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Establishment of Reference Interval and Decision Limits (C-RIDLs) for routine and special Biochemical Parameters in healthy Nepalese volunteers aged (18-65) years

Ram Vinod Mahato[1*], Ananta Madhab Dutta[2], Madhab Lamsal[3], Kiyoshi Ichihara[4]

[1]Ayurveda Campus, Institute of Medicine, Tribhuvan University, Kirtipur, Kathmandu, Nepal, [2]Assam Down town University, Guwahati, India, [3]B.P. Koirala Institute of Health Science, Dharan, Nepal, [4]Department of Laboratory Medicine, Graduate school of Medicine, Yamaguchi University, Ube, Japan

ABSTRACT

Background: A national multicenter study was planned to derive reference intervals (RIs) in Nepalese healthy volunteers for 30 commonly tested biochemical analytes and to explore sources of variation in reference values.

Materials and Method: Healthy subjects (n = 617), age ≥18 to ≤65 years old, were enrolled on the basis of the global study protocol. Blood samples were collected, serum was separated, and then sera were collectively analysed in Yamaguchi University, Department of Laboratory Medicine, Ube, Japan, using Beckman Coulter reagents and an AU 480 analyzer. Multiple regression analysis and two-level nested ANOVA were applied to derive reference intervals and analyse the source of variations.

Results and Discussion: Gender, age at ≤45 and ≥ 45 years old, and partial regression coefficient (rp) ≥ 0.2 were considered for derivation of RIs and calculation of source of variations. RIs for serum total cholesterol and fractional cholesterols, urea, and ALT were found to shift conspicuously to the lower side. Whereas, UL of RIs for LDH and CK were shifted to the higher side.

Conclusion: RIs valid for the Nepalese population were established for the majority of biochemistry parameters. Age and gender partitioning were required for some analytes. RIs derived from this nationwide study can be applicable for (18-65) age groups. It is not applicable for <18 and >65 years of age.

Keywords: healthy volunteers, biochemical analytes, reference value, reference interval, biological sources of variation.

***Corresponding author**
Ram Vinod Mahato
Assistant Professor
Ayurveda Campus, Institute of Medicine, Tribhuvan University
Kirtipur, Kathmandu, Nepal
Email: ramvinodmahato42@gmail.com

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INTRODUCTION

The terminology of ''reference value'' was introduced by Nils-Erik Saris and Ralph Grasbeck in 1968.[1] Seventeen years later, the International Federation for Clinical Chemistry (IFCC) published six guidelines.[2] In successive years, it was concluded that 'normal values' were not sufficient and partly inappropriate; 'therefore, reference values' came into application. There was very significant advancement and implementation from the 1990s to 2008.[3,4]

IFCC and Clinical Laboratory Standard Institute (CLSI) instituted the most crucial footstep in the development of RIs by publishing the C28-A3 guideline in 2008.[5,6] The guidelines 'Defining, establishing, and verifying reference intervals in clinical laboratories' guide essential stages for selecting healthy subjects and total testing procedures to establish RIs. The CLSI guideline states that RIs are ranges of central 95% of results. The values from lower limit to upper limit are referred to as RIs. For example, RI for calcium is from 9.1 to 10.3 mg/dL or from 2.27 to 2.57 mmol/L. These limits are used for the interpretation of a test result reported by laboratory personnel in clinical decision making. The test results

generated from clinical laboratories have no meaning in isolation without referring to RIs, which are indeed fundamental for clinical medicine.[7, 8]

The C28-A3 guideline describes procedures required to execute an RI study in detail under the heading of a priori segregation of healthy individuals and control of the pre-analytical phase (stipulations of sampling conditions, specimen processing, etc.). Analytical phases (standardisation to attain traceability of test results) and a well-explained QC program. According to EU directives, all in vitro diagnostic device manufacturers are requested to provide appropriate RIs with their reagents and assay platforms.[9] As stated by the International Organisation for Standardisation (ISO) 15189 standard for clinical laboratory accreditation, every clinical laboratory routinely re-evaluates their own RIs.[10] In spite of requirements and the issuance of the guideline by the Clinical Laboratory Standardisation Institute (CLSI), the operation of reference intervals in most medical laboratories is still inadequate. Therefore, the FCC, Committee for Reference Intervals and Decision Limits (C-RIDL), proposed to conduct a national-level study using an elaborated and harmonised protocol with stipulations of Standard Operating Procedures (SOPs) for the purpose in 2012.[11] Furthermore, IFCC and CRIDL launched a worldwide multicentric study on RVs.[12]

MATERIALS AND METHODS

Healthy subjects (n = 617) were carefully chosen from communities, colleges, hospitals, and clinical laboratories of five major cities, namely, Pokhara, Kathmandu, Janakpur, Biratnagar, and Dharan of Nepal, on the basis of the protocol explained in (C28-A3) provided by (IFCC/CRIDL).[5]

Information about the health status was collected through the questionnaire. The health questionnaire and consent form were approved by the Institutional Review Board (IRB) of NHRC (approval number 57/2014). Gender, age, height, weight, history of smoking, alcohol intake, and exercise of healthy subjects were included in the questionnaire.

Recruitment of Healthy Volunteers : 1.1 Inclusion criteria: a. apparently healthy volunteers; b. age between 18 and 65 years; c. who understood the objectives and importance of the research; and d. participant should not be on regular medication for any chronic disease.

1.2 Exclusion Criteria: a. Individuals suffering from chronic diseases like diabetes, liver diseases, kidney diseases, thyroid diseases, hypertension, gout, depression, dyslipidaemia, coronary graft, cardiovascular disease, cancer, and who are on regular drug therapy; b. Individuals with HBV, HCV, and HIV careers; c. within 2 weeks of recovery from acute diseases, which required surgery and hospitalisation for pregnancy or within one year of delivery; d. alcoholics, smokers, on hormone therapy; women on oral contraceptives; and blood donors who donated in the past three months.

Blood collection and sample processing and measurement: Excessive physical work was avoided before three days, and eating and drinking on the night before 10-12 hours was restricted among healthy subjects to collect samples in the fasting. Physical stress and postural influence were minimised by allowing the volunteers to rest for 30 minutes prior to blood collection.[13] Five ml of blood collected from (n = 110 ± 10) eligible individuals of each of the five major cities in the morning (7 a.m.–10 a.m.) in a plain vacutainer was centrifuged at 2000 g for 10 minutes to separate serum, which was further stored at -80^{0C} in well-sealed freezing containers. Samples were transported to Yamaguchi University, Department of Laboratory Medicine, in a 10x10 box of well-sealed cryo-vials in dry ice. Thirty biochemical analytes adopted for the study were measured in Beckman Coulter (AU-480). Summary is presented in **Table 1**.

Table 1: Summary of assay methods and analytical performance

Analyte	Method	Unit	CV
Total Protein	Biuret Reagent, end point - Photometric	g/L	4.6
Albumin	ALB1, Bromocresol Green - Photometric	g/L	2.8
Total Bilirubin	DPD - Photometric	µmol/L	7.7
Urea	URUK, Urease, kinetic - Photometric	mmol/L	2.4
Uric acid	Uricase/POD - Photometric	µmol/L	1.2
Creatinine	CRERB, Jaffe rate blanked - Photometric	µmol/L	1.6
Sodium	Ion Selective Electrode (indirect) - Potentiometry	mmol/L	1.3
Potassium	Ion Selective Electrode (indirect) - Potentiometry	mmol/L	3.8
Chloride	Ion Selective Electrode (indirect) - Potentiometry	mmol/L	1.4
Calcium	Arsenazo lll - Photometric	mmol/L	1.4
Gamma-glutamyl transferase	IFCC - Photometric	U/L	2.2
Glucose	Hexokinase - Photometric	mmol/l	1.9
Magnesium	MGXY, Xylidyl blue - Photometric	mmol/L	1.9
Phosphate	PHMD, Phosphomolybdate UV - Photometric	mmol/L	1.8

Table 1 (continued)

Lipase	1,2-Diglyceride Hydrolysis	mmol/L	8
Total cholesterol	CHO-POD - Photometric	mmol/L	1.2
Triglycerides	GPO-POD - Photometric	mmol/L	4.1
High density lipoprotein cholesterol	POD – Photometric	mmol/L	2
Low density lipoprotein cholesterol	Calculated by Friedwald equation	mmol/L	1.6
Aspartate aminotransferase	IFCC without Pyridoxal-5P - Photometric	U/L	5.6
Alanine minotransferase	ALTIF-Tris buffer with pyridoxal-5P- Photometric	U/L	28.1
Alkaline phosphatase	IFCC - Photometric	U/L	18.4
HSC reactive protein	Immuno-turbidimetric Assays	mg/L	1.8
Amylase	IFCC - Photometric	U/L	8.1
Creatinine kinase	IFCC Reference - Photometric	U/L	8.1
Immunoglobulin A	turbidimetric Assays	mg/dL	1.3
Immunoglobulin G	turbidimetric Assays	mg/dL	1.1
Immunoglobulin M	turbidimetric Assays	mg/dL	7
Compliment C3	turbidimetric Assays	mg/dL	6.7
Compliment C4	turbidimetric Assays	mg/dL	8.34
Transferrin	Turbidimentry Assays	g/L	2.6
Ferritin	Turbidimentry Assays	g/L	8.2

List of thirty biochemical parameters: Thirty serum biochemistry parameters were considered for analysis which were Total protein (TP), Albumin (Alb), Total Bilirubin (TBil), Urea nitrogen (UN, Uric acid (UA), Creatinine (CRE), Sodium (Na+), Potassium (K+), Chloride (Cl-), Calcium (Ca^{2+}), Iron (Fe), Glucose (Glu), Total cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) γ-glutamyl Transferase Lactate Dehydrogenase (LDH), Amylase (AMY), Creatine Kinase (CK), Immunoglobulin G (IgG) Immunoglobulin A IgA), Immunoglobulin M (IgM), Complement Component 3 (C3), Complement Component 4 (C4), C-reactive Protein (CRP) and Transferrin (Tf).

Total protein: Biuret method and principle: In alkaline solution, the cupric ion reacts with peptides and proteins (two peptide bonds) to produce a violet-coloured complex, which is the protein present in the sample. The colour complex of the solution is measured at 540/660 nm.[14]

Protein + Cu^{2+} + OH-® Blue Violet Complex

Clinical significance: Total protein is measured for the diagnosis and treatment of kidney, liver, bone marrow, and other metabolic complications. It is more useful to have particular knowledge of other components of proteins like albumins and globulins.[15]

RESULTS

Socio-demographic characteristics of the participants: A total (n = 617) of apparently healthy subjects (graduate students, laboratory medicine professionals, university employees, and community people) were enrolled in the study. There were five collection centres, one in each major city of the country, namely, Pokhara, Kathmandu, Janakpur, Biratnagar, and Dhahran. All the abnormal samples were omitted based on extreme values that are Triglyceride ≥7 mmol/L, Glu ≥13 mmol/L, ALT ≥200 U/L, CK ≥800 U/L, and GGT ≥300 U/L. The final number of participants for the subsequent analyses were five hundred forty (540) who were involved in the study. Out of them (n = 540), 102 (59 males, 43 females) from Dharan, 79 (48 males, 31 females) from Biratnagar, 116 (62 males, 54 females) from Janakpur, 186 (88 males, 98 females) from Kathmandu, and 57 (24 males, 33 females) from Pokhara (**Figure 1**).

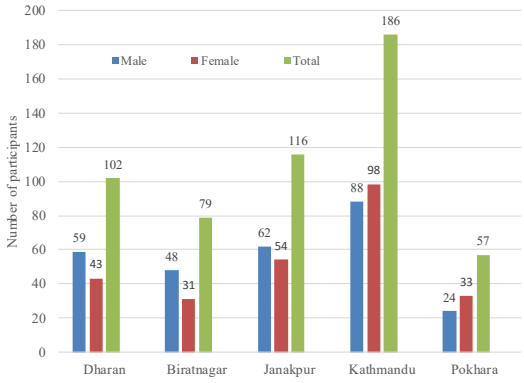

Figure 1. Citywise distribution of participants

There were 52% male and 48% female participants in the study. The distribution of gender has been shown in a pie chart (**Figure 2**).

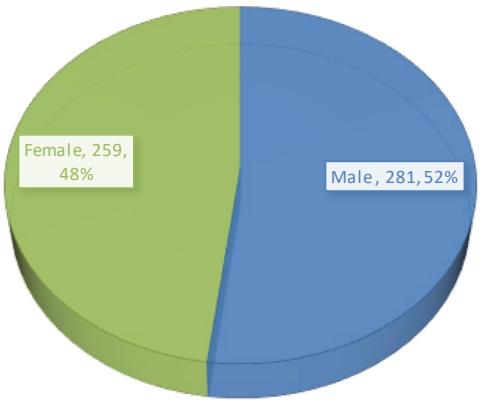

Fig 2: Genderwise distribution of participants

LAVE analysis : Table 2 shows food habits (vegetarian and non-vegetarian) (p = 0.001), regular exercise (p = 0.002), and blood group (p = 0.0001) are significant differences among the five cities.

Table 2: Descriptive characteristics of participants

Item	City	Biratnagar	Dharan	Janakpur	Kathmandu	Pokhara	P value
Age	n	79	100	124	191	60	P = 0.054
	Mean	37.9	41.8	40.6	38.9	36.8	
	SD	14.3	12.1	11	12.7	8.6	
Sex	F	29 (36.7)	39 (39.0)	55 (44.4)	99 (51.8)	33 (54.1)	P = 0.059
	M	50 (63.3)	61 (61.0)	69 (55.6)	92 (48.2)	28 (45.9)	
BMI	n	79	100	124	191	60	P = 0.043
	Mean ± SD	23.51 ± 4.26	25.09 ± 3.8	25.25 ± 4.94	24.48 ± 4.22	24.19 ± 4.02	
Abd Circ	n	79	100	124	190	59	P = 0.051
	Mean	85.97	88.33	90.02	88.36	91.24	
	SD	11.35	10.08	11.9	11.34	11.86	
Bld Grp	A	21 (28.4)	32 (34.8)	22 (17.7)	59 (32.2)	13 (23.2)	P = 0.0001
	AB	15 (20.3)	6 (6.5)	13 (10.5)	17 (9.3)	6 (10.7)	
	B	16 (21.6)	11 (12.0)	51 (41.1)	45 (24.6)	20 (35.7)	
	O	22 (29.7)	43 (46.7)	38 (30.6)	62 (33.9)	17 (30.4)	
DrkLvl	0	68 (86.1)	72 (72.0)	113 (91.1)	165 (86.4)	58 (95.1)	P = 0.046
	1	10 (12.7)	10 (10.0)	8 (6.5)	20 (10.5)	3 (4.9)	
	2	0 (0.0)	4 (4.0)	2 (1.6)	4 (2.1)	0 (0.0)	
	3~4	1 (1.3)	14 (14.0)	1 (0.8)	2 (1.0)	0 (0.0)	
SmkLvl	0	75 (94.9)	90 (90.0)	110 (88.7)	185 (96.9)	57 (93.4)	P = 0.758
	1~2	3 (3.8)	10 (10.0)	14 (11.3)	6 (3.1)	4 (6.6)	
ExerLvl	0	59 (74.7)	84 (84.0)	87 (70.2)	166 (86.9)	33 (54.1)	P = 0.002
	1	5 (6.3)	7 (7.0)	21 (16.9)	3 (1.6)	14 (23.0)	
	2~3	15 (19.0)	7 (7.0)	16(12.1)	25 (11.5)	14 (23.0)	
	4~7	0 (0.0)	2 (2.0)	1 (0.8)	0 (0.0)	0 (0.0)	
Veg	1	11 (13.9)	52 (52.0)	53 (42.7)	143 (74.9)	3 (4.9)	P = 0.000
	0	68 (86.1)	48 (48.0)	71 (57.3)	48 (25.1)	58 (95.1)	

[F=Female M=Male, Body mass Index (BMI), Abdominal Circumference (Abd Circ)

Bld Grp: Blood Group, DrkLvl: alcohol consumption; (0 =None, 1= Social drinker, 2-3 days per week, 4-5 days/per week, 5-7 days per week Smk Lvl:

smoking habits; Exer Lvl: regular exercise;

(0= none, 1= once a week, 2-3 days a week, 4-7 days a week, and Veg: vegetarian food, 1 = veg, 0 = non-veg)

RIs derived from comparing these data in 4 ways: parametric (P), non-parametric (NP), latent abnormal value exclusion LAVE (+), and LAVE (-) methods, LAVE (+) with LAVE and LAVE (-) LAVE. The RI was partitioned by sex with or without the application of the LAVE procedure (**Table 3**).

Table 3: Reference interval for thirty Biochemical parameters

Item	Units	LAVE	BMI	Sex	Age	n	90% CI of LL		LL	Me	UL	90% CI of UL
TP	g/L	(-)	All	MF	18-65	550	68.0	69.2	69	76	85	84.1-85.6
Alb	g/L	(-)	All	MF	18-65	546	38.7	39.6	39	44	51	50.3-51.5
Glb	g/L	(-)	All	MF	18-65	553	25.2	25.9	26	31	39	38.3-39.9
TBil	µmol/L	(-)	All	M	18-65	298	1.9	3.6	2.8	9.9	23.4	21.5-25.3
		(-)	All	F	18-65	254	1.9	2.9	2.4	7.7	20.0	18.3-21.8
Urea	mmol/L	(-)	All	MF	<45	367	1.73	1.85	1.8	3.1	5.3	5.05-5.53
		(-)	All	MF	>45	180	1.95	2.37	2.2	3.5	6.2	5.74-6.58
UA	µmol/L	(-)	All	M	18-65	295	219	237	228	346	477	462-492
		(-)	All	F	18-65	252	135	168	152	266	377	355-399
Cre	µmol/L	(-)	All	M	18-65	296	55	61	58	79	102	99-105
		(-)	All	F	18-65	253	42	47	44	59	75	72-79
Na	mmol/L	(-)	All	MF	18-65	539	133.2	134.6	133.9	138.1	143.8	143.1-144.5
K	mmol/L	(-)	All	MF	18-65	530	3.64	3.78	3.71	4.47	5.43	5.34-5.51
Cl	mmol/L	(-)	All	MF	18-65	548	98.0	99.5	98.8	104.0	109.2	108.5-110.0
Ca	mmol/L	(-)	All	MF	18-65	552	2.11	2.14	2.12	2.32	2.52	2.50-2.54
Fe	µmol/L	(-)	All	M	18-65	300	8.1	9.5	8.8	16.7	29.2	27.8-30.6
		(-)	All	F	18-65	253	4.0	5.8	4.9	12.3	24.5	22.9-26.0
Glu	mmol/L	(-)	<26	MF	18-65	332	3.08	3.37	3.23	4.57	5.93	5.65-6.21
TC	mmol/L	(-)	All	MF	<45	368	2.40	2.71	2.55	3.88	5.87	5.67-6.06
		(-)	All	MF	>45	184	2.54	2.90	2.72	4.47	6.45	6.15-6.74
TG	mmol/L	(-)	<26	M	18-65	187	0.37	0.51	0.44	1.25	3.81	3.06-4.55
		(-)	<26	F	18-65	154	0.33	0.42	0.37	0.95	3.00	2.54-3.47
HDL-C	mmol/L	(-)	<26	MF	18-65	341	0.28	0.31	0.30	0.75	1.53	1.43-1.63
Non-HDL	mmol/L	(-)	All	MF	<45	367	1.63	2.02	1.83	3.08	5.01	4.78-5.24
		(-)	All	MF	>45	184	1.76	2.20	1.98	3.65	5.64	5.40-5.88
LDL-C	mmol/L	(+)	All	MF	<45	291	0.84	1.09	0.97	2.03	3.25	3.09-3.41
		(+)	All	MF	>45	145	1.20	1.42	1.31	2.47	3.93	3.74-4.13
AST	IU/L	(+)	All	M	18-65	245	9.2	12.0	10.6	24.1	43.5	40.2-46.7
		(+)	All	F	18-65	199	7.6	9.8	8.7	18.9	31.7	28.3-35.1
ALT	IU/L	(-)	All	M	18-65	292	2.98	4.9	4	18	46	41.7-50.5
		(+)	All	F	18-65	197	2.8	4.7	4	13	28	24.3-31.6
LDH	IU/L	(-)	All	MF	18-65	548	63	76	70	156	238	228-248
ALP	IU/L	(-)	All	M	18-65	300	117	127	122	210	386	361-411
		(-)	All	F	18-65	254	101	116	108	201	344	324-364
GGT	IU/L	(-)	<26	M	18-65	186	8.8	12.7	11	26	100	78-122
		(-)	<26	F	18-65	151	7.5	9.9	9	15	32	23.7-40.1

CK	IU/L	(-)	All	M	18-65	298	12	20	16	87	295	250-341
		(-)	All	F	18-65	254	8	15	12	59	178	152-203
AMY	IU/L	(-)	All	M	18-65	296	37	44	41	82	150	138-163
		(-)	All	F	18-65	253	33	39	36	71	132	118-145
CRP	mg/L	(+)	<26	MF	18-65	299	0.11	0.14	0.13	0.85	7.83	5.93-9.73
IgG	g/L	(-)	All	MF	18-65	530	8.91	9.67	9.3	13.5	19.7	19.08-20.21
IgA	g/L	(-)	All	MF	18-65	525	1.08	1.26	1.17	2.14	3.94	3.79-4.09
IgM	g/L	(-)	All	M	18-65	298	0.31	0.42	0.36	1.00	2.53	2.25-2.81
	g/L	(-)	All	F	18-65	255	0.46	0.62	0.54	1.47	3.24	3.00-3.48
C3	g/L	(-)	All	MF	18-65	554	1.00	1.09	1.04	1.44	1.96	1.92-2.0
C4	g/L	(-)	All	MF	18-65	553	0.12	0.15	0.14	0.27	0.54	0.50-0.58
TF	g/L	(-)	All	M	18-65	299	2.04	2.13	2.09	2.67	3.40	3.31-3.50
		(-)	All	F	18-65	192	2.07	2.32	2.20	2.96	3.91	3.81-4.02

The need for partitioning by sex was primarily considered on the basis of SDRsex. The shades on the bar represent 90% CI for the limits of the RI predicted by the bootstrap method of 100 repetitive computations. The results of (TG, LDL-L, AST, ALT, GGT, and CRP) six representative analytes for M and F (**Figure 3**) and comparison of RIs for Nepalese with other collaborating countries (**Figure 4**).

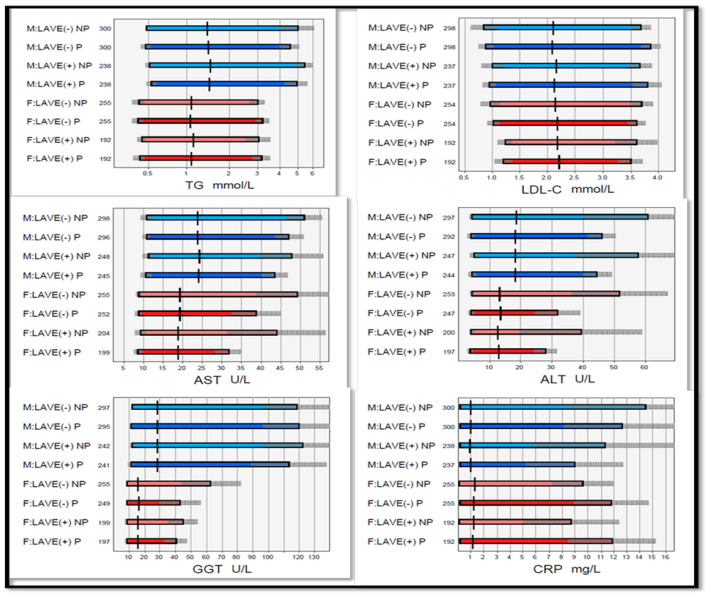

Fig 3: RIs and 90% CI derived in four w

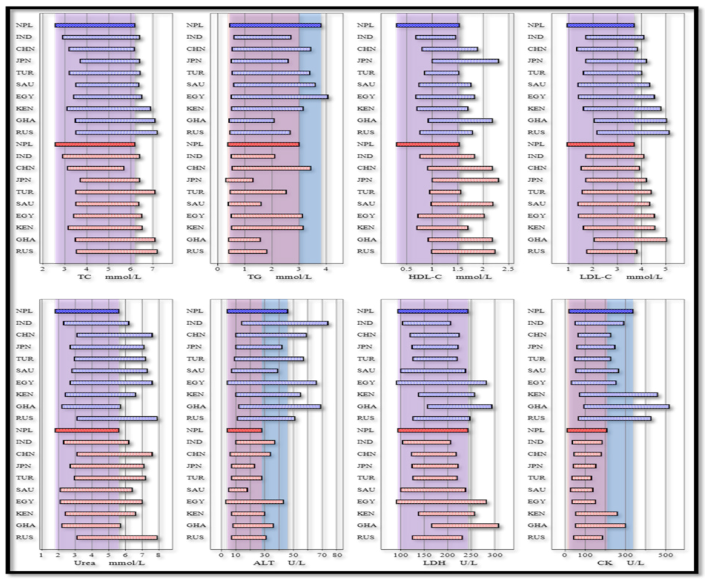

Fig 4: Comparison of RIs for Nepalese with other collaborating countries

Reference interval derived for thirty major biochemical parameters: Reference interval was calculated by parametric and non-parametric after application of secondary exclusion based on the LAVE method (**Table 3**). RIs calculated narrower by the parametric method than observed by the non-parametric, as shown for six representative analytes (**Figure 4**). Therefore, the parametric method was applied for all analytes to derive RIs except for those analytes that do not produce a Gaussian distribution by power transformation. Figures showed that efficacy of LAVE in improving the reference limits was seen in a limited number of nutritional markers: for example, AST, ALT, and GGT for both sexes, LDL-C for females, and CRP for males.

DISCUSSION

Reference intervals (RIs) are crucial information for the clinical decision-making process. Due to the lack of literature on RIs for Nepalese, the present study was designed. Derivation of country-specific RIs for thirty major biochemical parameters was the primary objective of the study, which was accomplished by conducting a multicenter study following IFCC C-RIDL's harmonised protocol. A common value-assigned serum panel was used for the measurement of each analyte; therefore, the RIs derived

in a standardised way can be applied in any Nepalese laboratory, as long as the laboratory standardised their assay procedure with the certified reference material (CRM). The standardised RIs of the other counties were compared with the RIs derived in this study.

In this study, the two-parameter Box-Ox formula for the parametric method was applied as suggested by the CLSI C28-A3 guidelines. It achieves Gaussian transformation for precise expectation of central 95% intervals.[16] Advantages of parametric methods were: 1. a reliably narrow CI of 90% RI limits; and 2. a narrow range of reference intervals in comparison to non-parametric methods.

The results of this study showed that a substantial number of unusual results are present at the extremities of the data distribution. This may be due to insufficient fasting glucose (Glu) and triglycerides (TG), due to the inclusion of subjects having predominant metabolic syndromes (Glu, TG, GGT, and ALT with very close relation to BMI), and TP, IgA, IgM, IgG, C3, and C4 due to concurrent inflammation and muscular exertion (AST, CK, and LDH) prior to the sample collection. A high proportion of outliers have unacceptable influence by the non-parametric but not much influence by the parametric method. The parametric method includes reference values from the centre of the distribution as well as from the third exclusion step, which truncates data outside the mean ±2.81 SD.[17] This fact is depicted in **Figure 4** by the small decrease in data size by the parametric method regardless of whether the LAVE method is applied or not. This characteristic of the parametric method underlines the importance of the non-parametric method for the purpose of RI.

Coping with the inevitable inclusion of hidden abnormal values among apparently healthy individuals, the latent abnormal value exclusion method was adopted to reduce the influence of latent diseases.[17] This study showed partial efficacy of the LAVE procedure. The LAVE procedure was effective for only four analytes: AST, ALT in females, and GGT, CRP in males, which contradicts the other studies like Turkey, Saudi Arabia, Russia, and China.[18,19]

BMI restricted to ≤ 26 kg/m^2, and the upper limits of TG, GLU, LDL-C, GGT, and CRP were lowered. This information shows that there are a substantial number of subjects with metabolic syndrome, and thus the application of LAVE improves its efficacy. As a matter of fact, in our trial of applying the LAVE method, allowing no abnormal results in any biochemical parameters, the ranges of RIs were appreciably narrowed and the number of reference values greatly reduced for the derivation of RIs.

The result showed that the stages of associations among 6 parameters in Nepal were weak when compared with other countries. Therefore, it is assumed that, despite a report of high incidence of metabolic syndrome in Nepal compared to other countries,[20] severity is less with weaker relations among nutritional parameters, which results in a in a lack of notable effect of the LAVE method.

Calculated RIs were compared with similar other studies and also compared with clinical decision limits suggested by NCEP. The derived reference intervals mirror the exact health status of a local population, contrary to CDLs, which are well-defined by agreement amongst doctors and destined for stoppage early interference of diseases.[21]

RIs for renal function and liver function parameters were found to be almost similar to other studies.[18] It has been noted that the upper limit for ALT (28 U/L) in Nepalese females is significantly lower than the study done in India (50–74 U/L)[22], the values are comparable to Chinese but higher than Saudis and Turkish.[23] The unstable concentration of liver enzymes among different countries may be due to vitamin supplements or differences in platforms used for measurements of enzymes.[24]

The study showed that the possibility of differences in assay platforms was because of careful standardisation of test results among the nations. Therefore, differences in dietary habits and susceptibility of liver cells to the consequence of obesity seem to be the most likely cause of large fluctuations in liver enzymes. The LL for UA was lower, while the UL was comparable to most populations except for Chinese.[25] Also, the upper limits of creatinine were comparable with India, Asian studies, Turkey, and China but lower than Saudis.[26]

RI for inflammatory parameters like CRP is a little lower, whereas IgG and IgA are comparable to other studies. RIs of C3 and C4 were comparable, but IgM is higher in Nepalese than the Asian studies. [25] Our finding is consistent with an Asian study done by the IFCC, which revealed a strong regional variation of CRP, IgG, IgA, C3, and C4, which indicates that countries adjoining the equator are inclined to have higher blood concentrations of those markers. It may be due to the higher microbial activities in the region near the earth's equator.[27] RIs for the following parameters (Cl, Ca, IP, IP, Fe, Tf, AMY, LDH, Na, and K) were analogous with other studies excepting CK, whose lower limit and upper limits were higher than Turkish, Chinese, Asians, and Saudis[28], which may be due to longer daily hours of standing among Nepalese.

For the requirement of different reference intervals of age and gender, a two-level nested ANOVA was applied. A threshold for SDR ≥0.4 was adopted for partitioning by age and sex.[29] SDRsex was considerably high (≥0.40) for 5 analytes: UA, Cre, Fe, GGT, and IgM, while SDR-age was significant for TC, TG, and LDL-C, non-HDL-C, and CRP. A one-way ANOVA was also performed after the separation of data by sex. Albumin, alkaline phosphatase, creatinine, GGT, glucose, LD, LDL-C, TG, urea, and uric acid showed unmatched SDRage between genders, respectively. Further study is required to explore the factors for variations of these parameters.

RIs derived in this study were standardised with a value-assigned serum panel and transferred to another Nepalese laboratory for nationwide relevance and applicability. Test results were standardised after measurement in a fully automated Beckman Coulter AU480. Results of a one-way ANOVA show no significant regional differences for most of the analyte across the five cities.

Therefore, the results of our study and statistical analysis of

findings show that any clinical laboratory in Nepal can adopt them as long as the analytical results are standardized. The practice of standardisation is a must for any laboratory that needs to import the reference intervals determined in other studies. Standardisation is to make the study results comparable across different assay systems by testing value-assigned certified reference material (CRM), which can be purchased from associated manufacturers, and recalibrate the analyzers. Therefore, any Nepalese laboratory that aims to use the RIs derived in this study can do so after recalibrating their lab's test results with the CRM.

Our study is an attempt to create acceptable data on RIs considering five major cities of Nepal and will definitely encourage other laboratories of the country to establish standardised RIs for better health prospects of Nepalese. A nationwide effort of concerned authorities with a large number of subjects' recruitment from various societies and geographical regions is required for the derivation of RIs.

LIMITATION OF THE STUDY

RIs derived are not applicable for subjects under 18 and above 65 years of age.

CONCLUSION

The reference intervals (RIs) reported in this study for thirty biochemical parameters can be used in all clinical laboratories of Nepal for the diagnosis and treatment of patients.

RECOMMENDATIONS

A wider study encompassing a large number of subjects from all sections of society is an urgent need. This type of study not only establishes the RIs but also enlightens clinicians for better health care of Nepalese people.

ETHICAL CONSIDERATION

The health questionnaire, consent form, and study design were approved by the Nepal Health Research Council's ethical review board. Approval number 57/2014.

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CONFLICT OF INTEREST

In this observational prospective study, recruitment of healthy subjects, blood sample collection, analysis, and result reporting were carried out according to the Declaration of Helsinki.

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