regarding BMI.

Derivation of reference intervals

The RIs for the immunological parameters were derived using the parametric method. Based on the SDR threshold of 0.40, RIs for IgM were partitioned by sex only (Table 2). The RIs of IgM had shifted to a lower side in males than females (Figure 1). A summary of all the RIs for various measured parameters in relation to sex is shown in Table 3.

Saudi RIs in comparison to other countries

This is the first study that has used the IFCC global protocol for determining RIs for FLCs. Only a few studies reported

Table 1: A standardized partial regression coefficient (rp) between different sources of variation in free κ , free λ , FLCR and immunoglobulins (IgG, IgM, IgA) in total number of subjects.

Parameters	n	R	Sex	Age	BMI	Smk
Free κ	177	0.404	0.066	0.387	0.047	0.019
Free λ	177	0.314	0.043	0.266	0.113	0.058
FLCR	177	0.269	0.042	0.260	–0.076	–0.056
IgG	173	0.255	0.001	–0.111	–0.016	–0.229
IgA	175	0.276	–0.102	0.236	0.062	–0.012
IgM	174	0.499	0.450	–0.177	–0.025	–0.038

Partial regression coefficients (rp) were calculated using the multiple regression analysis. The $|rp|$ values that exceed 0.2 are shown in bold font and two graded background colors as moderate ($0.2 \leq |rp| < 0.30$) and highly significant ($|rp| \geq 0.3$). n, the number of subjects after secondary exclusion; free κ (kappa); free λ (lambda); FLCR, free light chains ratio; BMI, body mass index; Smk, level of smoking habits.

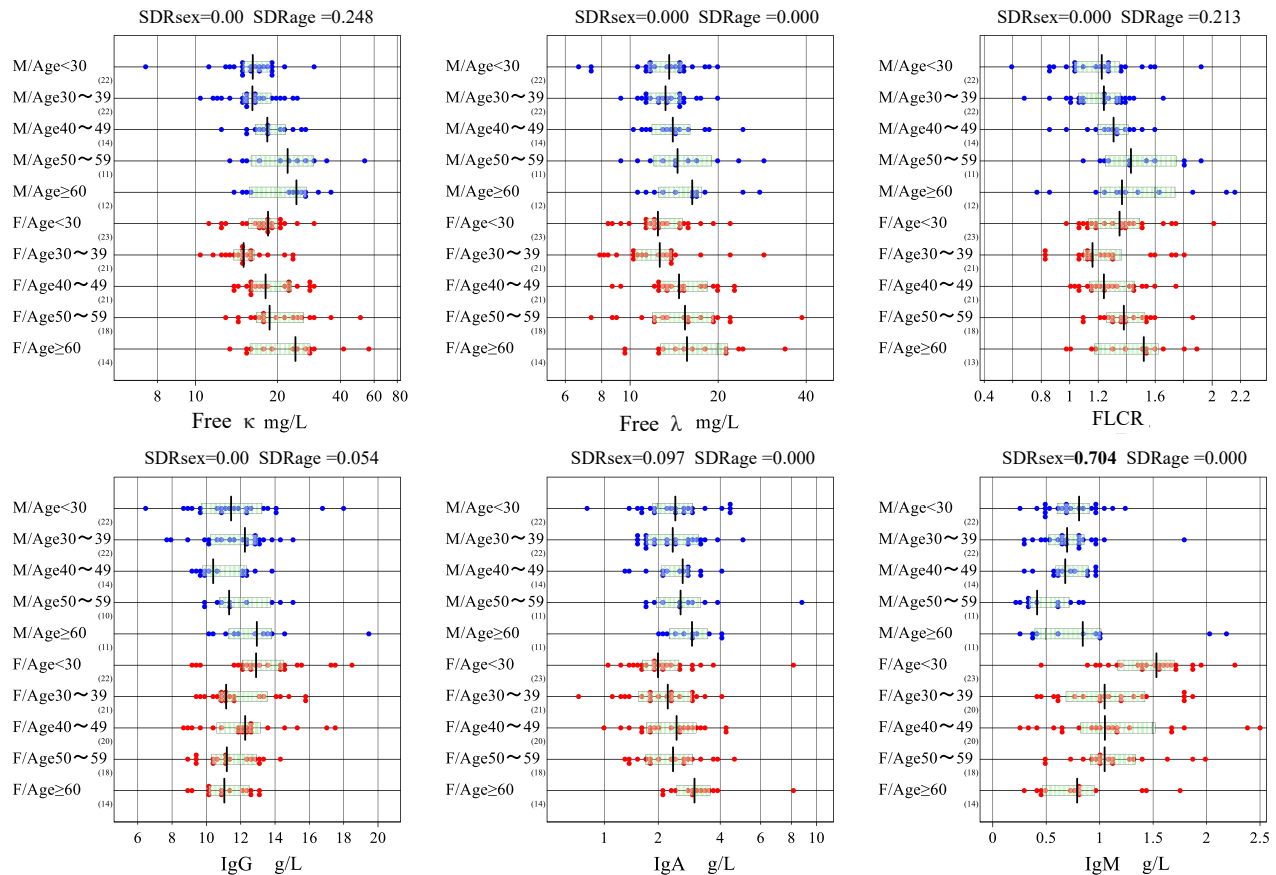


Figure 1: The effect of sex and age factors on RV for free light chains and immunoglobulins. RVs of FLCs and immunoglobulins classified in different subgroups of sex and/or age are shown (<30, 30–39, 40–49, 50–59, ≥60 years). The box in each scattergram represents the central 50 % range of values and the vertical bar in the middle represents median RVs. On top of each panel, the magnitudes of between-sex and between-age variations are shown as the standard deviation ratio for sex (SDRsex) and age (SDRage). Data points for male (M) and female (F) subjects were plotted in blue and red, respectively. No secondary exclusion was performed in plotting data. Free κ (kappa); free λ (lambda); FLCR; free light chains ratio.

FLC RIs, which we compared by bar chart, as shown in Figure 2. The RI limits (LL, UL) for free κ of the Saudi population were higher than those compared to the other countries: i.e., LL (11.4 mg/dL) and UL (38.5 mg/dL) of free κ, while free λ RI limits were compatible to most of the other countries except China.

RIs for FLCR, showed wider variations between-countries (Figure 2). It was shown that FLCR (0.85) of Saudi had the highest LL compared to the other countries. The RI designated for USA actually represents the Katzmann’s RI adopted by the manufacturer which was quite different from the RI of this study.

RIs validation for FLCR

In addition to our 180 healthy subjects, the FLCs assays were validated by comparing the results of the healthy subjects with abnormal levels of gamma-globulins. The FLCs results

Table 2: Standard deviation ratios (SDR) for the magnitude of sex and age effects on the immunoglobulins and free light chains tests.

Parameters	n	SDRsex	SDRage
Free κ	177	0.000	0.248
Free λ	177	0.000	0.000
FLCR	176	0.000	0.213
IgG	173	0.000	0.054
IgA	175	0.097	0.000
IgM	174	0.704	0.000

Nested ANOVA was applied for between-sex and -age differences. $SDR \geq 0.4$ (bold font) interpreted as a significant difference between subgroups. Free κ (kappa); free λ (lambda); FLCR, free light chains ratio; SDRsex, between-sex; SDRage, between-age.

of healthy subjects were compared with polyclonal hypergammaglobulinemia (n=33) and hypogammaglobulinemia (n=27) subjects. All these subjects used for validation showed no evidence of MM and were negative for monoclonal components at the time of analysis. The minimum

Table 3: Summary of RIs with 90 % confidence interval (CI) for various measured parameters in relation to sex.

Parameters	Sex	n	90 % CI		Reference interval			90 % CI UL	
					LL	Me	UL		
Free κ, mg/L	MF	177	9.9	11.7	11.4	17.5	38.5	34.0	41.6
Free λ, mg/L	MF	177	7.7	8.7	8.1	13.8	25.3	23.6	28.6
FLCR	MF	176	0.76	0.89	0.85	1.30	1.90	1.82	1.97
IgG, g/L	MF	173	7.83	8.76	8.6	11.8	17.0	16.30	17.76
IgA, g/L	MF	171	0.99	1.26	1.19	2.40	4.45	4.59	5.93
IgM, g/L	M	80	0.20	0.31	0.24	0.67	1.62	1.28	1.92
	F	95	0.28	0.46	0.35	1.14	2.20	2.09	2.30

CI, confidence interval derived from parametric methods; LL, lower limit; UL, upper limit; Me, median; free κ (kappa); free λ (lambda); FLCR, free light chains ratio; MF, male and female; M, male; F, female.

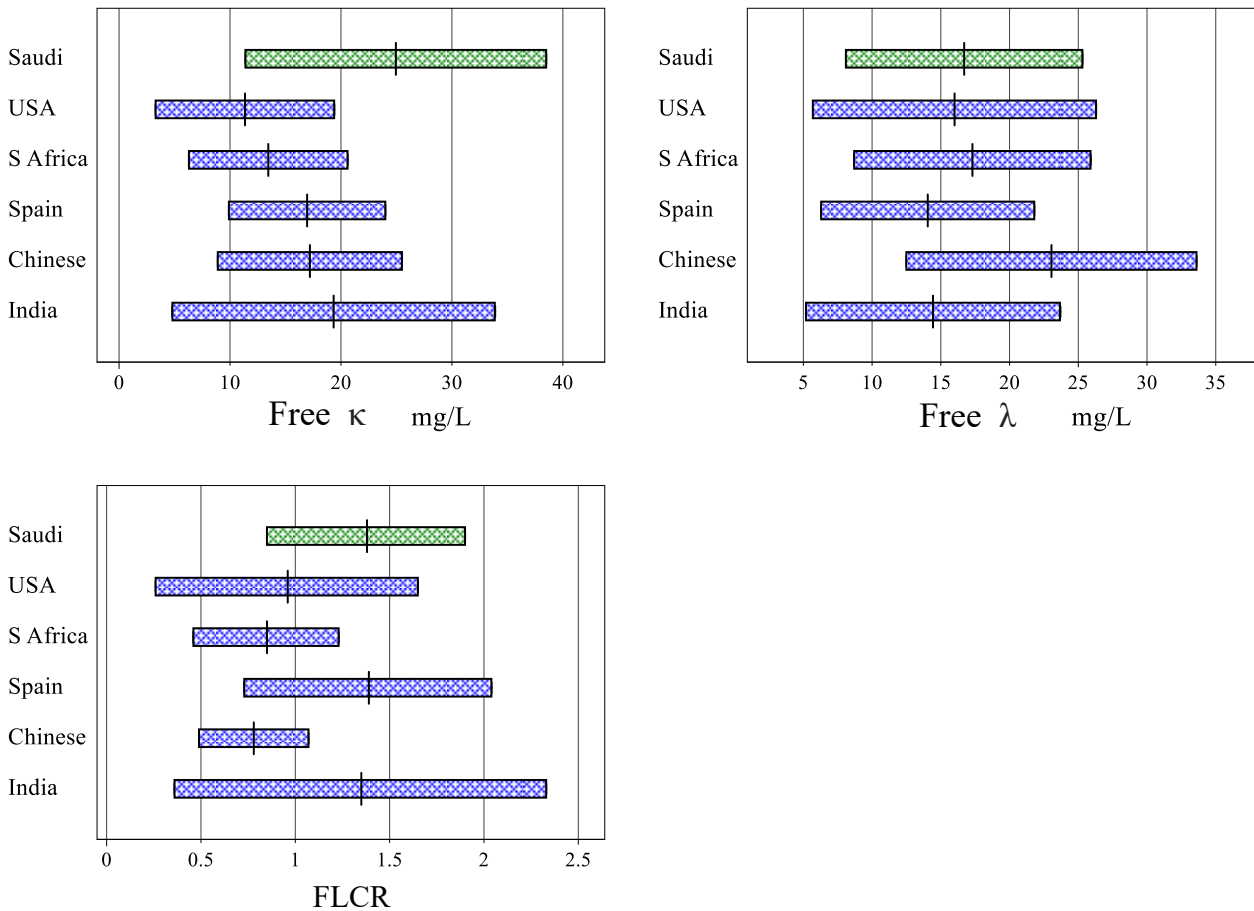


Figure 2: Saudi RIs for FLCs compared to other countries RIs. RIs of five countries (USA, South Africa, China, Spain and India) were compared by bar-chart for free κ, free λ and FLCR. The green bar represents the RI of this study. Free κ (kappa); free λ (lambda); FLCR, free light chains ratio.

and maximum readings for the gamma zone in healthy subjects were from 8.0 to 15.8 % and for subjects with hypo and hyper gamma-globulins were 1.4–7.6 % and 16.7–31.4 %, respectively.

The main aim of this study was to validate the diagnostic ability of our RI for FLCR, which can be used as an index for monoclonal proliferation. When applying these

RIs of FLCR, we found that 2.8 % of total healthy subjects were out of the established RI range (0.85–1.90). This percentage of outliers represented <5 % of the total results, which were considered acceptable. The same percentage for FLCR outliers was found among patients with polyclonal hyper- or hypo-gammaglobulinemia. When Katzmann’s RIs for FLCR were considered (0.26–1.65), the

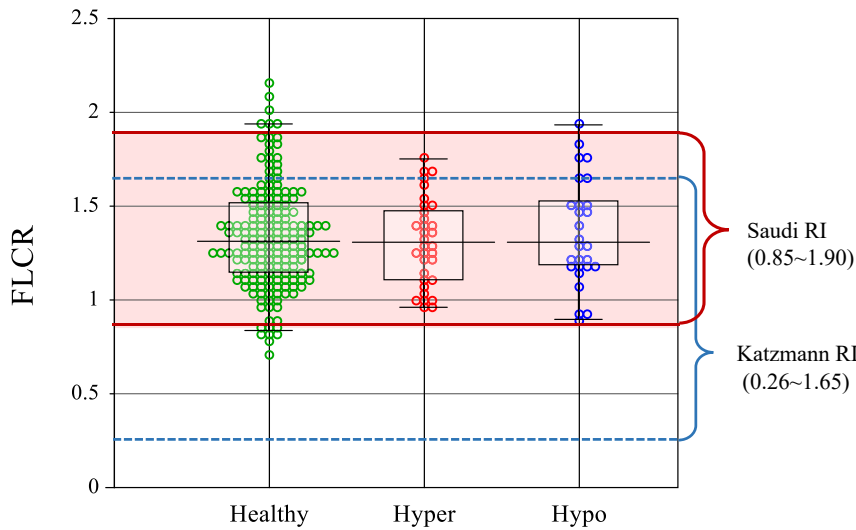


Figure 3: Box plot distribution of FLCR values obtained from healthy, polyclonal hypergammaglobulinemia and hypogammaglobulinemia subjects. The red lines represent Saudi RI (0.85–1.90); the blue dotted line represents Katzmann et al., RI (0.26–1.65) [7]. The rectangle box represents the middle 50 % of the values. The middle horizontal line within the rectangle box represents the median value. The top and bottom ends of the box represent the upper and lower quartiles of the values. The short horizontal lines below and above the box of each group represent 2.5 and 97.5 percentile, respectively. FLCR; free light chains ratio.

percentage of outliers increased in healthy (11.2 %), hypo- (15.4 %) and hyper-gammaglobulinemia (9.1 %) subjects (Figure 3). No statistically significant differences ($p > 0.05$) between-groups in terms of the mean of FLCR were observed by the Mann-Whitney test.

Discussion

Most of the commonly used RIs for free κ , free λ , and FLCR in clinical laboratories use Katzmann RIs as a reference interval (free κ : 3.30–19.40 mg/L; free λ : 5.71–26.30 mg/L; FLCR: 0.26–1.65). These values were based on the Caucasian population in the year 2002. The FLCR is important because it is used as an index for monoclonal proliferation. Therefore, we measured the RIs for the FLCR in our Saudi population to see whether they differ from the manufacturer or other population. It is recommended that each laboratory should establish their own RIs for its local population, and this was the main objective of our study.

In Figure 2, although we used the same methods and materials, one cannot ignore the contribution of other factors, such as statistical methods applied. In this study, the parametric method was employed to calculate the RIs, but no difference was observed in the upper and lower limits when compared to the nonparametric method.

In this study, we found that the upper and lower limits of Saudi RIs were significantly higher than the RIs adopted by the manufacturer [7]. Our free κ limits, LL and UL were considerably higher (245 and 98 %, respectively), while the free λ lower limit was higher 42 %; and for FLCR, the LL and UL limits were also higher than the manufacturer (227 and 15 % respectively). The shift in the FLCR UL from 1.65 to 1.90 and LL from 0.26 to 0.85 has almost certainly

improved the diagnostic and management decisions for MG in Saudis. The new UL for FLCR will reduce false positive cases for free κ while the new LL will reduce false negative cases for free λ in healthy Saudi population in comparison to the one adopted from the manufacturer, thereby enabling the clinician to avoid both patient misdiagnosis and unnecessary investigations for MG as well as financial burden.

We also observed that FLC concentrations increased with age in both males and females (Figure 1). Unfortunately, due to the small number of participants in our study, we could not determine specific RI for each subgroup or category. The increase in FLC concentration may be caused by a decrease of physiological eGFR with an increase in age, as reported in a previous study [20]. In addition, we found smoking to be associated with a significant decrease in serum IgG concentration. This outcome is compatible with the published studies [17, 18, 21, 22].

When FLC was compared by sex, no statistically significant differences were observed. For this reason, we considered obtaining a unified RI for both genders, and for immunoglobulins (IgG and IgA) but not for the IgM, in which case the RIs should be partitioned by sex. Our partitioning by sex for immunoglobulins was similar to the IFCC globally published studies, Ghana [23], India [24], Kenya [25] and Russia [26]. The IgM levels in females was noticed to be higher compared to males in all the countries and required sex-specific RIs. This may be linked to the higher estrogen level in females, which is involved in enhancing humoral immunity [27].

A study of Ichihara et al., stated that the closer the country or region was to the equator, the higher the serum concentrations of inflammatory markers such as IgG and CRP [28]. This could be due to the higher rate of infectious environment closer to the equator.

One of the limitations of this study was that the SPE test performed on subjects for validation only. However, health status of the subjects was determined by biochemical and hematological screening tests.

In comparison to other populations studied that utilized the same source of reagents (Freelite®), our study showed a higher level of free κ light chains for Saudis. Some studies have demonstrated an upward drift in Freelite® reagents for free κ measurement over time [29, 30].

In conclusion, we observed that lower and upper reference limits of FLCR are higher in the Saudi population than the limits adopted by the manufacturer. Therefore, establishing an appropriate population-specific RIs is important for a reliable diagnosis and management of patients with monoclonal proliferation.

Acknowledgments: On behalf of the Saudi Society for Clinical Chemistry (SSCC), we are grateful to King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences (KSAU-HS) for their support, and the collaborating laboratory technologists at King Abdulaziz Medical Cities for their valuable contributions to the success of the study. The authors are also grateful to The Binding Site company for providing the necessary materials, including instruments and reagents needed in all stages of the study.

Research ethics: The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Research Ethics Committee, King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences (KSAU-HS), King Abdulaziz Medical City, Ministry of National Guard, Saudi Arabia (NRJ21J/154/06).

Informed consent: Informed consent was obtained from all individuals included in this study.

Author contributions: AB, KI, SS contributed to the conception and designed of the project. Recruitment of subjects, sample collection and measurements were conducted by AA, AM and WT. The initial draft of the manuscript was written by AB and KI. SS, AM, and WT reviewed the draft manuscript and made comments and suggestions. The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The author(s) state(s) no conflict of interest.

Research funding: None declared.

Data availability: The raw data can be obtained on request from the corresponding author.

References

- Kyle RA. Sequence of testing for monoclonal gammopathies. *Arch Pathol Lab Med* 1999;123:114–8.
- Bradwell AR, Carr-Smith HD, Mead GP, Harvey TC, Drayson MT. Serum test for assessment of patients with Bence Jones myeloma. *Lancet* 2003;361:489–91.
- Palladini G, Russo P, Bosoni T, Verga L, Sarais G, Lavatelli F, et al. Identification of amyloidogenic light chains requires the combination of serum-free light chain assay with immunofixation of serum and urine. *Clin Chem* 2009;55:499–504.
- World Health Organization. Cancer Country Profile, 50. World Health Organization; 2014:1–4 pp.
- Bazarbashi S, Al Eid H, Minguet J. Cancer incidence in Saudi Arabia: 2012 data from the Saudi cancer registry. *Asian Pac J Cancer Prev* 2017;18: 2437–44.
- Alaskar A, Alsaeed A, Alsharif F, Alhashmi H, Alghamdi M. Multiple myeloma in Saudi Arabia: consensus of the Saudi lymphoma/myeloma group. *J Appl Hematol* 2019;10:37–44.
- Katzmann JA, Clark RJ, Abraham RS, Bryant S, Lymp JF, Bradwell AR, et al. Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. *Clin Chem* 2002;48:1437–44.
- Dispenzieri A, Kyle R, Merlini G, Miguel JS, Ludwig H, Hajek R, et al. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. *Leukemia* 2009;23:215–24.
- Fink SL, Wener MH, Rudolf JW, Nwosu A, Tacker DH, Kadkhoda K, et al. A universal reference interval for serum immunoglobulins free light chains may be outdated. *Clin Chem Lab Med* 2023. <https://doi.org/10.1515/cclm-2023-0473>.
- Zemlin AE, Rensburg MA, Ipp H, Germishuys JJ, Erasmus RT. Verification of serum reference intervals for free light chains in a local South African population. *J Clin Pathol* 2013;66:992–5.
- Liang YF, Chen WM, Wang QT, Zhai YH, Yang YJ, Liu JY, et al. Establishment and validation of serum free light chain reference intervals in an ethnic Chinese population. *Clin Lab* 2014;60:193–8.
- Morales-Garcia LJ, Pacheco-Delgado MS. Serum free light chain reference intervals in an Optilite and their influence on clinical guidelines. *Clin Biochem* 2021;92:54–60.
- Borai A, Ichihara K, Al Masaud A, Tamimi W, Bahijri S, Armbuster D, et al. Establishment of reference intervals of clinical chemistry analytes for the adult population in Saudi Arabia: a study conducted as a part of the IFCC global study on reference values. *Clin Chem Lab Med* 2016;54:843–55.
- Borai A, Ichihara K, Masaud A, Tamimi W, Bahijri S, Armbuster D, et al. Establishment of reference intervals for immunoassay analytes of adult population in Saudi Arabia. *Clin Chem Lab Med* 2020;58:1302–13.
- Borai A, Ichihara K, Bahijri S, Almasoud A, Tamimi W, Abdulhadi W, et al. Establishment of reference intervals for hematological parameters of adult population in the western region of Saudi Arabia. *PLoS One* 2023; 18:e0281494.
- Ozarda Y, Ichihara K, Barth JH, Klee G, Committee on Reference I, Decision Limits IFCC, et al. Protocol and standard operating procedures for common use in a worldwide multicenter study on reference values. *Clin Chem Lab Med* 2013;51:1027–40.
- Ichihara K, Kawai T. Determination of reference intervals for 13 plasma proteins based on IFCC international reference preparation (CRM470)

- and NCCLS proposed guideline (C28-P,1992): trial to select reference individuals by results of screening tests and application of maximal likelihood method. *J Clin Lab Anal* 1996;10:110–7.
18. Ichihara K, Itoh Y, Lam CW, Poon PM, Kim JH, Kyono H, et al. Sources of variation of commonly measured serum analytes in 6 Asian cities and consideration of common reference intervals. *Clin Chem* 2008;54: 356–65.
 19. Ichihara K, Boyd J, IFCC Committee on Reference Intervals and Decision Limits (C-RIDL). An appraisal of statistical procedures used in derivation of reference intervals. *Clin Chem Lab Med* 2010;48:1537–51.
 20. Campbell L, Simpson D, Shields A, Ferry B, Ramasamy K, Sadler R. In-house age-specific reference ranges for free light chains measured on the SPAPLus(R) analyser. *Ann Clin Biochem* 2020;57:138–43.
 21. Tarbiah N, Todd I, Tighe PJ, Fairclough LC. Cigarette smoking differentially affects immunoglobulin class levels in serum and saliva: an investigation and review. *Basic Clin Pharmacol Toxicol* 2019;125: 474–83.
 22. McMillan SA, Douglas JP, Archbold GP, McCrum EE, Evans AE. Effect of low to moderate levels of smoking and alcohol consumption on serum immunoglobulin concentrations. *J Clin Pathol* 1997;50:819–22.
 23. Bawua SA, Ichihara K, Keatley R, Arko-Mensah J, Ayeh-Kumi PF, Erasmus R, et al. Derivation of sex and age-specific reference intervals for clinical chemistry analytes in healthy Ghanaian adults. *Clin Chem Lab Med* 2022;60:1426–39.
 24. Shah SAV, Ichihara K, Dherai AJ, Ashavaid TF. Reference intervals for 33 biochemical analytes in healthy Indian population: C-RIDL IFCC initiative. *Clin Chem Lab Med* 2018;56:2093–103.
 25. Omuse G, Ichihara K, Maina D, Hoffman M, Kagotho E, Kanyua A, et al. Determination of reference intervals for common chemistry and immunoassay tests for Kenyan adults based on an internationally harmonized protocol and up-to-date statistical methods. *PLoS One* 2020;15:e0235234.
 26. Evgina S, Ichihara K, Ruzhanskaya A, Skibo I, Vybornova N, Vasiliev A, et al. Establishing reference intervals for major biochemical analytes for the Russian population: a research conducted as a part of the IFCC global study on reference values. *Clin Biochem* 2020;81:47–58.
 27. Taneja V. Sex hormones determine immune response. *Front Immunol* 2018;9:1931.
 28. Ichihara K, Ceriotti F, Tam TH, Sueyoshi S, Poon PM, Thong ML, et al. The Asian project for collaborative derivation of reference intervals: (1) strategy and major results of standardized analytes. *Clin Chem Lab Med* 2013;51:1429–42.
 29. Murray D, Dispenzieri A, Kumar S, Gill H, Vachon C, Snyder M, et al. Free light chain assay drift: potential for misdiagnosis? *J Appl Lab Med* 2020; 5:1411–3.
 30. Rindlisbacher B, Schild C, Egger F, Bacher VU, Pabst T, Leichtle A, et al. Serum free light chain assay: shift toward a higher kappa/lambda ratio. *J Appl Lab Med* 2020;5:114–25.