ESTABLISHMENT OF REFERENCE INTERVALS OF LABORATORY TESTS USING HOSPITAL PATIENT DATA*

Mustafa Toprakçı, Ph.D. / Kaya Emerk, Ph.D.*****

* *Department of Biochemistry, School of Medicine, Kadir Has University, Istanbul, Turkey.*

** *Department of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey.*

ABSTRACT

Objective: In this study, hospital patient data was used to derive reference intervals for selected clinical laboratory tests.

Methods: Data were obtained indirectly using our hospital database including both sexes. No selection criteria have been applied. The data has been partitioned into only three age groups as, 3-20, 21-60 and 61 to older in order to prevent age related grouping in the distribution. The distributions have been checked by normality analysis using Kolmogorov-Smirnov test. Nonparametric percentile estimate method was used to obtain reference intervals in the age groups of 21-60 and 61 to older. In age group 3-20, the number of data were below 120 in most tests so, GraphROC for Windows, a statistical package which performs a robust modified nonparametric method, was used to find reference intervals.

Results: Most of the test data did not show Gaussian distribution form and parametric analysis of these data has failed. Instead, nonparametric analysis has succeeded in establishing the intervals in three age groups.

Conclusion: The results resembled the characteristics of our hospital patient population. Especially, protein and lipid parameters showed clear differences in our population, compared to the reference values of the manufacturer, which are currently used. This study has been a clear evidence indicating the importance of determination of reference intervals and the analysis of indirectly selected hospital patient data using nonparametric statistical techniques.

Key Words: Reference interval, Hospital patient data, Nonparametric.

INTRODUCTION

Reference intervals are routinely used in clinical trials and constitute a major part in the evaluation of the diseased individual. In his study Benson (1) investigated the concept of reference interval and described it as the most intractable problem that limits the usefulness of laboratory data. The course of identifying reference values utilises many statistical procedures; from this point of view, it depends on the methods selected to analyse the data (2,3). Besides, it needs a clear

(*) In this study we obtained reference intervals for our laboratory using hospital patient data.

(Accepted 23 January, 2002)

Correspondance to: Mustafa Toprakçı, Ph. D. - Department of Biochemistry, School of Medicine, Kadir Has University, Gayrettepe, Istanbul, Turkey.
e.mail address: mtoprakci@ttnet.net.tr

definition and selection of data from a target population, which is to be modelled (4,5).

The goal of this study is to indicate the importance of reference interval study and to establish the methodology for evaluating hospital patient data for reference interval analysis. At first, clear distinction should be made between normal population and hospitalised population. Otherwise unimodality in data will be difficult to preserve. The first problem is to find the right population for determination of laboratory test reference intervals. This actual population can be modelled by sampling smaller populations from it. A group of investigators prefer to use clearly defined and selected data from hospital populations, which is called direct sampling. Whereas, others collect laboratory data, without applying any selection criteria (6-8). This latter method is called indirect sampling. In our study, this second approach was used and the collected data was grouped into three age groups as: 3-20, 21-60 and 61 and older. We selected our laboratory tests to minimize the possible effects of sex distribution. Biochemical tests which would be least affected from gender related groupings were mainly chosen.

These are: Albumin (ALB), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Blood Urea Nitrogen (BUN), Calcium (CA), Cholesterol (CHOL), Creatinine (CREA), Direct Bilirubin (DBIL), Phosphorus (PHOS), Total Bilirubin (TBIL), Total Protein (TP), Triglyceride (TRIG). Several well-defined criteria could be applied in the selection and the partition of the test data (9). To find the most suitable statistical procedure, which would best analyse the selected population data, parametric and nonparametric methods were checked. Use of parametric methods decreases the requirement for large number of data, but also requires carefully selected data. Well-defined criteria must be applied to the data and the distribution should follow Gaussian form. It was shown that mostly data from hospital populations do not follow Gaussian form; even when strict selection criteria had been applied (10).

Nonparametric methods produce better results in non-Gaussian distributions. Even indirectly selected hospital patient data can be used to derive reference intervals. Nonparametric percentile estimate method which is a more practical way to determine reference intervals on a strong statistical methodology was used in this study.

MATERIALS AND METHODS

Patient data have been collected from the hospital database. The laboratory data had been produced by Dade Behring Dimension XL analyser using the test kits supplied from the same manufacturer. The calibrations and the internal quality controls, during the test period were carried out by the materials supplied by Dade Behring. Precision and accuracy of the test were checked also by external quality control program carried by the Turkish Biochemistry Association.

The measurements were carried out on the serum materials collected from the patients admitted to the out-patient clinics and those who were hospitalised in various departments. Emergency patients were excluded from the study. The blood specimens were collected according to the general phlebotomy rules. They were separated to serum and supplied to the analyser in 20 minutes. These procedures were adapted according to the rules given in the NCCLS-C28-A document (9).

Two nonparametric methods have been used in the statistical analysis of the test groups; these were nonparametric percentile estimate (6,9) and the robust nonparametric method applied by the GraphROC program (11). The first method was applied to the test groups in the 21-60 and 61-older ages. The second method was applied to the 3-20 age group. The selection of these nonparametric methods was based on the number of data obtained in the age groups. Application of nonparametric percentile estimate method is proposed by NCCLS document for indirectly selected hospital laboratory data.

The data were curtailed to exclude illness-related values. It should be noticed that the high values might be greater in number, especially in indirectly selected data from hospital population. Because there were enough data in 21-60 and 61-older ages' test groups; these test groups were curtailed using ±3 SD limits. In age group 3-20 there were not enough data to apply nonparametric percentile estimate method, because at least 120 data is needed to perform this analysis (9,12). For this reason a robust nonparametric method defined by Kairisto (11) in GraphROC statistical program was preferred for this age group tests. This method curtails the data using ±4 SD limits which enables wider reference data. The test values in this age group show large variations and the data number is small which could cause extreme disturbances during statistical analysis.

In essence the data number closely depends on the statistical procedure chosen. Several investigators proposed different numbers on different statistical methods (12). Extreme values were handled using Dixon's method. All data were rechecked and extreme values were omitted. Dixon's method was applied to data manually after ±3 SD curtail in 21-60 and 61 to older ages and also by the GraphROC program in the 3-20 ages' data (13).

In descriptive statistical analysis, the SPSS PC program was used (SPSS PC). Other data sorting and charting applications were carried by MS Excel (Microsoft Windows).

In evaluating the distribution of data normality analysis was performed using the Kolmogorov-Smirnov test. In non-Gaussian distribution Log(10) transformation of data was performed. After transformation the distributions were reevaluated with normality analysis (14,15).

RESULTS

The data in each test group were analysed using descriptive statistics. The number of data and the distribution type are the principle elements, which are used to select the statistical method to analyse the reference intervals. In the age group 3-20 the data numbers vary greatly and are well below the critical level of 120 to apply classic nonparametric tests.

The normality analysis were carried with the Kolmogorov-Smirnov test. It was observed that, in most of the test groups the data did not follow normal distribution. In some tests (ALT, AST, CHOL, TRIG) the standard deviations were high. To exclude the illness-related data, the distributions were curtailed by omitting the data lying outside the ±3 SD limits. This way we could exclude the test results that were directly related to the pathology. This procedure lowered the amount of data in all test groups. In age group 3-20 due to lack of enough data, no ±3 SD curtail was applied. GraphROC for Windows program produced meaningful results in this age group using raw data. Additionally Dixon's analysis for extreme values had been performed to all test data in all age groups. The descriptive statistical analysis of curtailed data in the age groups 21-60 is given in Table I.

Table I: Descriptive statistical analysis of 21-60 age group after±3 SD curtail.

	Mean	SD	Distribution
ALB	3.80	0.398	Gaussian
ALP	81.68	28.74	Non-gaussian
ALT	39.98	9.65	Non-gaussian
AST	23.22	6.39	Non-gaussian
BUN	13.07	3.35	Gaussian
CA	9.51	0.54	Gaussian
CHOL	201.7	31.59	Non-gaussian
CREA	0.85	0.14	Non-gaussian
DBIL	0.144	0.056	Non-gaussian
PHOS	3.482	0.64	Non-gaussian
TBIL	0.63	0.25	Gaussian
TP	7.38	0.58	Gaussian
TRIG	107.7	50.31	Non-gaussian

As seen from Table I, most of the test data did not follow normal distribution. At this step transformation of data was performed. Normality analysis was applied to the transformed data and most of the test distributions did not obey Gaussian form.

In Fig. 1a raw and transformed data distributions are seen. In Fig. 1b output of GraphROC program is seen on the ALT 3-20 age group data. This method regroups the data and produces a new distribution. One can choose parametric or nonparametric analysis of this new distribution. In this study nonparametric analysis was performed.

The data in all test groups were evaluated with nonparametric methods. Table II gives the

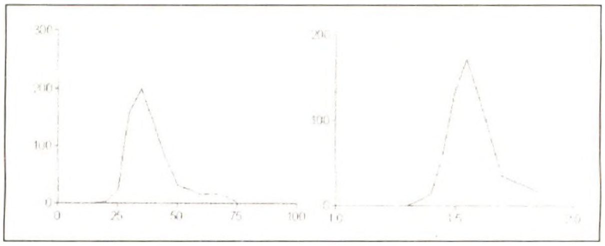

Fig. 1a: ALT distributions are given. In the left plot raw data is shown to present skewness to the left side. In the right plot Log transformation is applied to the same data and recovery to Gaussian distribution is obviously seen.

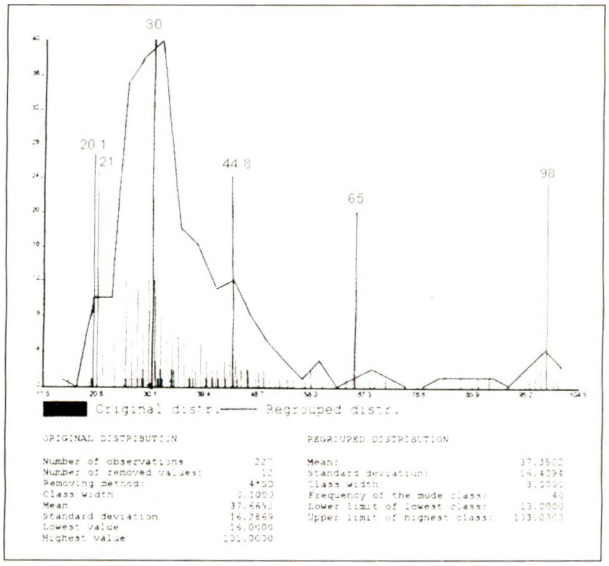

Fig. 1b: ALT 3-20 age group distribution. Reference interval was determined as 20.1-44.8 mg/dl, where reference interval of the manufacturer was 30-65 mg/dl. The 21.0-98.0 mg/dl. interval was obtained by parametric analysis of this same distribution. This shows clearly the inappropriate result of parametric analysis on indirectly selected data.

results of our study; the first age group (3-20) intervals were obtained with nonparametric indirect method used in GraphROC for Windows program. The intervals of the two later age groups (21-60 and 61 to older) were obtained with the nonparametric percentile estimate method.

DISCUSSION

The results of the study show that reference intervals from three age groups bear considerable difference. This is especially evident between the 3-20 age group and the two later age groups.

The most prominent differences were seen in lipid parameters. CHOL and TRIG values were considerably higher in our population, in all age groups. In this age group we could not obtain a meaningful value for TRIG in both methods.

The causes of differences can be various, but mostly they result from the reference population and the statistical method used. There are many other studies in which hospital laboratory data were used as reference population (16,17). We carried indirect sampling during data collection from the hospital database. This kind of approach will be different from direct sampling of the data where a well-criticized selection is applied. Number of the data is also an important factor and greatly affects the results especially when nonparametric methods are used. The

Table II: Reference intervals produced in this stuay are given. In age group 3-20 no meaningful result could be obtained for TRIG.

	3-20 age N	Reference Interval	21-60 age N	Reference Interval	61 to older N	Reference Interval	Current reference intervals	Units
ALB	74	2.33-4.96	282	3.04-4.5	143	3.1-4.56	3.4-5.0	g/dl
ALP	146	51-267	551	37-144	334	37-146.8	50-136	U/L
ALT	240	20.1-44.8	700	26-66.7	302	25-65.6	30-65	U/L
AST	241	12.1-39.4	724	13.4-39.3	313	13.5-39.1	15-37	U/L
BUN	100	6.9-17.8	562	7-19.3	268	8-20	7-18	mg/dl
CA	91	9.05-10.6	380	8.5-10.6	250	8.5-10.6	8.8-10.5	mg/dl
CHOL	91	125-240	519	126-246	228	128-249	0-200	mg/dl
CREA	142	0.5-1.0	532	0.6-1.2	383	0.6-1.4	0.6-1.3	mg/dl
DBIL	70	0.02-0.3	128	0.05-0.3	104	0.08-0.3	0.0-0.3	mg/dl
PHOS	53	2.4-6.1	312	2.3-5.1	207	2.4-5.1	2.5-4.9	mg/dl
TBIL	75	0.27-1.03	197	0.2-1.2	120	0.2-1.17	0.0-1.0	mg/dl
TP	69	6.4-8.2	247	6.1-8.4	155	6.0-8.4	6.4-8.2	g/dl
TRIG	70	—	591	38-230	291	36-231	30-200	mg/dl

number should be well over 120 in order to obtain meaningful results. We have carried ±3 SD curtail to omit illness related values in age groups 21-60 and 61 to older, if selection criteria were applied previously in direct sampling, ±4 SD could be chosen as limits in these age groups. It is also shown that transformation of data did not correct the distribution characteristics.

Nonparametric percentile estimate analysis is very powerful in obtaining the lower and upper values in skewed distributions. In addition, regrouping the data makes the distribution of populations, which have less than 120 data number, more suitable for nonparametric analysis. This method had been applied in 3-20 age group using GraphROC program. This had changed the reference intervals significantly and produced better results (Fig. 1b). The nonparametric reference interval analysis should be preferred in indirectly selected data as shown in our study. In previous studies it is shown that it is difficult to obtain a Gaussian distribution from hospital patient data, even when selection criteria are applied.

The higher values obtained for CHOL and TRIG also needs further investigation. Larger data pools should be obtained from several different hospital locations and lipid values should be established clearly for our population. As a result it can be said that, hospital patient data together with nonparametric analysis is a practical and effective method to determine reference intervals.

ACKNOWLEDGEMENTS

In this study we thank Marmara University Hospital, Biostatistics Department for their kind help. We are especially grateful to our co-workers who have helped by contributing the production of test results and their collection.

REFERENCES

1. *Benson ES. The concept of normal range. Hum Pathol 1972;3:152-157.*
2. *Reed H. Influence of statistical method used on the resulting estimate of normal range. Clin Chem 1971;17:275-284.*
3. *Lumsden JH. On establishing reference values. Can J Comp Med 1978;42:293-301.*
4. *Young DS. Determination and validation of reference intervals. Arch Pathol Lab Med 1992;116:704-709.*
5. *Harris EK, Wong ET. Statistical criteria for separate reference intervals: Race and gender groups in Creatinine Kinase. Clin Chem 1991;37:1580-1582.*
6. *Massod MF. Nonparametric percentile estimate of clinical normal ranges Am J Med Tech 1977;43:243-252.*
7. *Kouri T, Kairisto V. Reference intervals developed from data for hospitalised patients: Computerised method based on combination of laboratory and diagnostic data. Clin Chem 1994;40:2209-2215.*
8. *Kairisto V, Hänninen K. Generation of reference values for Cardiac enzymes from hospital admission laboratory data. Eur J Clin Chem Clin Biochem 1994;32:789-796*
9. *NCCLS Document C28-A.1995 Vol 15 No.4*
10. *Horn PS. A robust approach to reference inerval estimation and evaluation. Clin Chem 1998;44:622-631.*
11. *Kairisto V, Poola A. Software for illustrative presentation of basic clinical characteristics of laboratory tests: GraphROC for Windows. Scand J Clin Lab Invest Suppl 1995;222:43-60.*
12. *Lott JA, Mitchell LC. Estimation of reference ranges: How many subjects are needed? Clin Chem 1992;38:648-650.*
13. *Dixon WJ. Processing data for outliers. Biometrics 1953;9:74-80.*
14. *Stephens MA. EOF statistics for goodness-of-fit and some comparisons. J Am Stat Asso 1974;69:730-737.*
15. *Solberg HE. Satistical treatment of the reference values in laboratory medicine: Testing the goodness-of-fit of an observed distribution to the Gaussian distribution. Scand J Clin Lab Med Suppl 1986;46:125-132.*
16. *Collins PP. Determination of normal values from a hospital population. Am J Med Tech 1975;41:25-31.*
17. *Ünsal İ, Enünlü CT. Hasta örneklerinden derlenen veri kullanılarak laboratuvar testleri için, "Sağlıkla ilişkili sınırlar" ın saptanması. Biokimya Dergisi 1998; 13: 6-11.*