ejpmr, 2016,3(6), 368-372

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

"COMPARISON OF COVERAGE INTERVAL WITH REFERENCE INTERVAL FOR SOME COMMON LABORATORY ANALYTES IN A TERTIARY CARE HOSPITAL OF KOLKATA"

Dr. Indranil Chakraborty[1], Dr. Sanghamitra Chakraborty[2*], Dr. Chinmoy Ghosh[3], Dr. Sharmistha Chatterjee[4] and Dr. Mayukh Chakraborty[5]

[1]Professor and Head of Department of Biochemistry, Sagar Dutta College of Medicine, West Bengal, India
[2]Demonstrator in the Department of Biochemistry, Nilratan Sircar Medical College & Hospital, Kolkata, West Bengal, India.
[3]Assistant Professor in the Department Of Biochemistry, Nilratan Sircar Medical College & Hospital, Kolkata, West Bengal, India
[4]Assistant Professor in the Department Of Biochemistry, Sagar Dutta College of Medicine, West Bengal, India
[5]Medical officer in Dr.J.R Dhar Subdivisional Hospital Bongaon, West Bengal.

***Corresponding Author: Dr. Sanghamitra Chakraborty**
Demonstrator in the Department of Biochemistry, Nilratan Sircar Medical College & Hospital, Kolkata, West Bengal, India.

Article Received on 09/04/2016 **Article Revised on 30/04/2016** **Article Accepted on 21/05/2016**

ABSTRACT

The reference intervals serve as sheet anchor in clinical diagnosis. The reference intervals of laboratory analytes differ from one population to another due to differences in age, gender, genetic make-up, dietary habits, ethnicity, life style and socio-economic strata. The objective of this study is to compare the 'coverage interval' obtained from the few reference values with that of 'reference interval' calculated from 120 reference values.This study has been undertaken to establish coverage interval of some common analytes with 71 study subjects in Bengali population and to highlight the presence of differences if any with reference intervals using 120 subjects as per IFCC guidelines.Analysis of the analytes was done using automated and semi-auto analyser. The values of biochemical parameters were analysed using IBM SPSS 16 statistical software. The determination of reference interval of the anaytes is a priori for "good laboratory practice". The coverage interval obtained were 103-217 mg/dl, 27-44 mg/dl and 56-163 mg/dl for total cholesterol, high density lipoprotein cholesterol and triacyglycerol respectively. While on the other hand there was no significant difference ($p>0.05$) of mean values for glucose for the study subjects used for coverage interval and reference interval estimation. But the upper limit was 5.6mmol/L lower than the theoretical cut-off of 6.1mmol/L for diabetes diagnosis. This implies that pre-diabetic in Indian population is increasing which is indeed the truth. Moreover, both reference interval and coverage interval of liver enzymes, bilirubin was quite different from the reporting interval in literatures.

KEYWORDS: Reference values, reference interval, coverage interval.

INTRODUCTION: In recent times with the advent of laboratory technology and diagnostic investigations, clinical diagnosis has become more dependent on the laboratory tests. The laboratory scientist guides the clinician in interpreting the test results by correlating with appropriate reference values. When a clinical practitioner orders a laboratory test, he is more concerned whether the test result is within the reference limits than the test value itself. Grasbeck and Harris first suggested the concept of reference interval.[1] Reference values defines normative values of laboratory analytes used by the clinical laboratories for clinical diagnosis and the difference between the limits of reference values defines reference interval.[2] Normal and abnormal values often overlaps as most disease and biological analytes change in a continuing fashion. As biological data from a reference sample is skewed; the use of the normal terminology may misinterpret that the distribution is bell-shaped symmetrical Gaussian distribution.[3] According to International Federation Of Clinical Biochemistry (IFCC) recommendations every country or clinical laboratory must establish its own reference interval for healthy homogeneous population with a measurement of at least 120 samples.[4,5] It is not feasible in a big country like India with heterogeneous population burden to have 120 healthy reference individuals for all analytes measurement maintaining the stringent selection criteria and cost burden of methodology. Because the reference values will obviously vary depending on genetic pattern, diet, life style, physiological states like pregnancy etc. International Union Of Pure And Applied Chemistry (IUPAC) have coined the term *'Coverage interval'* which refers to population based reference values obtained from healthy group of reference individuals.[6]

The coverage interval provides a meaningful interval even with few reference values and also provides information related to precision of the estimated interval. Literature review did not yield any finding regarding the comparison of coverage and reference intervals of Bengali population, thus necessitating the urge to estimate the reference interval in our population. In this instance, we decided to establish the reference interval of some common laboratory analytes using standard protocol and compare with coverage interval using 71 individuals to see whether "coverage interval" can give an estimate of reference interval in realistic sense.

METHODOLOGY: In this study, total 385 healthy volunteers were enrolled and finally 120 healthy adult individuals were found fit as study subjects. Selection of subjects within the given age interval (18-37 years), according to IFCC recommendation and pre-defined inclusion and exclusion criteria, was random. Subjects with hypertension, hypercholesterolemia, diabetes, obesity, genetically determined risks, habits of smoking, alcohol consumption, any history of drug intake for treatment of disease or suffering like antiepileptic, anti-tubercular drugs (ATD), Chemotherapy, oral contraceptive pills(OCP), any variation in physiological status like pregnancy, stress, excessive exercise were excluded from the study The subjects for the study were considered free from any physical and mental disabilities, as excluded by history, physical examination and relevant investigation. The mental state was assessed by GHQ (WHO) questionnaire. Following overnight fasting, peripheral venous samples were taken into evacuated fluoride and plain tubes, centrifuged and plasma and sera were separated. The analyses of the analytes were done in accordance with the standards of the Ethical Committee of Medical College, Kolkata. Estimation of plasma glucose, creatinine, liver enzymes, cholesterol triglycerides, total bilirubin, conjugated bilirubin, protein and albumin were done in automated analysers (TRANSASIA XL 600) using standard methods. High Density lipoprotein cholesterol (HDL-C) was estimated in semi auto-analyser (CHEM V SEMIAUTOANALYSERS) and calibrated using cholesterol calibrator. The remaining chemistries were calibrated using multicalibrator (CFAS from ROCHE). Analysis of the biochemical analytes were done simultaneously with two levels quality control samples in each batch of run. Moreover, as a part of external assurance our accredited laboratory is enrolled in proficiency testing survey of Biorad. The values of biochemical parameters were analysed using IBM SPSS 16 statistical software, checked for normal distribution by using Kolmogorov Smirnov test and parametric and nonparametric methods were used accordingly.

RESULTS AND DISCUSSION

The mean age and weight of the study population 28.49 $\pm$ 9.49 years and 62.9 $\pm$ 11.58 Kilograms respectively. The median age for both the study population included in determination of coverage and reference population was 27years with inter quartile range (IQR) of 7 years.

Table 1: Table showing "Reference interval" and "coverage interval" of the test analytes. Where, P>0.05 Parametic intervals were estimated and for P<0.05. Non-Parametric intervals were calculated.

		For Reference interval n=120				For Coverage Interval n= 71			
Sl.no	**Analyte**	**mean**	**Median**	**Kolmogorov Smirnov test**	**Reference interval**	**Mean**	**Median**	**Kolmogorov Smirnov test**	**Coverage interval**
1.	**Fasting plasma glucose(mg/dl)**	87.73	88	.200*	72-101	87.80	88	.200*	73-104
2.	**Total bilirubin(mg/dl)**	0.71	0.64	.000	0.3-1.71	0.77	0.7	.001	0.42-1.7
3.	**Conjugated bilirubin(mg/dl)**	0.27	0.25	.010	0.1-0.57	0.29	0.27	.001	0.1-0.57
4.	**Alanine aminotransferase (U/L)**	24.52	24	.007	10-38	25.59	26	.200*	13-37
5.	**Aspartate aminotransferase (U/L)**	27.1	25.5	.037	12-50	29.48	28	.022	12-50
6.	**Alkaline phosphatase(U/L)**	173.38	171.5	.200*	87-281	192.33	192	.200*	101-334
7.	**Total protein (gm/dl)**	7.26	7.3	.200*	6.3-8.3	7.33	7.4	.200*	6.3-8.5
8.	**Albumin (gm/dl)**	4.37	4.4	.034	3.6-5	4.45	4.5	.018	3.7-5
9.	**cholesterol (mg/dl)**	147.65	147.5	.200*	95-199	147.9	147	.200*	103-217
10.	**High Density lipoprotein(mg/dl)**	42.08	41.5	.000	36-49	42.42	42	.061	27-44
11.	**Triglyceride(mg/dl**	104.82	103.5	.010	60-151	99.33	97	.200*	56-163

It is evident from table 1, that the upper limit of both reference interval and coverage interval was 5.6mmol/L(101mg/dl) and 5.7 mmol/L (104mg/dl) in contrast to the WHO and American Diabetic Association (ADA) theoretical limit of 6.1 mmol/L.[7] Diabetes is the seventh leading cause of death in the United States, with an increase in prevalence from 8.3% in 2010 to 9.3% in 2012. Moreover, according to International Diabetes foundation, India has more diabetics further supported by ADA reports that India will have highest number of diabetic patients by 2030[8,9]. The upper reference limit is almost in agreement with the upper reference limit, calculated in 605 Nigerian subjects of Port Harcourt Teaching Hospital , 5.7 mmol/L.[7] Moreover, there was no significant correlation with age in our study population and our reference interval was quite narrower than the recommended reference interval of our kit insert i.e. 74-110mg/dl and also from the consolidated reference interval of fasting plasma glucose calculated by Ashavaid et al in 4466 Indian subjects[10]. This could be due to difference in population demographics, diet, life style etc. The second category consisted of evaluation of lipid profile, where there was an elevation in the coverage interval of cholesterol i.e. 103-217 mg/dl. The upper limit of coverage interval was much higher than the recommendation of National Cholesterol Education Program (NCEP) and is consistent with the fact that there has been an increment in total cholesterol by 25mg/dl with age.[13] The median of serum cholesterol and triglyceride was much lower than values obtained in Assamese population.[12] however the median of high-density lipoprotein cholesterol was higher than Assamese population. Moreover, there was a downward swing of the upper reference limit of HDL-C even lower than our reporting values of 35-59 mg/dl. This finding agrees with the Coronary disease Among Indians Study observation suggesting that only 14% men and 5% women had optimal serum HDL-C concentrations.[14] Moreover, various factors like age, ethnicity, diet, genetic and gender differences have substantial effect on lipid profile parameters. The HDL-C upper limits were significantly lower than the North-Indian subjects i.e for male and female 33-64 and32-58 mg/dl respectively. This could be attributed to the huge difference of diet in Bengalis and North Indians, due to high fish intake in Bengali population.[15] The mean of cholesterol, triglyceride and HDL cholesterol is also significantly lower than the level observed by Goswami and Bandyapadhay in Middle class Bengali population.[16]

Table 2: Table showing comparison between "Reference interval","coverage interval" and "Reporting interval".

Analytes	reference interval	Coverage interval	Reporting interval as per kits
Total bilirubin(mg/dl)	0.3-1.71	0.42-1.7	<2
Conjugated bilirubin(mg/dl)	0.1-0.57	0.1-0.57	<0.2
Alanine aminotransferase (U/L)	10-38	13-37	5-35
Aspartate aminotransferase(U/L)	12-50	12-50	5-45
Alkaline phosphatase(U/L)	87-281	101-334	108-316
Total protein (gm/dl)	6.3-8.3	6.3-8.5	6.4-8.3
Albumin (gm/dl)	3.6-5	3.7-5	3.5-5.2

In the third category, of screening for liver enzyme and bilirubin were quite different from the reporting intervals as in Table 2. However, the upper limits of both "coverage interval" and "reference interval" of conjugated bilirubin was higher than the reporting value of our laboratory (<0.2mg/dl) and cannot be explained as none of the study subjects had history of jaundice and liver ailment in recent past. Now comparing the coverage interval and reference interval, 0.95 parametric reference interval with 120 samples is wider than coverage interval. Moreover, it is also evident from figure number 1 that there is no significant difference of mean between cholesterol and triglyceride as calculated from 120 and 71 individuals. Similarly, table 3, shows no significant difference between mean for analytes used for reference interval and coverage interval except for alkaline phosphatase.

Table 3: Showing the comparison of mean between the individuals of reference interval and coverage interval.

Name of analyte	no.of subjects	Mean	sig (t-test)
Glucose	120 71	87.78 87.80	0.986
Total bilirubin	120 71	0.71 0.79	0.190
Direct bilirubin	120 71	0.27 0.29	0.289
SGOT/AST	120 71	24.52 24.59	0.33
SGPT/ALT	120 71	27.10 29.47	0.13

ALP	120 71	173 192	0.03 (P<0.05)[significant]
Total protein	120 71	7.26 7.33	0.38
Albumin	120 71	4.37 4.42	0.14

According to IFCC, both the reference interval and the coverage interval recommended here can be estimated either non-parametrically or parametrically. In general, the parametric coverage intervals are narrower and more useful than non-parametric coverage interval. Coverage interval can be constructed on both one-sided and two sided. However, the application of one-sided intervals may be of more practical interest when only the upper limit is of toxicological interest, or when a fraction of the reference values is below the detection limit of the measurement method.[17] The coverage uncertainty, an estimate of the reliability of coverage interval, is independent of the standard deviation, but depends on level of expectation, level of confidence and number of values.[18] Moreover; no measurement is perfect due to anticipated measurement of uncertainty. Due to randomness of uncertainty, some assumptions are made regarding distribution of statistical error and it is seen that with a coverage probability of 0.95 a coverage interval covers 0.95$\pm\delta$ of the measurand (where δ is uncertainty Figure2) Oden et al. have published exhaustive tables on coverage uncertainty including different levels of expectation and confidence.

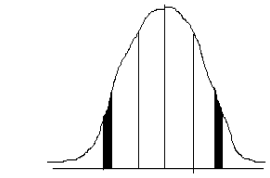
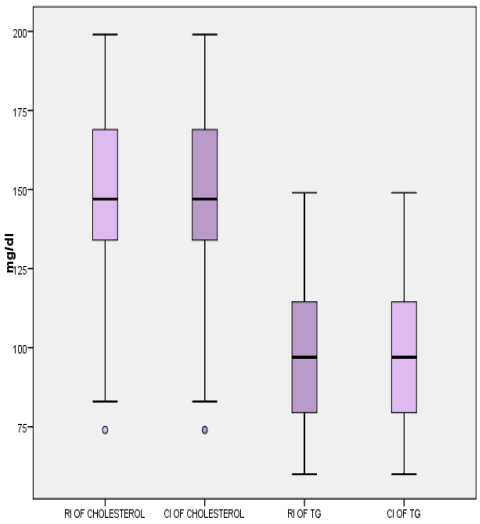


Figure2: showing concept of coverage uncetainty

Figure: Showing box plot



Small coverage uncertainty demonstrates high precision in the estimate of the limits of the coverage interval.[19]

CONCLUSION

So, coverage interval is a predictor of reference interval. Recently, the interest of laboratory scientist, doctors and above all the patients have shifted towards the quality of the methodology. So to establish a near accurate reference interval the technical qualities of methodology should also be accounted. Analytes like trace elements, antiepileptic drugs, antioxidants have reference methods which are very costly. Now it is really a hurdle to establish reference interval for such analytes with huge number of samples with the cost burden. In such cases, coverage interval may be a good alternative.

ACKNOWLEDGEMENT

The present work is inspired by an IUPAC work on serum lead concentration in Denmark population.The authors of this article acknowledge the inspiration and help received from the scholars whose articles have been cited in the reference section. The authors pay their gratitude to authors/editors/publishers of all those articles/journals/books from where the reviews and literatures for the discussion have been collected.

REFERENCE

1. Ichihara k, Boyd Jc; IFCC Committee on Reference and Decision Limits (C-RIDL) An appraisal of statistical procedures and in derivation of reference intervals. ClinChem Lab Med. 2010; 48: 1537-51.43.
2. Grasbeck R, Saris NE. Establishment and use of normal values. Scand J Clin Lab Invest. 1962; 26 (Suppl 110): 62-63.
3. Solberg HE, Grasbeck R. Reference values. AdvClinChem 1989; 27: 1-79.
4. Clinical and laboratory Standards Institute. Defining, Establishing and Verifying Reference

Intervals in the Clinical Laboratory; Approved Guideline. CLSI Document C28-A3c. Wayne, Pa: Clinical and laboratory Standards Institute, 2010.
5. Solberg HE,A guide to IFCC recommendations on reference value,Jint Fed clin chem.:1993; 5: 160-4.
6. PoulsenOM,HolstE,Christensen JM, Calculation and application of coverage interval for biological values; Pure and applied chemistry,1997; 69(7): 1601-1611.
7. AA Ejilemele, AC Ojule, CG Orluwene, Plasma glucose in a reference population in Port Harcourt, Nigeria Port Harcourt Medical Journal Vol. 2 (3) 2008: pp. 228-232.
8. Gale, Jason (November 7, 2010). "India's Diabetes Epidemic Cuts Down Millions Who Escape Poverty". Bloomberg. Retrieved 8 June 2012.
9. Wild, Sarah, Gojka Roglic, Anders Green, Richard Sicree, and Hilary King. "Global Prevalence of Diabetes." Diabetes Care. American Diabetes Association, 26 Jan. 2004. Web. 22 Apr. 2014.
10. Tester F. Ashavaid, Seema P. Todur, Alpa J. Dherai Establishment Of Reference Intervals In Indian Population Indian Journal of Clinical Biochemistry, 2005; 20(2): 110-118.
11. Gupta, R. and Singhal, S. Epidemiological evolution, fat intake, cholesterol levels and increasing coronary heart disease in India: paper presented at National Symposium on Hyperlipidaemia, 1997,March 21, New Delhi: NHS.
12. Das M and Saikia M. ESTIMATION OF REFERENCE INTERVAL OF LIPID PROFILE IN ASSAMESE POPULATION Indian Journal of Clinical Biochemistry, 2009; 24(2): 190-193.
13. Enas, E.A., Davidson, M.A., Garg, A., Nair, V.M. and Yusuf, S. (1991) Prevalence of coronary heart disease and its risk factors in Asian Indian migrants to the United States. Proc. Int. Symp. Atherosclerosis, Rosemont, IL, Oct.6-11.
14. Yadav D1, Gupta M, Mishra S, Sharma P Reference interval for lipid profile in North Indian population from Rajasthan according to various partitioning criteria. Clin Chim Acta. 2013 Nov 15; 426: 145-51.
15. Goswami K, Bandyopadhyay A. Lipid profile in middle class Bengali people of Kolkata. Ind J Clin Biochem 2003; 18(2): 127-30.
16. Christensen JM, Holst E, Bonde J.P & Knudsen: Determination of chromium in blood and serum: Evaluation of Quality control procedures and estimation of reference values in Danish subjects, Sci. Tot. Environ.1993; 132: 11-25.
17. Odeh RE, Youn-Min C & Owen DB: Sample size determination for two-sided β – expectation tolerance intervals for a normal distribution. Technometries, 1989: 31: 461-468.
18. Poulsen OM,Holst E,Christensen JM, Calculation and application of coverage interval for biological values; Pure and applied chemistry,1997; 69(7): 1601-1611.
19. Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV,Minchenela J et.al "Current databases on biologic variation: pros, cons and progress" Scand J Clin Lab Invest.1999; 59" 491-500.