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Original Research Article

Analysis of observed values of glucose, creatinine, bilirubin and ALT examinations in Blood (Serum, Plasma) in hospital population

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Abstract

Objectives: Find reference range by Hoffman's method and compare it with published reference range in standard textbooks.

Method: Observed values (results) of glucose (FBS, RBS and PP2BS), creatinine, bilirubin (total and direct) and ALT were collected from LIS of biochemistry section of New civil hospital surat laboratory services. Data were analysed using computarised hoffman's method as described by Katayev *et al*. Reference range derived by computarised hoffman's method were compared with published reference range in standard textbooks.

Results and conclusion: The study shows that Hoffman method is useful for verification of reference range determined by direct methods when large numbers of data are available, distribution is more or less Gaussian and analyte in question is not etiologically responsible for a disease.

Keywords: Reference range, Glucose, ALT, Bilirubin, creatinine

1. Introduction

In the era of diagnostics, physician use laboratory results and reference range given by laboratory to interpret of laboratory report[1]. As Stated in clause 5.8.3 of ISO 15189:2012 Laboratory report must include reference range in report preferably established in own laboratory[3].

Most laboratory uses reference range published in standard textbook and kit inserts provided by manufacturers. But that reference range is established in different population with different instrument and different method for measurement of analyte. To use manufacturer's reference range, laboratory must verify by them by suitable methods. It can be done by analyzing observed values in 20 healthy individuals as per NCCLS guideline[4].

One of the method for determination of reference range is direct one; requiring healthy volunteers. Health is a condition lacking universal definition and recruiting healthy subject is costly[2]. Direct method includes recruiting healthy subject by various inclusion and exclusion criteria. So that method is cumbersome. Indirect methods are also available for reference range determination. Indirect method is easy to use. In indirect method reference range is determined by stored data in LIS of laboratory.

Computerized hoffman's method decribed by Alex katayev and colleagues is most simple technique for determination of reference range in hospital population.

2. Materials and methods

The study was conducted in following steps.

- Laboratory data stored in LIS were retrived by SQL query
- Observed value (results) of glucose, creatinine, ALT and bilirubin are exported from LIS database MySQL to libreoffice calc spreadsheet.
- Data for period of September 2013 to April 2014 were analysed.
- Observed values were classified in to OPD, IPD and ICUs groups.

- Average, SD and CV% of all Observed values were calculated using libreoffice calc equations.
- Probability of each observed value was calculated using "NORMDIST()" inbuilt function in libreoffice calc.
- If probability of occurrence of any observed value is less than "1/2N" where N=total number of observed values then that observed values becomes outlier in our data and these values were excluded in further calculations[2].
- Frequency data classes for each examination were prepared.
- Frequency and cumulative frequency of each member of data classes were calculated.
- Cumulative frequency of each member of data classes were calculated after excluding outliers.
- Find % of cumulative frequency by following equation.

  % cumulative frequency

  $$= \frac{\text{cumulative frequency}}{\text{Sample size (N)}} \times 100$$

- Draw curve of % cumulative frequency versus values of results (observed values).
- X axis = % cumulative frequency
- Y axis = values of results (observed values)

Figure 1 (Illustration 1): Example of ALT cumulative frequency distribution in OPD samples

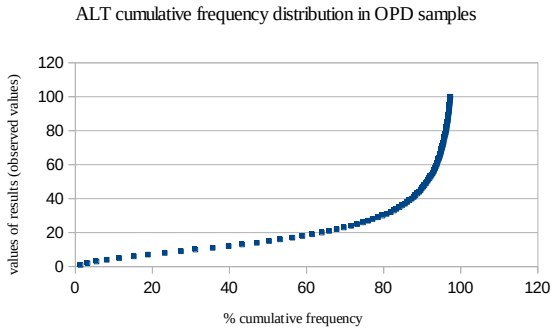
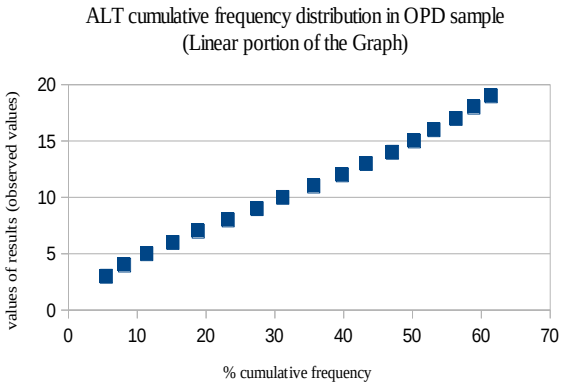


Figure 2 (Illustration 2): Example of ALT cumulative frequency distribution in OPD samples (Linear portion of the Graph)



- Find out Linear area of curve and find linear equation from above data.
- Equation is like Y=aX+b.
- Lower reference limit calculated using X=2.5
- Upper reference limit calculated using X=97.5

Table 1 (Illustration 3): Example of ALT reference ranges derived from OPD samples

Example of ALT reference ranges derived from OPD samples		
Linear equation	f(X) = aX+b	0.27x+1.65
Lower reference limit	0.27x**2.5**+1.65	2 U/L
Upper reference limit	0.27x**97.5**+1.65	28 U/L
ALT reference range derived from OPD samples		2-28 U/L

Reference ranges given in standard textbooks were compared with reference limit found in the study.

3. Results

Table 2 (Illustration 4): Number of observed values in study for all parameters

Parameters	Number of observed values in the study			
	OPD	IPD	ICUs	All samples
ALT	13285	34265	1952	47542
Creatinine	15159	39251	2893	54410
FBS	6570	7323	158	14199
RBS	7847	23258	112	33095
PP2BS	3107	4928	539	8270
Total bilirubin	13013	35758	2904	48771
Direct bilirubin	12575	34505	2840	47079

Table 3 (Illustration 5): Comparision of reference values observed in study with reference values published in Tietz textbook of clinical chemistry

Parameters (Unit)	Tietz textbook of clinical chemistry 5th ed	Reference Ranges observed in the study			
		OPD	IPD	ICUs	All sample
ALT (U/L)	<45	2-28	2-32	3-44	2-30
Creatinine (mg/dl)	0.9-1.3	0.6-1.6	0.5-1.4	0.5-1.4	0.5-1.4
FBS (mg/dl)	>126	75-127	69-151	78-176	74-135
RBS (mg/dl)	>200	76-120	70-138	50-190	70-134
PP2BS (mg/dl)	>200	62-286	79-211	-	79-233
Total bilirubin (mg/dl)	0 – 2.0	0.2-0.7	0.2-1.0	0.2-2.0	0.2-0.8
Direct bilirubin (mg/dl)	0 – 0.2	0.1-0.3	0.0-0.4	0.0-1.0	0-0.2

4. Discussion

The study shows that for ALT reference range derived from hospital data using Hoffman's method gives upper reference range lower than standard upper reference range given in Tietz textbook of clinical chemistry (5th ed).

Thus, the reference ranges stated in standard textbooks are not uniformly verified in various studies using various methods of deriving reference ranges. Probably, the values given in Tietz textbook of clinical chemistry (5th ed) is derived from western population and many not be applicable to India. The wide variations in reference range derived from various sources indicate need for efforts to derive local reference ranges for ALT. While most western countries and rest of the India (other than Gujarat) allow 60 ml of alcohol for inclusion of subject into the study, our study is likely to have abundance of non-drinking population as shown by lower upper reference limit.

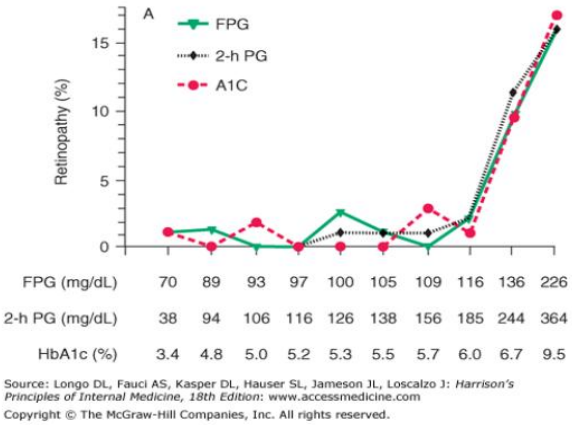
The study shows that for creatinine reference range derived from hospital data using Hoffman's method gives overall reference range 0.5-1.4 mg/dl. While the standard reference range given in Tietz textbook of clinical chemistry (5th ed) is 0.9-1.3 mg/dl. Thus,lower reference limit observed by this study for creatinine is 0.4 mg/dl lower than that mentioned in Tietz textbook of clinical chemistry (5th ed).

The study shows that for fasting blood glucose reference range derived from hospital data using Hoffman's method gives OPD reference range 75-127 mg/dl. While the standard upper reference range given in Tietz textbook of clinical chemistry (5th ed) is >126 mg/dl for diagnosis of diabetes mellitus.

The reference range for fasting glucose is not derived from study of healthy population because even a healthy person is likely to suffer from health related complication in future for fasting glucose beyond certain limit. The reference ranges for glucose are recommended on the basis of association of fasting glucose with prevalence of cataract, retinal complication and renal complication.

According to Harrison's principle of internal medicine "*DM is defined as the level of glycemia at which diabetes-specific complications occur rather than on deviations from a population-based mean*"[8]. (Illustration 6)

Figure 3 (Illustration 6): Relationship of diabetes-specific complication and glucose tolerance



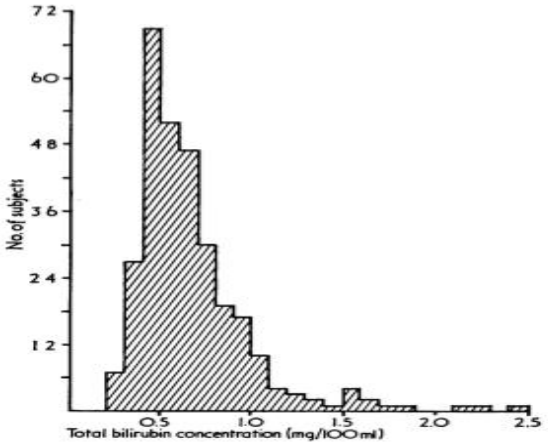
The study shows that for post prandial blood glucose reference range derived from hospital data using Hoffman's method gives OPD reference range 62-286 mg/dl. While the standard upper reference range given in Tietz textbook of clinical chemistry (5th ed) is >200 mg/dl for diagnosis of diabetes mellitus.

Post prandial blood glucose is generally is not used for diagnosis of Diabetes mellitus. Majority of post prandial blood glucose is done in hospital settings for knowing control of hyperglycemia and titrating the dose. Hence, it is not expected for Hoffman method to give correct reference range. In addition, internationally accepted reference ranges are derived from association of post prandial glucose with clinical complications of Diabetes mellitus rather than distribution among apparently healthy individuals. Similar interpretation can be made for random blood glucose.

The study shows that for Total bilirubin reference range derived from hospital data using Hoffman's method gives overall reference range 0.2-0.9 mg/dl. While the standard reference range given in Tietz textbook of clinical chemistry (5th ed) is 0.2-2.0 mg/dl. The frequency distribution graph for Total bilirubin is skewed to right. While majority of the population have bilirubin within a narrow range found in the study , significant proportion of population have Gilbert's syndrome where there is elevation of indirect bilirubin up to 2 mg/dl with all other liver function test normal and patient is completely healthy[6][7].

Illustration 7 shown below shows distribution of bilirubin in healthy population of liverpool[7].

Figure 4 (Illustration 7): Distribution of bilirubin in healthy population of Liverpool



The study shows that for direct bilirubin reference range derived from hospital data using Hoffman's method gives overall reference range 0.0-0.3 mg/dl. While the standard reference range given in Tietz textbook of clinical chemistry (5th ed) is 0.0-0.2 mg/dl. It appears that Gaussian distribution of direct bilirubin in healthy population is responsible for values found in the study matching with published standards.

5. Conclusions

Due to accumulation of large number of examination results in LIS at biochemistry laboratory of NCHSLS, New Civil Hospital, Surat, It was possible to analyze cumulative data statistically.

The analysis shows that reference ranges found by Hoffman's statistical method agrees with scientifically accepted reference ranges for creatinine and Direct bilirubin, While there is considerable difference for fasting glucose, ALT and Total bilirubin.

It appears that near Gaussian distribution of observed values in hospital population is responsible for agreement between Hoffman's method and scientifically accepted reference ranges for creatinine and direct bilirubin. Because reference ranges for fasting glucose are etiological reference range Hoffman method perform poorly in verification of reference range for fasting glucose.

Lack of regular alcohol intake in study population may be responsible for lower reference range for ALT observed in the study. Non Gaussian distribution for Total bilirubin value due to contribution from gilbert's syndrome may be responsible for lower reference range for Total bilirubin observed in the study.

The LIS of laboratory do not have detailed information about patient demographics and diagnosis, which could have help eliminate patients suffering from certain diseases and help stratification of data according to age and sex.

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