Original article

Establishing ranges of clinical normal limits and comparison with adopted limits for adult population

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Abstract: It is important to know the normal limits for each test in each laboratory. In most cases the normal limits established by others have been adopted and used as reference values. In view of this an attempt is made in this paper to establish ranges of clinical normal limits for adults. Eight determinations SGOT, SGPT, ALP, BILD, BILT, FBS, UREA, and CREATININE were included in the study. Normal limits were established based on a validated statistical method. Comparision was made with adopted normal limits in use in laboratories. For most tests notable differences in limits, particularly from the side of abnormal values, have been observed which resulted in high misclassification of laboratory test values. [*Ethiop. J. Health Dev.* 1997;11(2):97-101]

Introduction

Factors including sex, age, exercies, diet, emotion, posture, tourniquet, drugs, and fluid intake are examples of things known to affect certain tests (1). Surveys have shown that the normal ranges which laboratories report have little relation to results of their tests of survey specimens since they adopted ranges established for different populations. It is important for a laboratory to know what normal limits of each procedure are in its hand. In most instances the normal limits established for different populations have been adopted, even though problem of standardization and idiosyncrasies of individual laboratory may make these quite erroneous (2).

The concept of 'normal ranges' evolved as statistical methods were applied to laboratory medicine since 1951. In 1951, Wooton applied statistical methods for calculating normal range estimates of healthy volunteers (3). Following this several writers have devised different methods of estimating normal ranges. Basically there are two approaches in establishing normal limits. One can establish normal limits from healthy individuals which is considered as a gold standard procedure.

Data can be collected from healthy individuals and normal ranges could be established. However, the procurement and analysis of specimens from a large number of representative normal subjects is expensive and difficult. Experience has shown that for most clinical laboratories and most type of tests the great bulk of the routine samples for which analysis is requested are usually within normal limits. In addition, the great majority of abnormal values tend to be on a single side of the normals for most tests (4). It has been suggested that the accumulation of values for healthy persons is unnecessary and the clinical limits can be established from routine data by a purely statistical method which is regarded as a second approach for establishing normal limits.

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presented a method for estimating mean and standard deviation of a Gaussian distribution in the presence of one sided 'contamination', the side of contamination being known. He extensively explored the properties of estimates made by such methods under various known conditions of contamination by means of computer simulation and he has proved the estimation procedure to be robust for practical application. He, pointed out, however, that his contribution was in establishing and validating a statistical method, not in testing whether the underlying assumptions, were valid in actual application. In view of this a study involving 18 determinations to validate his procedure has been done. The study concluded that in using his method no evidence for systematic bias in characteristic value, dispersion, or position of limit on side of contamination has been seen when

Becktel developed a method for establishing normal limits from routine laboratory data. He compared with those limits derived from healthy individuals (2). Therefore, his method has been proved and recommended as a valid and useful method for setting normal limits in the laboratory. The normal limits used by the laboratories in this country are specified by proprietors elsewhere in the world. The applicability of these limits for Ethiopian population is questionable since several factors influence normal limits. Thus, It is worth trying to establish normal limits for the Ethiopian population and compare it with adopted ones, to determine the need to establish local normal limits.

Methods

Data was obtained from records of the Clinical Chemistry Laboratory of the Ethiopian Health and Nutrition Research Institute. For each clinical test under study a random sample was selected from the routine data of the year 1995. In the analysis only those aged over 20 were included. All tests under study were performed on Coulter KEM-O-MAT2, Phase 2 HP85, by kinetic assay measurement as part of the routine diagnostic service. The normal reference limits in use in laboratories are adopted from Coulter reagent kit, except for the enzymes, SGOT and SGPT, the reference values of which were obtained from Boehringer Manheim reagent kit.

The method employed to estimate the normal limits based on the routine data is a validated statistical procedure for estimating the mean and standard deviation of a Gaussian distribution in the presence of one sided 'contamination' which is developed by Becktel - Becktel's Method. Carefully selected samples of about 40 to 50 specimens yield an acceptable estimate from presumed normals (5). The Bektel procedure, however, requires about 2.5 times as many hospital subjects to achieve the same precision of estimates as can be obtained with a given number of presumed normals (2). Thus, a sample size of at least 125 will be required to apply the method. In general large sample sizes would be more preferable. All the tests under consideration satisfy the required sample size. For each test, sample size was computed according to the weight (frequency of occurrence) of each test. The most frequently occurring test, ALP, has for example the largest sample size compared to others. On the other hand, the least frequently occurring test, BILT, has the smallest sample size (Table 2). It is assumed that the bulk of the observations are from a Gaussian distribution and it is also assumed that the bulk of abnormal values are not too near the mean of those values from 'healthy' individuals for that test. In particular the latter assumption demands estimation of the mean either by the point of greatest concentration of observed values (the mode) or by the median of the observed values, depending on which is farther from the end known to contain the abnormal values, if any. In the estimation of the mode, the first step involved is to rank the observations in order of magnitude (X_1 to X_N). From the total number of observation, N, we find J, the largest odd number not greater than N/2. Then we calculate the difference $X_J - X_1$, $X_{(J+1)} - X_2$,..., $X_N - X_{(N-J+1)}$. If there is a unique smallest difference, then the mode is the observation corresponding to the average of the ranks giving the smallest difference. If the average of the ranks giving the smallest difference is greater than (N+1)/2 (if N is odd) or ((N/2) +((N/2)+1))/2 (if N is even), then the mean is estimated by the median.

When the Mode can be used as estimate of the mean of normal values encompassed, the number of such 'uncontaminated' observations, N^l, is estimated based on the presence of equal numbers (of 'normal' values) below and above the mean. If, however, the data are such that the mean is estimated by the median of the sample values, then $N^l = N$. Based on the estimated number of 'uncontaminated' or 'normal' values, N^l, percentiles will be calculated. For tests with high abnormal values, the 5^{th}, 10^{th}, 15^{th}, 20^{th}, 25^{th} and 30^{th} percentiles will be used and for those tests with low abnormal values the percentiles used are the 70^{th}, 75^{th}, 80^{th}, 85^{th}, 90^{th} and the 95^{th}. Linear interpolation will be employed when the desired percentile lies between two different values. The standard deviation is then estimated by the difference between the estimated mean and the average

of the six specified percentiles. Finally estimates of a 95% normal range for each of the tests were computed.

For many laboratory tests the distribution of values in normal subjects tends to be more nearly loggaussian than arithmetic gaussian (4). For those tests with coefficient of variation (sd/mean) more than 20% logarithmic transformation is required and the final limits are transformed back to the original scale. The coefficient of variations of all the test were found to be more than 20% and logarithmic transformations were performed for all of the tests under study (Table 2). Data entry and analysis was performed using EPI-INFO and LOTUS-123 computer programmes.

Result

Laboratory test results of the eight determination under study have been presented according to the adopted standard (Table 1). For all of the tests, except for FBS, the highest proportion (more than 70%) of the test results are in the normal range and in most of these tests the proportion of abnormal results found to be less than 20 %. In addition few results fall below the lower limit of the range. Therefore, one can easily understand that most of the results are within the normal limits of the adopted standard. Though most of the patients for whom analysis requested are those who are unhealthy and there are few Ethiopians who seek medical check up, the great majority of the routine samples are within the normal ranges of the adopted

Table 1: **Laboratory Results by the adopted standard**

	Normal range		Result		
Tests	sex		Normal	Abnormal	below
SGOT	M	9-30	212(73.4%)	73(25.3%)	4(1.4%)
	F	7-26	219(77.7%)	62(22.0%)	1(0.4%)
SGOT	M	8-42	229(79.8%)	54(18.8%)	4(1.4%)
	F	6-27	227(80.5%)	55(19.5%)	NONE
ALP	M + F	80-220	441(80.2%)	108(19.6%)	1(0.2%)
BILD	M + F	0.2-0.8	383(90.3%)	34(8.0%)	7(1.7%)
BILT	M + F	0.5-1.5	349(85.3%)	37(9.0%) 2	23(5.6%)
FBS	M + F	60-110	254(49.4%)	253(49.2%)	7(1.4%)
UREA	M + F	13-45	471(87.2%)	59(10.9%) 1	10(1.9%)
	M	0.7-1.6	200(85.8%)	25(10.7%)	8(3.4%)
CREAT	F	0.4-1.2	233(81.5%)	52(18.5%)	NONE

standards. In addition, most of the abnormal results are concentrated on the upper side of the limit (Table 1). Therefore, the data at hand satisfies all the necessary conditions to apply the Becktel's method. The presence of abnormal results is always expected, however, the most important thing is that the majority of these abnormal values should concentrate on the upper side of the limit.

Table 2: **Sample size, Mean, Standard deviation and coefficient of variation of each of the tests**

	Sex	N	Mean*	SD	CV**
SGOT	M	289	36.41	68.34	187.69
	F	282	26.06	32.99	126.59
SGOT	M	287	36.11	73.84	204.48
	F	282	23.60	37.02	156.86
ALp	M & F	550	194.78	157.87	81.05
BILD	M & F	424	0.57	1.28	224.56

BILT	M & F	409	1.11	1.81	163.06
FBS	M & F	514	154.05	98.06	63.65
UREA	M & F	540	32.05	27.28	85.11
CREATE	M	233	1.47	1.51	102.72
	F	286	1.21	1.28	105.78

* Arithmetic mean** CV (Coefficient of Variation)

= (SD/MEAN) *100

Arithmetic Mean, Standard deviation (SD) and Coefficient of Variation (CV) of estimates is presented in Table 2. All the tests under study have CV larger than 20 % and the distribution of values tends to be more nearly log-Gaussian than arithmetic Gaussian. Thus

Table 3: **Estimated Mean and standard deviation of the logarithmic transformed data and number of uncontaminated observations (N^l) based on the method**.

	Sex	N^l	Estimated Mean	Estimated[1] SD
SGOT	M	202	1.063257*	0.140863
	F	206	1.02328*	0.152811
SGOT	M	212	1.20412*	0.188812
	F	170	0.91046*	0.149811
Alp	M & F	409	2.12385*	0.108705
BILD	M & F	424	-0.45593**	0.17609
BILT	M & F	250	-0.22185*	0.09533
FBS	M & F	313	1.98227*	0.07871
UREA	M & F	535	1.41497*	0.18180
CREAT.	M	202	0.04139*	0.09099
	F	286	0.00000*	0.09919

* mean estimated by mode ($N > N^l$)

** mean estimated by median ($N = N^l$)

1 SD= estimated mean - average of the six percentiles

logarithmic transformation was performed on the original data. According to the method means and standard deviations of the transformed data were computed for each test. Except for BILD, where the mean estimated by the median, it is the mode which served as an estimate of the mean. In the case where the mode is used to estimate the mean the number of uncontaminated values, N^l, were computed and found to be less than the sample size (Table 3). The percentiles used in the method are the 5th, 10th, 15th, 20th, 25th, and 30th, since all of the tests under consideration have high abnormal values.

Only for three of the tests normal limits were presented by sex. The estimated limit for SGOT were found to be almost similar for both sexes, though, minor difference has been observed particularly from the lower side of the limit. Limits of SGPT have shown notable differences by sex, in particular, from the upper side of the limit (38.2 Vs 23). Such difference, though not very high has also been counted for Creatinine from both sides of the limit (Table 4). Adopted limits which are currently in use in laboratories in our country have been presented for comparison purpose. In three of the tests, SGOT for male, ALP, and Creatinine for male almost similar limits

Table 4: **Estimated and Adopted Ranges of normal values by sex, and percent misclassified**

	Sex	Side of Abnormal Value	Estimated Normal * Limit	Adopted Normal** Limit	percent Misclassified
SGOT	M	high	8.4 -30.6	9 -30	NONE
	F	high	7.4 -30.3	7 -26	4.6 %
SGOT	M	high	6.7 -38.2	8 -42	3.8 %
	F	high	5.8 -23	6 -27	7.1 %
ALP	M & F	high	80.6 -220	80 -220	NONE
BILD	M & F	high	0.13 -0.67	0.2 -0.8	1.9 %
BILT	M & F	high	0.39 -0.93	0.5 -1.5	11.0 %
FBS	M & F	high	67 -139	60 -110	13.0 %
UREA	M & F	high	11.3 -60	13 -45	5.7 %
CREAT.	M	high	0.72 -1.67	0.7 -1.6	NONE
		high	0.64 -1.58	0.4 -1.2	10.5 %

* Normal limits in original scale

** Normal limits currently in use in laboratories

SGOT= Serum Glutamate Oxaloacetate Transaminase - unit (iu/l)

SGPT = Serum Glutamate Pyruvate Transaminase - unit (iu/l)

ALP= Alkaline Phosphatase - unit (iu/l)

BILD= Bilirubin Direct - unit (mg/dl)

BILT= Bilirubin Total - unit (mg/dl)

FBS= Fasting Blood Sugar - unit (mg/dl)

UREA= Urea - unit (mg/dl)

CREAT= Creatinine - unit (mg/dl)

have been observed and no laboratory test value was misclassified (Table 4). In the rest of the tests discrepancies have been noticed. When compared to the estimated normal limits 13 % (67/514) of laboratory test values have been misclassified in FBS and were false positive, particularly from the upper side of the limit (138 Vs 110). In the case of BILT, the misclassification is also high where 11 % (45/409) of the test values were false negatives which occurred from the upper side of the limit (0.93 Vs 1.5). For Creatinine 10.5 % (30/286) of the laboratory test values have been misclassified for females and were false positive. Such misclassification can also be seen in limits of SGPT for female (7.1 %), UREA (5.7 %), SGOT for female (4.6 %), SGPT for male (3.8 %) and BILD (1.9 %) particularly from the upper side of the limit (Table 4). Except for three of the tests, the estimated and adopted normal limits under study have shown dissimilarities particularly from the upper side of the limit where the misclassification occurred. In general the adopted limits were found to misclassify laboratory test values either as false positive or false negative.

Discussion

Though normal limits could be established from a large group of presumed normals experiences have shown that it is an expensive and difficult task. Taking this fact into account, a validated statistical method has been used to establish normal limits from routine laboratory records. The method has been proved to be a workable, relatively unbiased, and simple for estimating normal limits from routine clinical experience of a laboratory and recommended for clinical use (2). Therefore, any laboratory could establish its own normal limits at no extra cost other than the computation time involved. Moreover, repeated determinations of normal limits over a period of time can serve as a form of quality control in laboratories.

In general, the adopted normal limits for most of the tests do not represent the population under study. The 95% confidence limit nearely overlap one another only in three of the tests. However, most of the limits under examination show major discrepancies. For most of the tests the adopted limits are found to misclasify laboratory test values either as false positive or as false negative. This clearely shows the urgency and need of establishing normal limits for Ethiopians. In this study normal limits were established only for eight of the tests performed in clinical laboratories, although there are other such tests which need to be studied. One can consider this as an attempt towards establishing normal limits for Ethiopian populations at large. The aim of this study is not to give explanation for the dissimilarities obeserved between the adopted and the established normal limits. It is rather to bring to one's attention the fact that, given the urgency for each laboratory to establish its own normal limits, there exists a simpler, clinically validated and statistically sound method for estimating normal limits from routine laboratory data. The need for such a less expensive and less difficult method would be more evident when one considers the fact that normal limits should not only be established but should also be verified and updated from time to time. Regarding the biochemical and other possible scientific explanations for the observer differences between the established and adopted limits, this study has nothing to contribute. It is important, however to note that normal limits can affect patient's treatment and therefore, problems associated with misclassification of laboratory test values would be real and important.

Data for this study is taken from records of one laboratory, therefore, the established normal limits might not represnt the population of Ethiopia. Thus, it is recommended to carry out such studies in the diferent regions of the country in order to have good representation of the population. The inclusion of other laboratory tests which are not examined in this paper would be essentail.

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