Normal Range Estimates of Serum Chemistry Values in Adult West Malaysians

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RESULTS OF clinical laboratory investigations can only be properly interpreted when relevant normal values are available for comparison. Few published normal biochemical values of the adult population in this region are readily available to clinicians except for the reports on serum ceruloplasmin (17), cholesterol (2, 3, 6, 7, 11), lipids (2, 3), magnesium (13), and some of the steroid hormones (18). Changes in analytical techniques also tend to invalidate the use of some older data. With, the introduction of automation in clinical laboratories analytical techniques are now mostly standardized. This not only makes laboratory analysis and acquiring of normal values less tedious, but also allows for inter-laboratory comparison of results.

This paper presents data from a preliminary survey of normal healthy adults carried out in our laboratory mainly with automated methods. Nineteen biochemical values were investigated and the results analysed.

Materials and Methods

The sample consisted of 577 subjects, drawn from 55 laboratory staff, 175 medical students and 347 blood donors. The blood donors were divided into two groups. One group of 193 had blood samples taken from one of the anticubital veins of the opposite arm before the blood donation. In the second group of 154 the samples were taken from the tubing of the blood donation set.

There were 504 male and 73 female subjects. Their ages ranged from 17 to 55 years, 81% of whom were between 20 and 35. Twenty-seven of the subjects were below the age of 20 years, their ages ranging from 17 to 19. They have been grouped as adults because biochemical values for this age group do not significantly differ from those of adults (4, 5, 9, 12, 15, 19). The distribution by ethnic group and sex of the 577 subjects studied is shown in Table 1.

Table I

Composition of Samples by Ethnic Group and Sex

Ethnic Group	Male	Female	Total
Chinese	218	50	268
Malay	140	13	153
Indian	138	9	147
Others	8	1	9
Total	504	73	577

Blood samples were drawn in the morning, after overnight fasting, from the laboratory staff and medical students. Collections were made at all times of the day from blood donors, the majority of whom had no food for at least 2 hours. Samples intended for glucose and urea were collected into bottles containing fluoride and oxalate. Plain universal bottles were used for samples intended for all other estimations. Separation of plasma or sera from cells were carried out within half an hour of sample collection. Analyses were performed within 48 hours of collection, along with routine hospital samples, by methods currently in use in this laboratory (Table II). Automated methods were performed on the Technicon Auto Analyzer (Technicon Corporation, Tarrytown, New York, U.S.A.).

Table II

Methods Used and Their References

	Method	Reference
Blood glucose	AutoAnalyzer Potassium Ferricyanide	Technicon AutoAnalyzer Method N-16b I/II
Cholesterol	AutoAnalyzer-FeCl3HsSO4	Technicon AutoAnalyzer Method N-24a
Uric acid	AutoAnalyzer-Phosphotungstate	Modification of SMA 12/60 Method VIII
Total proteins	AutoAnalyzer-Biuret	Technicon AutoAnalyzer Method N-14b I/II
Albumin	AutoAnalyzer-Bromcresol Green	Beng and Lim, Amer. J. Clin. Path. 59: 14, 1973
Total bilirubin	AutoAnalyzer-Jendrassik-Grof	Simmons, N. A., J. Clin. Path 21: 196, 1968
SGOT	AutoAnalyzer-Morgenstern	Technicon AutoAnalyzer Method N-25b I/II
SGPT	Manual-Reitman-Frankel	Micro-Analysis in Medical Biochemistry Woot-ton; J & A Churchill, 1964, p. 112
Alkaline phosphatase	Manual-King-Armstrong	Mciro-Analysis in Medical Biochemistry Woot-gon; J & A Churchill, 1964, p. 101
Urea	AutoAnalyzer-Diacetyl Monoxime	Technicon AutoAnalyzer Method N-16b I/II
Creatinine	AutoAnalyzer-Alkaline Picrate	Modification of Technicon AutoAnalyzer Method N-11b
Calcium	Atomic Absorption	D. L Trudeau & E. F. Freier Clin. Chem. 2: 101, 1967.
Magnesium	Atomic Absorption	D. L Trudeau & E. F. Freier Clin. Chem. 2: 101, 1967
Inorganic phosphate	AutoAnalyzer-Phosphomolybdic Acid	Technicon AutoAnalyzer Method N-4c 1/II
Sodium	AutoAnalyzer-Flame II	Modification of Technicon AutoAnalyzer Method N 84 I/II
Potassium	AutoAnalyzer Flame II	Modification of Technicon AtoAnalyzer Method N-84 I/II
Chloride	Chloridometer (Buchler-Cotlove)	Cotlove, E., Trautham, H. V., and Bowman, R. L. J. Lab. Clin. Med. 50: 358, 1958.

Each set of data was examined for conformity to gaussian or log-gaussian distribution by plotting the percentage cumulative frequency on probability paper (10) and by the Chi-square test (1). Normal range estimates were calculated by the percentile method recommended by Reed et al (14), and the medians calculated according to standard formula. In all cases, the normal range estimates were calculated to include 95% of the target population with limits set at the 2.5 and 97.5 percentiles.

Results

The percentage cumulative frequency plots on probability paper for the 19 biochemical values are shown in figures 1 to 4. On normal probability paper only the plots for serum total proteins, albumin and globulins gave straight-line graphs (Fig. 1), indicating their frequency distributions to be gaussian. Chi-square test confirmed gaussian distribution for these values. The Chi-square test and plots on log-probability paper (Fig. 2) showed that 7 of the biochemical values conformed to log-gaussian distribution. These included the A : G ratio, serum magnesium, cholesterol, urea, inorganic phosphate, potassium and calcium. Curved-line graphs were obtained when data for the remaining 9 biochemical values were plotted on both normal and log probability papers (Fig. 3 and Fig. 4). The Chi-square test when applied to their data and the respective log values also showed they do not conform to either gaussian or log-gaussian distributions. Table III compares the frequency distributions of the 19 biochemical values observed in this study with those reported by Roberts (16) and Wootton (20). Only 6 of the 19 biochemical values conformed to the frequency distribution as reported by either of these authors. Three of 7 biological values previously reported to have gaussian distribution were found to have log-gaussian distribution, the other 4 did not conform to either gaussian or log-gaussian distributions. Non-conformation to gaussian or log-gaussian distribution was also found in 3 values previously reported to have log-gaussian distribution.

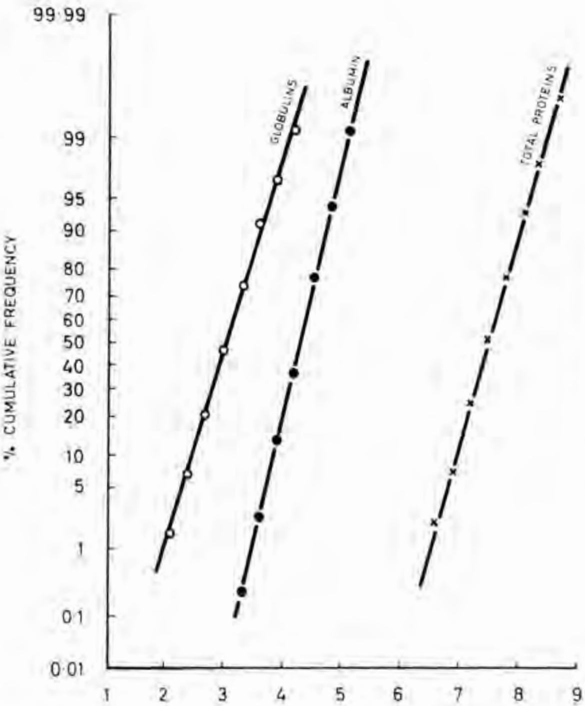

Fig. 1

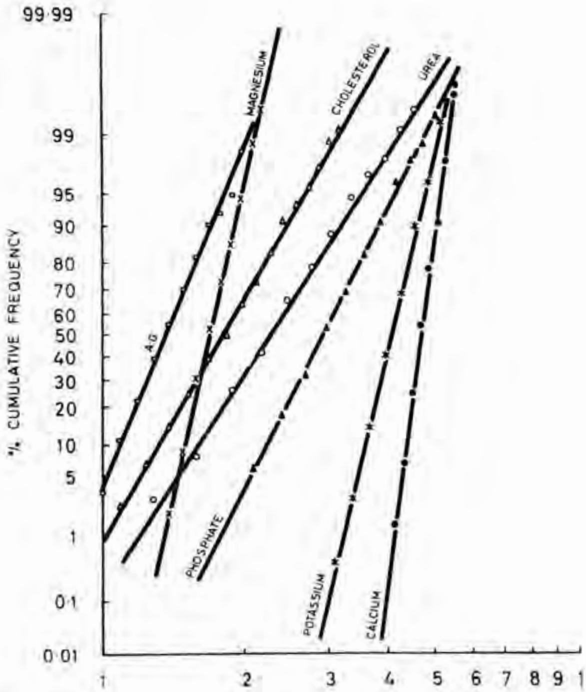

Fig. 2

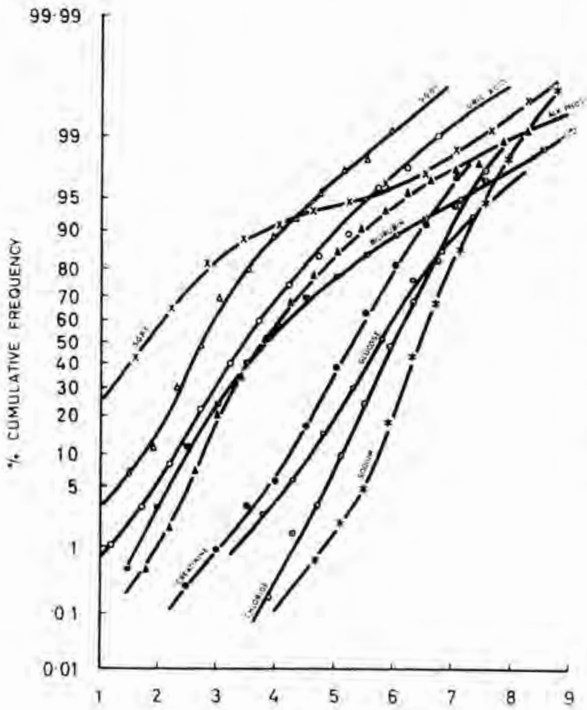

Fig. 3

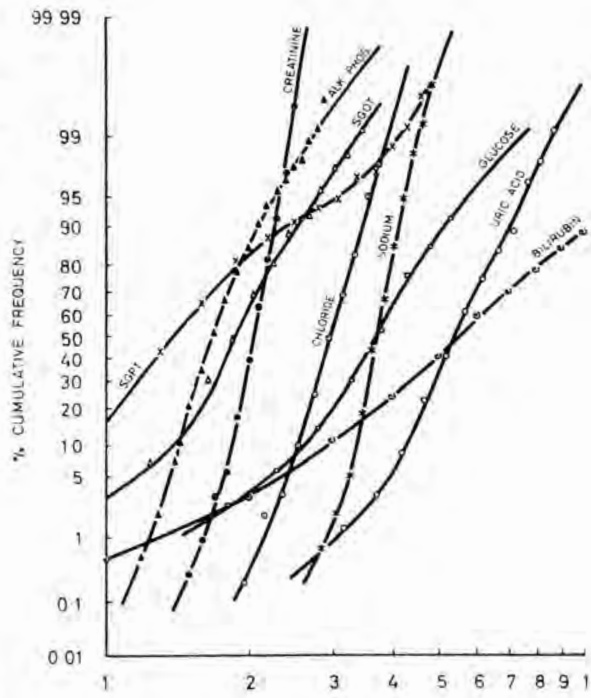

Fig. 4

Table IV shows the normal range estimates of the 19 biochemical values for all ethic groups. No attempts were made to compare the values between sexes as the number of female subjects was small. Only data from male samples were used for calculating the serum uric acid and creatinine values.

No significant ethnic group differences were observed in the normal range of 18 biochemical values. Serum uric acid was the exception, and the values in the 3 main ethnic groups are shown in Table V. Identical lower limits were obtained for all ethnic groups, but the upper limit for Chinese males was significantly higher than for Malay or Indian males ($p < 0.02$).

Table III

Frequency Distribution as reported by:

	Roberts	Wootton	Observed Distribution
Total Proteins	Gaussian	Gaussian	Gaussian
Albumin	Gaussian	Gaussians	Gaussian
Globulins	Gaussian	—	Gaussian
Calcium	Gaussian	Gaussian	Log-Gaussian
Magnesium	Gaussian	Gaussian	Log-Gaussian
Inorganic Phosphate	Gaussian	—	Log-Gaussian
A: G Rratio	—	—	Log-Gaussian
Urea	Log-Gaussian	Log-Gaussian	Log-Gaussian
Potassium	Gaussian	Log-Gaussian	Log-Gaussian
Cholesterol	—	Log-Gaussian	Log-Gaussian
Sodium	Gaussian	Gaussian	Neither
Chloride	Gaussian	Gaussian	Neither
Blood Glucose	—	Gaussian	Neither
Uric Acid	Gaussian	—	Neither
Creatinine	Log-Gaussian	Log-Gaussian	Neither
Total Bilirubin	Log-Gaussian	Log-Gaussian	Neither
Alkaline Phosphatase	Log-Gaussian	Log-Gaussian	Neither
SGOT	—	—	Neither
SGPT	—	—	Neither

Table IV

Normal Values for All Ethnic Groups

	Units	Number Examined	Median	Range
Blood glucose (Fasting)	mg/100ml	280	88	68 – 110
Cholesterol	mg/100ml	460	195	120 – 300
Uric acid (Males)	mg/100ml	453	5.5	3.8 – 8.8
Total proteins	g/100ml	372	7.6	6.7 – 8.5
Albemin	g/100ml	372	4.5	3.7 – 5.1
Globulins	g/100ml	372	3.2	2.3 – 4.1
A : G ratio		372	1.4	1.0 – 2.0
Total bilirubin	mg/100ml	382	0.6	0.2 – 1.4
SGOT	I.U./1	365	10	2 – 22
SGPT	I.U./1	360	6	2 – 27
Alkaline phosphatase	K.A.U./ 100ml	365	7	4 – 16
Urea	mg/100ml	436	25	14 – 41
Creatinine (Males)	mg/100ml	321	1.1	0.7 – 1.5
Calcium	mEq/1	291	4.8	4.2 – 5.5
Magnesium	mEq/1	189	1.7	1.45 – 2.16
Inorganic phosphate	mgP/100ml	342	3.1	2.0 – 4.4
Sodium	mEq/1	448	138	132 – 145
Potassium	mEq/1	822	4.3	3.5 – 5.1
Chloride	mEq/1	452	101	93 – 108

Table V

Uric Acid Values for Males According to Ethnic Group

	Uric Acid mg/100 ml		
	No. Examined	Median	Range
Chinese	203	5.6	3.8 – 9.2
Malay	123	5.4	3.8 – 8.5
Indian	127	5.6	3.8 – 8.3
All Ethnic Group	453	5.9	3.8 – 8.8

The frequency distributions of serum potassium values obtained from pre-donation and volunteer samples (group 1), and those obtained from post-donation samples (group 2) are shown in Figure 5. A range of 3.5 – 5.1 mEq/1 was obtained for group 1, while group 2 gave a range of 3.2 – 4.9 mEq/1. This difference is significant ($p < 0.01$). These two groups did not show significant differences for other biochemical values.

DISTRIBUTION OF SERUM POTASSIUM

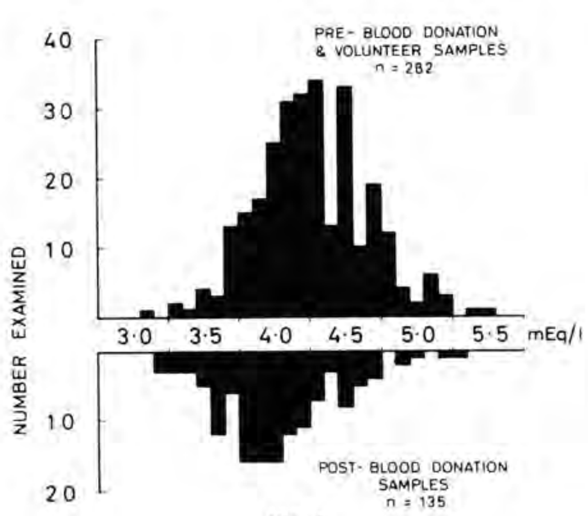

Fig. 5

Discussion

Normal range estimates are commonly derived from the expression, mean + 2 standard deviations, to include approximately 95% of the normal population. This technique, however, is based on the assumption that frequency functions of data are either gaussian or log-gaussian. Reed et al. (14) have pointed out that such an assumption is not true for many biological values. Our finding of 9 of the 19 biochemical values which are niether gaussian nor log-gaussian supports their observation. They have shown that in such instances the normal range estimates are more accurately derived by non-parametric statistical methods, which do not depend on frequency functions of data, and have recommended the use of percentile estimates together with non-parametric confidence intervals for the true percentile. This method has also been shown to be as accurate as estimates that assume the frequency functions to be gaussian or log-gaussian when the assumptions happen to be true. Non-skewed and skewed distributions are often assumed to be gaussian or log-gaussian respectively, as done by Roberts in his report. However, visual assessment of frequency functions of data is far too inaccurate. Statistical testing of the frequency functions of 7 biochemical values, previously assumed to be gaussian by visual assessment, showed 3 to be log-gaussian and 4 to be neither gaussian nor log-gaussian. Similarly, the frequency functions of 3 biochemical values previously assumed to be log-gaussian, were shown to be neither gaussian nor log-gaussian. Further the frequency distribution of serum potassium found by us and that reported by Wootton did not agree with that reported by Roberts (Table III). Calculation of normal range estimates by parametric statistical methods based on assumed gaussian or log-gaussian distributions could therefore lead to inaccuracies. All these difficulties and uncertainties can easily be avoided by using the non-parametric method recommended by Reed et al.

Variations in the normal range estimates due to ethnic group differences would be expected as the sample population is heterogeneous. However, significant ethnic group difference was observed only for serum uric acid, where Chinese males had a wider range than Malay or Indian males. This is because of the higher upper limit found among Chinese males. Seven values among the Chinese samples exceeded 9.0 mg/100ml. The highest value obtained for Chinese was 10.8 mg/100ml, for Indians 8.9 mg/100ml, and for Malays 9.1 mg/100ml.

Sampling donors at the end of blood donation is the easiest way to obtain samples for normal range studies. Flynn et al (8) have pointed out that this common practice can lead to errors, especially in the case of serum potassium values. The finding of lower serum potassium values in the post-blood donation samples in this study supports their observation. This not only emphasizes the need to exclude post-blood donation samples in the study of normal serum potassium values, but also the need for care when evaluating the serum potassium values of patients after acute haemorrhage.

It is often difficult to compare biochemical results obtained from separate laboratories due to differences in analytical methods. Most of the analytical methods used in this laboratory are well established and universally accepted, especially those adapted to the Technicon AutoAnalyzer. With increasing use of this automated equipment in clinical laboratories in this region, the data presented are applicable not only to the University Hospital, Kuala Lumpur but to all hospitals similarly equipped.

Acknowledgement

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References

1. Alder, H. L. and Roessler, E. B. in "Introduction to Probability and Statistics" W. H. Freeman and Co., San Francisco p. 192, 1964.
2. Chong Y. H. Serum lipids and lipoproteins in healthy Malaysians Med. J. Mal. 16, 136, 1962.
3. Chong, H. H., Soh, C. C., Ho, G. S., Rajaratnam, R., and Nonis, P. Serum low desnity lipoproteins, triglycerides and cholesterol levels in Malaysia. Clin. Chim. Acta 34, 85, 1971.
4. Christiansson, G. and Josephson, B. The uric acid concentration in serum from children, newborn infants and mothers after delivery. Acta Paediat. 49, 633, 1960.
5. Clarke, L. C. and Beck, E. Plasma alkaline phosphatase activity J. Pediatrics 36, 335, 1950.
6. Danaraj, T. J. Coronary artery disease in Malaya. Cardiologia Practica 13, 212, 1962.
7. Davis, T. A. L. and Willsher, J. D. Plasma Cholesterol levels in first, second and third generation Chinese and Tamil Indians in Singapore. Med. J. Mal. 15, 97, 1961.
8. Flynn, F. V., Gracia-Webb-Sharp. P., Piper, K. A., Healy, M. J. R. and Troop, J. Some immediate effects of a blood donation on plasma Chemistry. Clin. Chim. Acta 24, 51, 1969.
9. Hare, R. S. Endogenous Creatinine in serum and urine. Proc. Soc. Exp. Biol. Med. 74, 148, 1950.
10. Hoffman, R. G. Statistics in the practice of medicine. J. Amer. Med. Assoc. 185, 864, 1963.
11. Lau, K. S., Lopez, C. G. and Gan, O. M. Serum Cholesterol levels in Malays, Indians and Chinese in Malaya. Med. J. Mal. 16, 184, 1962.
12. Oberman, J. W. Gregory, K. O., Burke, F. G., Ross, S. and Rice E. C. Electrophoretic Analysis of serum proteins in infants and children. New Eng. J. Med. 255, 743, 1956.
13. Paul, F. M., and O'Brien, D. Serum magnesium levels in acute gastroenteritis in childhood. Singapore Med. J. 7, 98, 1966.
14. Reed, A. H., Henry, R. J. and Mason, W. B. Influence of statistical method used on the resulting estimate of normal range. Clin. Chem. 17, 275, 1971.
15. Reinhold, J. G. in Standard methods of Clinical Chemistry. Vol. 1 Academic Press N. Y. p. 70, 1953.
16. Roberts, L. B. The normal ranges, with statistical analysis for 17 blood constituents. Clin. Chim. Acta 16, 69, 1966.
17. Vella, F. Ceruloplasmin levels in body fluids in four ethnic groups. Med. J. Mal. 12, 456, 1957.
18. Wang, D. Y., Bulbrook, R. D. and Shanmugaratnam K. The plasma levels of dehydroepiandrosterone sulphate, androsterone sulphate, cortisol and transcortin in Chinese, Indian and Malay males. S'pore. Med. J. 10, 18, 1969.
19. Wilkinson, J. H. in "An Introduction to Diagnostic Enzymology" Edward Arrold (Pub.) Ltd., London, p. 118, 1962.
20. Wootton I. D. P. in Micro-analysis in medical biochemistry 4th Edition J. & A. Churchill Ltd. London 1964.