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Original Article

Derivation of Reference Intervals for commonly tested Biochemical analytes from five major Cities of Nepal.

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ABSTRACT

Introduction:

Reference interval (RIs) is the range of values provided by laboratory scientists in a convenient and practical form to support clinician for diagnosis, treatment and monitoring of a disease. Clinical laboratories in Nepal uses RIs, provided in the kit inserts by the manufacturers or from the scientific literature, established for western/European population. It is well known that population across the globe differs physiologically, genetically; ethnically, food habits and diet which have great impact on the reference values. Thus, it is inappropriate to use RIs that is not derived for local population. This approach highlights for establishing reference values for Nepalese population using the IFCC-CRIDL guidelines published in (C28-A3) **Methods:** Reference individuals were selected from healthy volunteers according to the IFCC/C-RIDL protocol in (C28–A3). After exclusion of abnormal samples, a total of 555, age and sex matched apparently healthy subjects of 18-65 were enrolled in the study. Blood samples were collected, serum were separated and stored in well-sealed cryo vials and finally measured collectively in Beckman Coulter AU480, a fully automatic chemistry analyzer. The sources of variations and need for partitioning were analyzed by multiple regression analysis and two-level nested ANOVA respectively. **Results:** We adopted a threshold of SDR $\geq$0.4 for city wise partitioning. The SDRs for between-city differences (SDRcity) were calculated, which revealed that there is no significant differences for most of the analytes among the cities,

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except for K, LDH and CK. Total protein, DrkLvl, and Standing Position also shows association but not significant to derive separate RIs. Sex-related changes were typically noted by the criterion of either SDR or BR for UA, Cre, Fe, GGT, IgM, and Tf. From the result, city partitioning was required for 3 analytes, K, LDH, and Drklvl, in male and 4 analytes in TP, K, CK, Standing position in female. **Conclusions**: The reference intervals for common biochemical parameters in five major cities were derived. Source of variation and need for partitioning of RI was calculated.

Keywords: Reference interval, Nepalese population, common biochemical parameters

Introduction

In 20th century the term **"**Reference Value "was first introduced by Ralph Grasbeck, Fellman and Nils-Erik Saris **[1]**. They published a paper entitled 'Normal Values and Statistics' as an initial study in the field of reference intervals (RIs) **[2].** In subsequent years it was realized that the terminology of 'normal values' was not adequate and even partially incorrect, so the term 'reference values' came into use. From 1987 to 1991, the International Federation of Clinical Chemistry (IFCC) published a series of 6 papers, recommending that each laboratory should produce its own reference interval following the IFCC-CRIDL and CLSI guidelines **[3].**

In spite of immense clinical importance of RVs, most laboratories across many developing countries including Nepal refers either from kit inserts provided by the manufacturers or from the scientific literature, which are based on Western/European population **[4].** It is well known that population across the globe differs physiologically, genetically, ethnically, geographically, lifestyle and food habits (frequency and type of food) which have great impact on the various biochemical analytes. Therefore, it is inappropriate to use RIs that do not represent the local population.

Nations around the world like (Argentina, Bangladesh, China, Egypt, Ghana, India, Japan, Kenya, Malaysia, Nigeria, Pakistan, Russia, Saudi Arabia, South Africa, the Philippines, the UK, Turkey, USA) have participated in the international multicenter collaborative project initiated by the C-RIDL of IFCC, followed the standard protocol for derivation of country specific RIs **[5].** Due to lack of study on Nepalese population specific RIs, this study is designed to determine RI for biochemical parameters in healthy Nepalese volunteers from five major cities of Nepal.

Materials and Methods

1. Study subjects

A total of 617 reference individuals were selected from apparently healthy volunteers from community, colleges, hospitals, and clinical laboratories of five developmental region of Nepal, according to the IFCC/C-RIDL protocol in (C28 –A3) **[6]**. The study design was approved by Nepal Health Research Council (NHRC), Institutional Review Board (IRB). Age, sex, height, weight, abdominal circumference, smoking history, alcoholic history and exercise habits are included in the general health questionnaire.

2. Inclusion Criteria/ Exclusion Criteria

Inclusion: Healthy volunteers aged 18-65 years who understood the objective and importance of the study were selected as reference individuals. **Exclusion:** i) Individuals on regular drug therapy for chronic disease (diabetes, hypertension, thyroid disorder, dyslipidemia, gout, depression, renal disease, cardiovascular diseases, coronary bypass graft ii) within two weeks' recovery from acute disease requiring hospitalization, or surgery iii) pregnancy or within one year of delivery iii) smoker, alcoholic, hormone therapy, women on oral contraceptive. Volunteers were requested to avoid excessive physical exertion/exercise/excessive eating and drinking and fast overnight for 10-12 hour **[6]**

3. Sample Collection and handling

The fasting blood samples were collected from 120 subjects from each five centers between 7:00-10:00 am, serum were separated and refrigerated in a cryo-vials. Serum samples were measured by fully automated biochemistry analyser, Beckman Coulter (BC480) in the Clinical Laboratory [7].

Fig. 1: shows the sample collection sites (Biratnagar, Dharan, Janakpur, Kathmandu, Pokhara

4. Analytes to be measured

Total protein (TP), albumin (Alb), total bilirubin (TBil), urea, uric acid (UA), creatinine (Cre), sodium (Na), potassium (K), chloride (Cl), calcium (Ca),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-glutamyltransferase (GGT), lactate dehydrogenase (LDH), amylase (AMY), creatinine kinase (CK), immunoglobulin G, A, and M (IgG, IgA, IgM), complement component 3 and 4 (C3, C4), C-reactive protein (CRP), transferrin (TF) by use of Beckman-Coulter/Olympus AU480 biochemistry auto-analyzer. The reagents, calibrators and quality control sera were proprietary to the manufacturer. Computed parameters were globulin (Glb) as (TP–Alb), and non-high density lipoprotein cholesterol (nonHDL) as nonHDL = TC–HDL-C.

Results

1. Study subjects

A total of 617 serum samples were included in the analysis process, 62 samples were excluded applying Latent Abnormal Value Exclusion (LAVE). Remaining 555, Male 300 and Female 255 were analyzed by parametric and non-parametric methods for calculation of RIs. The age of reference individuals ranged from 18-65 years. Average age was 38.73±12.24yrs.

Gender wise ratio of participants were 54:46% as shown in the pie chart fig 1. Out of which, 79 (50 male, 29 female) from Biratnagar, 100 (61 males, 39 females) from Dharan 124 (69 male, 55 female) from Janakpur, 191 (99 male, 92 female) from Kathmandu and 61 (28 male, 33 female) from Pokhara, male female ratio and citiwise distribution of participants has been shown in **Fig 2 and 3** respectively.

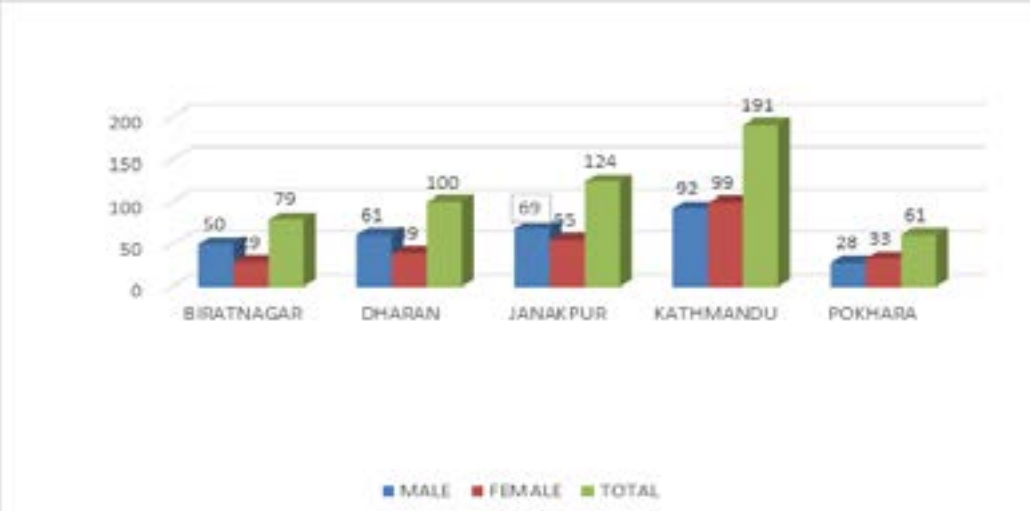

Fig. 3: Shows distribution of Male and Female recruited in RI study

Table 1: Demographic Profile of Participants

	City	Biratnagar	Dharan	Janakpur	Kathmandu	Pokhara	
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	1	29 (36.7)	39 (39.0)	55 (44.4)	99 (51.8)	33 (54.1)	P=0.059
	0	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	23.51	25.09	25.25	24.48	24.19	
	SD	4.26	3.8	4.94	4.22	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)	

2. Demographic Profile of Participants

Abd Circ: abdominal circumference; BMI: body mass index; Bld Grp: Blood Group, DrkLvl: alcohol consumption; (0= None, 1=social drinker, 2-3 days per week, 4-5 days/week, 5-7 day/per week) SmkLvl: smoking habits; ExerLvl: regular exercise; (0=None, 1= once a week, 2-3 days a week, 4-7 days a week, and Veg: vegetarian food. [0=male, 1= female]. P values in Table 1 for food habits (vegetarian and non-vegetarian, p=0.000) ExerLvl: regular exercise, p=0.002) and BldGrp: Blood Group, p=0.0001) shows significant difference among the five cities.

Table 2: Reference intervals on the basis of magnitude of between-city differences SDRcity

Analyte	SDRsex	SDRcity	SDRcityM	SDRcityF
TP	0.000	**0.311**	0.187	**0.418**
Alb	**0.402**	0.227	0.098	**0.312**
Glb	**0.480**	0.242	0.172	**0.321**
TBil*	0.298	0.242	0.265	0.205
Urea*	0.272	0.095	0.000	0.151
UA	**0.900**	0.092	0.113	0.054
Cre*	**1.396**	0.132	0.046	0.208
Glu*	0.000	0.188	0.155	0.233
TC	0.072	0.109	0.136	0.043
TG*	**0.310**	0.155	0.113	0.204
HDL-C	0.085	**0.355**	**0.319**	**0.389**
LDL-C	0.000	**0.301**	**0.343**	0.226
nonHDL-C*	0.162	0.000	0.000	0.000
Na	0.000	0.227	0.145	0.294
K	0.000	**0.553**	**0.596**	**0.502**
Cl	0.295	0.100	0.125	0.059
Ca	0.079	0.218	0.144	0.276
Fe	**0.600**	0.106	0.156	0.000
AST*	**0.318**	0.275	0.277	0.274
ALT*	0.263	0.292	**0.309**	0.262

Analyte	SDRsex	SDRcity	SDRcityM	SDRcityF
LDH*	0.000	**0.398**	**0.450**	**0.303**
ALP*	0.048	0.295	0.259	**0.338**
GGT*	**0.658**	0.068	0.000	0.147
CK*	**0.322**	**0.419**	**0.420**	**0.417**
AMY*	0.297	0.137	0.000	0.239
CRP*	0.000	0.127	0.000	0.189
IgG	0.224	0.215	0.153	0.272
IgA*	0.000	0.070	0.125	0.000
IgM*	**0.527**	0.000	0.000	0.000
C3	0.000	0.229	0.230	0.227
C4*	0.000	0.142	0.144	0.139
Tf	**0.461**	0.155	0.113	0.178
Age	0.203	0.171	0.000	0.291
BMI	0.000	0.144	0.000	0.215
Abd Circ	0.110	0.110	0.058	0.148
DrkLvl	0.284	**0.397**	**0.418**	0.078
SmkLvl	0.263	0.148	0.157	0.079
ExerLvl	0.000	0.206	0.215	0.190
Stand	0.000	**0.302**	0.120	**0.449**
Sit	0.000	0.263	0.290	0.219

SDRsex is calculated for male and female and each city by 2N-ANOVA. SDR ≥ 0.3 is indicated by bold font, and SDR ≥0.4 was marked by background color in two grades (<0.6: light orange; ≥ 0.6: orange). * indicates that SDRsex, and SDRcity were computed after excluding individuals with BMI ≥26 kg/m2 to adjust for confounding influence of sex related change in BMI.

3. Partitioning of reference interval on the basis of magnitude of between-city SDR city.

To judge the need for partitioning of reference values by city, SD ratio based on two-level nested ANOVA (2N-ANOVA) was calculated. We adopted a threshold of SDR ≥0.4 for city wise partitioning of reference intervals. The SDRs for between-city differences (SDRcity) were calculated as listed in **Table 2** which revealed that there is no significant differences for most of the analytes among the cities, except for K, and CK in both sex. While total protein and standing position, in female and LDH and DrkLvl in male also shows association but not significant to derive separate RIs. Sex-related changes were typically noted by the criterion of either SDR or BR for Alb, Glb, UA, Cre, Fe, GGT, IgM, and Tf as shown in **Table 2.** From the result, city partitioning was required for 3 analytes, K, LDH, and Drklvl, in male and 4 analytes in TP, K, CK, and standing position in female.

Table 3: Reference interval derived for male and female of five 5 major cities

Item	City	BRT		DRN		JNK		KTM		POK	
	Sex	M	F	M	F	M	F	M	F	M	F
TP	Me	74.3	77.5	72.6	71.2	74.6	74.0	73.5	73.8	74.3	74.8
	LL	71.6	74.0	70.3	68.8	72.2	72.1	70.8	71.5	71.6	72.4
	UL	78.2	81.3	74.2	72.8	76.0	76.7	76.0	76.7	76.0	77.2
Alb	Me	47.2	47.5	46.5	43.9	47.3	45.7	46.8	44.8	44.8	46.1
	LL	45.1	44.0	45.0	42.5	46.4	44.3	45.3	43.5	43.5	43.6
	UL	49.5	49.4	48.8	45.6	49.0	47.1	48.0	47.0	47.0	48.1
TBil	Me	6.8	5.1	10.0	6.7	10.3	7.0	11.3	8.1	8.1	9.3
	LL	5.4	4.0	7.1	4.5	7.5	5.6	8.2	5.6	5.6	7.7
	UL	10.4	10.8	12.4	10.5	13.2	9.1	14.5	10.9	10.9	13.8
Urea	Me	3.42	3.17	3.66	2.99	3.42	3.03	3.47	3.29	3.29	2.69
	LL	2.76	2.42	2.81	2.57	2.84	2.7	2.93	2.56	2.56	2.21
	UL	4.31	3.75	4.24	3.82	4.25	3.57	4.07	3.82	3.82	3.35
UA	Me	353	258	320	277	342	265	355	264	264	264
	LL	288	225	289	242	315	221	314	240	240	225
	UL	400	281	363	300	398	290	386	292	292	301
Cre	Me	79	57	78	58	75	56	80	60	60	61
	LL	73	52	68	54	70	51	74	56	56	56
	UL	86	62	84	65	85	62	86	65	65	65
Glu	Me	4.36	4.35	4.52	4.49	4.7	4.62	4.84	4.75	4.75	4.64
	LL	4.04	4.11	4.25	4.12	4.4	4.39	4.41	4.23	4.23	3.89
	UL	4.84	4.68	4.78	4.83	5.08	5.06	5.3	5.17	5.17	4.96
AST	Me	15	12	18	13	22	20	23	18	18	23
	LL	11	9	14	11	15	14	18	14	14	20
	UL	19	15	22	16	30	26	28	21	21	27
ALT	Me	10	8	11	9	18	16	24	16	16	25
	LL	8	6	9	8	12	10	17	11	11	20
	UL	15	11	17	12	33	28	32	18	18	33
ALP	Me	218	194	208	189	241	250	251	229	229	225
	LL	184	163	175	149	187	214	217	188	188	196
	UL	267	242	236	234	309	304	310	278	278	282
GGT	Me	24	14	25	17	26	15	27	16	16	19
	LL	18	11	15	13	20	13	17	12	12	15
	UL	42	18	44	20	53	23	41	21	21	25
LDH	Me	103	111	109	108	137	162	154	149	149	185
	LL	81	95	89	87	106	125	133	124	124	167
	UL	122	127	132	140	187	197	181	171	171	234
AMY	Me	82	68	78	60	88	79	85	74	74	66
	LL	66	52	61	49	70	62	67	59	59	52
	UL	99	81	102	73	106	98	109	86	86	80
CK	Me	67	31	100	63	64	70	118	73	73	114
	LL	37	19	66	42	44	34	81	42	42	82
	UL	98	53	150	95	133	101	172	97	97	158
TC	Me	3.69	3.79	4.12	3.92	3.90	4.29	4.29	3.98	3.98	4.24
	LL	3.30	3.16	3.39	3.29	3.37	3.56	3.41	3.36	3.36	3.69
	UL	4.55	4.67	5.07	4.94	4.78	4.79	4.96	4.44	4.44	4.50

Table 3: Reference interval derived for male and female of five 5 major cities

Item	City	BRT		DRN		JNK		KTM		POK	
	Sex	M	F	M	F	M	F	M	F	M	F
TG	Me	1.35	0.73	1.27	1.17	1.41	1.21	1.45	1.14	1.14	1.02
	LL	0.82	0.53	0.84	0.65	0.96	0.90	0.95	0.68	0.68	0.82
	UL	1.71	0.99	1.83	1.47	2.37	1.48	2.21	1.62	1.62	1.74
HDL-C	Me	0.60	0.52	0.79	0.82	0.58	0.80	0.79	0.83	0.83	1.03
	LL	0.41	0.40	0.70	0.62	0.49	0.62	0.59	0.61	0.61	0.91
	UL	0.89	0.92	0.99	0.96	0.96	1.18	0.97	0.99	0.99	1.19
LDL-C	Me	1.98	1.94	2.12	2.29	2.12	2.25	2.60	2.31	2.31	2.77
	LL	1.55	1.52	1.77	1.85	1.51	1.85	1.91	1.99	1.99	2.26
	UL	2.32	2.43	2.98	2.90	2.68	2.90	3.02	2.78	2.78	2.99
Ca	Me	2.31	2.32	2.31	2.27	2.36	2.29	2.34	2.31	2.31	2.29
	LL	2.24	2.25	2.22	2.15	2.29	2.23	2.28	2.26	2.26	2.23
	UL	2.42	2.53	2.36	2.32	2.40	2.37	2.40	2.37	2.37	2.35
Fe	Me	16.5	12.8	17	12.2	15.5	11.9	18	12.9	12.9	11.5
	LL	14.5	9.8	13.4	10.1	12.9	8.3	15.2	9.6	9.6	9.7
	UL	21.4	16.2	21.6	14.7	18.3	14.2	22.8	16.9	16.9	16.1
IgG	Me	13.8	16.0	12.8	13.6	13.5	14.2	12.9	13.7	13.7	14.6
	LL	12.5	13.6	11.0	11.4	12.3	12.6	10.9	12.0	12.0	12.8
	UL	16.0	18.5	14.5	15.2	15.0	16.2	14.6	15.8	15.8	15.5
IgA	Me	2.0	2.3	2.1	1.9	2.3	2.1	2.1	2.2	2.2	2.4
	LL	1.6	2.0	1.6	1.7	1.8	1.7	1.6	1.8	1.8	1.8
	UL	2.5	2.8	2.4	2.6	2.7	2.6	2.8	2.7	2.7	2.9
IgM	Me	0.86	1.65	1.12	1.35	1.06	1.50	1.06	1.47	1.47	1.57
	LL	0.66	1.20	0.69	1.07	0.88	1.12	0.71	0.98	0.98	1.14
	UL	1.11	2.05	1.55	1.96	1.28	1.99	1.44	1.78	1.78	2.01
C3	Me	1.29	1.33	1.18	1.31	1.27	1.28	1.23	1.21	1.21	1.27
	LL	1.18	1.20	1.12	1.17	1.19	1.16	1.07	1.11	1.11	1.13
	UL	1.40	1.44	1.34	1.49	1.40	1.46	1.35	1.35	1.35	1.32
C4	Me	0.28	0.27	0.26	0.23	0.29	0.28	0.25	0.26	0.26	0.27
	LL	0.24	0.21	0.22	0.19	0.23	0.23	0.20	0.20	0.20	0.21
	UL	0.34	0.32	0.31	0.33	0.35	0.36	0.32	0.33	0.33	0.32
CRP	Me	0.80	1.05	0.90	1.80	1.30	1.60	0.80	0.80	0.80	1.25
	LL	0.50	0.50	0.50	0.70	0.60	0.58	0.50	0.40	0.40	0.55
	UL	1.58	2.00	1.80	2.90	3.75	3.75	1.38	2.10	2.10	3.40
Tf	Me	2.79	3.04	2.61	2.75	2.71	3	2.65	2.87	2.87	3.09
	LL	2.57	2.61	2.35	2.41	2.56	2.78	2.4	2.59	2.59	2.77
	UL	3.04	3.37	2.88	3.06	2.99	3.19	2.87	3.22	3.22	3.6
Na	Me	138.5	139	138	137.2	138.2	139	138	137.2	137.2	138
	LL	137.3	137	136.3	135.2	136.4	136.6	136.6	136.2	136.2	136.3
	UL	142.5	142.6	139.1	138.6	139.9	141.1	139.3	138.8	138.8	138.6
K	Me	4.37	4.35	4.63	4.52	4.3	4.32	4.69	4.72	4.72	4.28
	LL	4.1	3.97	4.26	4.15	4.09	4.07	4.5	4.42	4.42	4.13
	UL	4.58	4.65	4.9	4.72	4.48	4.46	5.03	4.94	4.94	4.49
Cl	Me	104.1	104.4	103	103.9	103.2	105.5	103.7	104.9	104.9	104.1
	LL	102.1	101.7	100.5	102.3	101.6	103.2	102.1	103.7	103.7	102.8
	UL	105.4	107.2	103.9	105.2	105.4	107.2	104.9	105.9	105.9	105.5

BRT= Biratnagar, DRN=Dharan, JNK= Janakpur, KTM=Kathmandu, POK=Pokhara

LL= Lower Limit, UL= Upper Limit, Me= mean value

Discussion

Reference intervals are very essential for the diagnosis and treatment of disease. This study was designed for derivation of RIs in five major cities of Nepal. It was accomplished by conducting a multicenter study followed by CLSI/IFCC guidelines. This study applied modified (two-parameter) Box-Cox formula for the parametric method. It invariably succeeded in achieving Gaussian transformation for precise anticipation of central 95% intervals **[8, 9].**

According to result obtained, substantial number of abnormal results were present at the both ends of data distribution. This may be due to insufficient fasting, inclusion of subjects with metabolic syndrome, concurrent inflammation, and muscular exertion. High proportion of outliers have unacceptable influence on RIs by the non-parametric method but not much influenced by parametric method. Parametric method includes reference values from the center of the distribution and also includes third exclusion steps which truncated the values outside the mean ± 2.81 SD **[10].**

In coping with inevitable inclusion of hidden abnormal values, LAVE method was adopted to exclude the influence of latent disease and inappropriate samples **[11, 12].** To narrow down the RI for those analytes that have some association with the reference analytes is the advantage of LAVE method, whereas the RIs of analytes that have no relation with the reference analytes were not affected by this procedure. Partial efficacy of the LAVE procedure was found in this LAVE procedure, which contradicts from the other studies like Turkey, Saudi Arabia, and China **[13-15].**

The presence of between city differences SDRcity were calculated based on two-level nested ANOVA (2N-ANOVA). SD ratio was calculated and adopted a threshold of SDR ≥ 0.4 for city wise partitioning. The SDRs for between-city differences (SDRcity) is listed in **Table 2.** For most of the parameters no citiwise partitioning is required, because SDRcity value is lower than the threshold value SDR ≥ 0.4, except for K, and CK. A possible cause of which seemed to a difference in time before separating serum from blood by the location. TP, DrkLvl, and Standing Position also shows association but not significant to calculate RIs. Sex-related changes were typically noted by the criterion of either SDR or BR for UA, Cre, Fe, GGT, IgM, and Tf. In this study an attempt has been made to derive citiwise acceptable RIs considering five major cities of Nepal.

Conclusion

Reference Interval (RIs) from well-defined healthy Nepalese between 18-65 years of age were derived for thirty major biochemical parameters by the application of up-to-date statistical methods following the internationally harmonized protocol elaborated by IFCC, C-RIDL. This study for the first time systematically delivered information on RIs, source of variations (SVs) and partition criteria of reference values (RVs).

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