ESTABLISHMENT OF REFERENCE INTERVALS IN INDIAN POPULATION

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ABSTRACT

The central role of the laboratory scientist is to aid the clinician, in interpreting observed values, by providing relevant reference values in a convenient and practical form. In India, reference values used in laboratories have been established in the western population. But these can be questioned due to differences in genetic load, lifestyle, and diet. This review highlights the approach for establishing reference values in our population using the IFCC guidelines and our observations from our data as compared to the reported values in our laboratory.

KEY WORDS

Hypercholesterolemia, Reference limits, Reference values.

INTRODUCTION

Health of an individual is conceptually different in different countries, in the same country at different times and in same individuals at different ages. It is thus a relative and not an absolute state. This means that the condition of individuals must be related to or compared with reference data. On comparing the individuals data collected during the medical interview, clinical examination, and supplementary investigations with the reference data, the condition of individuals can be interpreted. A patient's laboratory result simply is not medically useful if appropriate data for comparison are lacking. It is thus the central role of the laboratory scientist to aid the clinician in interpreting observed values by providing relevant reference values and presenting them in a convenient and practical form (1).

A rational approach to providing a sound basis for interpretation of observed values calls for a theory, which describes the principles and procedures for selection of reference populations and definitions of reference values. The International Federation of Clinical Chemistry (IFCC), has coined some concise and well defined terms that permit unambiguous description and discussion of the subject of reference values (1):

- a. *Reference individuals* - individuals selected for comparison using defined criteria
- b. *Reference population* - consists of all possible reference individuals
- c. *Reference sample group* - subset of the reference population
- d. *Reference value* - value obtained by observation or measurement of particular type of quantity on an individual belonging to reference group
- e. *Reference distribution* - statistical distribution of reference values
- f. *Reference limit* - derived from reference distribution
- g. *Reference Interval* - interval between and including two reference limits
- h. *Observed values* - values obtained by observation or measurement of particular type of quantity, and produced to make a medical decision. They can be compared with *reference values, reference distributions, reference limits, or reference intervals*.

Strategies for selection of reference individuals

Of the strategies available for selection of reference individuals, only direct selection method (2) agrees with the reference values concept as recommended by the IFCC. In this, the individuals are selected from a parent population using defined criteria. However the disadvantages are the problems and cost of obtaining a representative group of reference individuals. In contrast, in indirect method (3) the individuals are not considered. The method assumes that the values of an

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analyte have a distribution with a preponderant central peak, which is composed, mainly of normal values. The normal interval can be estimated by extracting the distribution of normal values from this part of the distribution. However, the major disadvantages are the dependence of the lower and upper limits on the particular method used and varying of results not only across hospitals but also for the same hospital at different times depending on the characteristics of the hospitals patient group at that particular time. The other strategies are the *a priori* and the *a posteriori* sampling method (2, 3). The *a priori* (prospective) strategy is best suited for smaller studies and individuals fulfilling defined inclusion criteria are selected for sample collection. On the other hand, a *posteriori* (retrospective) method consists of database containing both analysis results and information on a large number of individuals. Values of individuals fulfilling defined inclusion criteria are selected.

Steps involved during establishment of reference values

While establishing reference values it is necessary that the individuals and methods of production of values be adequately described. Thus it is essential to specify the following factors:

1. Selection criteria

For health associated reference values, the IFCC has laid down certain guidelines (2). According to this the following individuals should be excluded:

- a) Pathophysiological states - renal failure, congestive heart disease, chronic respiratory diseases, liver diseases, malabsorption syndromes, and nutritional anemias.
- b) Systemic diseases - hypertension, diabetes
- c) Intake of pharmacologically active agents - alcohol, and tobacco, oral contraceptives, replacement or supplementation therapy eg. Insulin
- d) Modified physiological states - pregnancy, psychological and mental disorders such as severe stress and depression, exercise or physical training, feed intake prior to blood collection
- e) Other factors - obesity (BMI >30 kg/m²) (4).

The selected reference individuals may be further subclassified into more homogenous groups by stratifying them according to age and sex. Age may be categorized by equal intervals (e.g. by decades) or by intervals that are narrower in the periods of life where greater variation is observed.

2. Preparation of individuals and collection of specimens (3,5)

Ideally, specimens for the production of reference values for clinical use should be collected under conditions as similar as possible to those prevailing in clinical practice. As several factors cause increased variability of analytes, it is necessary to standardize the pre-analytical procedures. Important among biological factors are those that modify the metabolism of lipids, amino acids, and carbohydrates. Meals, prolonged fasting, pharmacologically active substances, hormonal supplementation therapy, stress, and physical exercise may have an effect on the metabolic state of the reference individual. Hemodynamic factors like change in posture (6), recent exercise, or tourniquet pressure can bring about an increase in the concentrations of proteins, calcium, fatty acids, and bilirubin. Intake of ethanol, anticonvulsant drugs may induce synthesis of liver enzymes (eg. gammaglutamyltransferase) and this may increase the clearance of many substances, thus affecting their concentration in serum.

3. Importance of Analytical Procedures and Quality control (7)

It is necessary to specify the essential components while establishing reference values:

- a) Analysis method (including information on equipment, reagents, calibration standards, type of raw data, and calculation method.
- b) Quality control
- c) Reliability criteria

The analyzer has to be calibrated with materials provided by the manufacturers. Changes in calibration curve and specificity of the analytical method can be detected by using a number of accuracy control specimens, at both normal and pathological levels of concentration, for the various analytes. During the course of the study there should be no change in the equipment, reagents, calibration standards, and controls. In order to maintain the required precision an adequate number of control specimens should be included at fixed or random positions in each analytical run (8). Ideally precision controls should be employed at different levels of concentration.

4. Determination of Reference limits

In clinical practice, one usually compares an observed patients value with the corresponding reference interval, which is bounded by a pair of reference limits (9). In cases, where the reference distribution of analytes shows Gaussian distribution (symmetrical), parametric methods are used. According to this, the determination of reference limits (percentile) would be

calculated as values two standard deviations below and above the mean. If the reference distribution shows another shape, one may use mathematical functions that transform data to approximately Gaussian shape. In the nonparametric method the percentiles are simply determined by cutting off the required percentage of values in each tail of the subset reference distribution.

Using the reference distribution the reference interval can be computed (10). Three kinds of reference intervals have been suggested: tolerance interval, prediction interval and interpercentile interval. The interpercentile interval is simple to estimate, more commonly used, and recommended by the IFCC. It is defined as an interval bounded by two percentiles of the reference distribution. It is an arbitrary but common convention to define the reference interval as the central 95% interval bounded by the 2.5 and 97.5 percentiles. The interpercentile interval can be determined by both parametric and non-parametric statistical techniques, as mentioned earlier.

5. Presentation of an observed value in relation to reference values (11)

An observed value (patients value) may be compared with reference values. The set of reference values can be made available to the clinicians in the form of tables, graphs or a few figures, either on result reports (laboratory sheets) or by separate publication. A printed set of reference data on reports is recommended only if one can ensure that it is relevant to the observed value to be reported. When necessary different sets should be given according to age, sex, activity, posture etc.

On the basis of the two reference limits of a reference interval, it is possible to classify an observed value as

- 'unusually low' when situated below the lower reference limit
- 'usual' if between or equal to either of the reference limits
- 'unusually high' when above the upper reference limit

On reports it is convenient to flag unusual results for e.g. by using 'L' for low and 'H' for high.

Establishing Reference Intervals

According to the IFCC it is necessary for every laboratory to have their own set of reference limits. However, in India most of the laboratories follow reference intervals established in the western population. The reference intervals can be questioned because of differences arising due to variations in diet, lifestyle etc. in western and Indian populations. So far we have not come across any literature on Indian

reference values, even an internet search did not yield any finding, thereby necessitating the need to establish the reference values in our population.

The BUPA Health Screening generated large amount of data and was used for establishing reference intervals for various analytes (12). We decided to conduct the study along similar lines for establishing the reference intervals in our population. The population chosen for this study comprised of individuals who had been to our hospital for a health check up program from the years 1996 -2001. The selection strategy in this study was direct and *a posteriori* (retrospective) method. A total of 39,940 medical folders were screened manually. The blood collection was done in fluoride, plain and EDTA tubes. While plasma from fluoride tube was used for estimation of glucose levels, whole blood from EDTA tube was used for WBC count, platelet count, and for measuring the hemoglobin concentration. Serum obtained from plain tube was used for analysis of BUN, Creatinine, Uric acid, Total Bilirubin, SGPT, Total Protein, Albumin, Alkaline Phosphatase, GGT, Total Cholesterol, Triglyceride, and HDL cholesterol. The preanalytical conditions (hours of fasting, morning and post prandial collection, body posture etc) were approximately the same in all the subjects. All those suffering from pathophysiological diseases, systemic diseases, on oral contraceptives, alcohol, smoking, documented depression, stress, etc were excluded. Among those excluded, from the population of 39,940 subjects, about 8986 subjects (22.5%) of the population were suffering from hypertension (HTN) BP>140/90. These subjects were already on treatment or were diagnosed to be hypertensive at the time of checkup. Apart from this 4.2% (n =1712) of the subjects showed the presence of slightly elevated BP. That the prevalence of HTN is high is highlighted by 2 studies- one in the urban population of north India where the prevalence was 31.5%, (13) and another in south Indian urban population, where the prevalence was 21.1% (14).

In our study diabetes was present in 14.2% (n = 5599) of our population. In addition, about 5.3% of the subjects showed the presence of glucose in urine. In a study conducted in the urban (n = 1806) populations of Moradabad, the prevalence of diabetes was 6.0% (15). Ramachandran *et al* assessed the prevalence of DM in 11,216 subjects in six major cities and found the prevalence of diabetes to be 13.5% among Chennai residents, in Bangalore - 12.4%, Hyderabad - 16.6%, Kolkata - 1.7%, New Delhi - 11.6%, and in Mumbai - 9.3% (16).

In our population, coronary artery disease (CAD) was found to be present in around 3.9% (n = 1542) of the subjects. These subjects had previously undergone angiography, angioplasty, or CABG. This correlates

with the finding in Jaipur, where the prevalence of CHD was reported to be about 4.5% (17). While 6% of the subjects in our study were positive for stress test, in about 0.7% of the subjects it was inconclusive. Additionally, 33% (n = 12861) of the patients had to be excluded as these were found to be suffering from ailments like anemia, asthma, urinary tract infection, or they were smokers, or alcohol consumers. As medication is known to affect the levels of certain analytes therefore patients on drug therapy (ayurvedic treatment, alternative medicine etc.) had to be excluded. Finally from the total of 39,940 subjects there were only 4466 subjects (11%) that could be termed as healthy and were used for establishing reference values.

All the biochemical and hematological parameters of these subjects were analyzed on automated analyzers using standard IFCC methods. During this period the equipments (Synchron Cx₇ and Coulter Counter from Beckman Coulter), reagents, controls etc were the same. Prior to analysis, the instruments were calibrated using calibrators and the controls run at normal and pathological concentration of the analyte. The instrument was calibrated using calibrators. Analytes like BUN, creatinine, glucose, and calcium were calibrated using Delta calibrator, bilirubin using Bilirubin calibrator, HDL cholesterol using HDL cholesterol calibrator. The remaining chemistries were calibrated with Multi Cal. Three levels of controls: Decision Level 1, 2, and 3 from Beckman coulter were run every 8 hours as a part of the internal quality control program. As part of External quality assurance our laboratory is enrolled in the proficiency testing surveys of the College of American Pathologists.

After filtering the data according to age, sex, and diet (vegetarians, non vegetarians), it was subjected to statistics using the SPSS-8.0 package. As many parameters displayed non Gaussian distributions, non parametric methods were used. The percentiles were calculated and the reference intervals reported as values corresponding to 2.5 percentile (r1) and 97.5 percentile (r2).

$$r1 = 0.025 (n + 1)$$

$$r2 = 0.975 (n + 1)$$

Along with this the corresponding 90% confidence interval (CI) was calculated for both the percentiles (18).

Analysis of 4466 subjects revealed that the consolidated intervals and the laboratory reporting intervals either showed minor variations or major variations (Table 1) causing the separation of the analytes into 2 categories. The first category comprised of analytes showing minor variations which included fasting and postprandial plasma glucose,

BUN, calcium, Total protein, albumin, alkaline phosphatase, creatinine, uric acid, bilirubin, SGPT, GGT, hemoglobin, WBC count and platelet count. In case of postprandial plasma glucose, high levels were found in the older age groups particularly in the 55-64yrs non-vegetarian females (64-175 mg/dl) as compared to our reporting values (70-140 mg/dl) [Graph 1]. The levels of BUN, calcium, total protein, albumin, WBC, and platelet, irrespective of diet did not vary much throughout the age groups. In case of creatinine, the consolidated intervals were not only higher than our reporting intervals (Table 1) but they were also higher in males than females. This could be because of storage of creatinine as a waste product in muscle mass and the presence of high muscle mass in males (19). Also the creatinine levels increased with increasing age (Graph 2). The consolidated interval from our current study also show the uric acid levels to be higher in males (3.8-7.8 mg/dl) than in females (2.6-6.3 mg/dl) as compared to our reporting values (males 3.4-7.0 mg/dl, females 2.4-5.7 mg/dl). However no differences were observed between the vegetarian (n=2780) and non-vegetarian groups (n=1686) at all ages in both males and females. The hemoglobin levels at the 2.5 percentile in males (12.3 g/dl) and females (11.0 g/dl) were lower than the levels at the 2.5 percentile in our reporting values (males 14.0 g/dl, females 12.0 g/dl). In males however, the levels at the 97.5 percentile were observed to be higher than our upper reporting limit (16.4 g/dl v/s 16.0 g/dl). Some of the other analytes showing variation with our reporting values were bilirubin, SGPT, GGT, and Alkaline phosphatase. The consolidated interval of bilirubin in our population (males 0.2-1.5 mg/dl, females 0.1-1.1 mg/dl) was found to be high as compared to our laboratory reporting value (0.2-1.0 mg/dl) and cannot be explained as none of these subjects show any history of jaundice or any other ailment affecting the liver. Though the subjects chosen for this study did not consume alcohol, yet elevated levels of GGT were seen in the 45-54 yrs non-vegetarian males and 55-64 yrs non-vegetarian females.

The second category consisted of lipids. In this category total cholesterol levels in both males and females showed an elevated consolidated interval (126-267 mg/dl and 123-263 mg/dl respectively) as compared to our laboratory reporting values (130-220 mg/dl). The median levels also showed an upward rise with increasing age in both the sexes irrespective of the diet. The high levels of total cholesterol in our population correlates with the finding that in the last 25 yrs there has been an increase in the total cholesterol by 25 mg/dl (20). Increased levels were also observed for LDL cholesterol (21) and triglycerides. There was not much of difference in the lipid and lipoprotein levels in both males and females irrespective of diet. This could be attributed to the sometimes infrequent consumption of meat and the vegetarian diet being rich

Table 1. Comparison of the reference values in the two groups

Reported Interval			Consolidated Interval LL (90%CI) - UL (90%CI) (LL-lower limit UL-upper limit)		
Total Protein (g/dl)					
Males (n = 2172)	6.7	- 8.2	6.4 (6.38-6.42)	-	8.0 (7.98-8.02)
Females (n = 2294)	6.7	- 8.2	6.3 (6.27-6.33)	-	8.0 (7.97-8.03)
Albumin (g/dl)					
Males (n = 2172)	3.2	- 5.5	3.5 (3.48-3.52)	-	4.9 (4.88-4.92)
Females (n = 2294)	3.2	- 5.5	3.4 (3.38-3.42)	-	4.8 (4.78-4.82)
BUN (mg/dl)					
Males (n = 2172)	5.0	- 25	5.0 (4.82-5.18)	-	17 (16.82-17.18)
Females (n = 2294)	5.0	- 25	5.0 (4.83-5.17)	-	16 (15.83-16.17)
Calcium (mg/dl)					
Males (n = 2172)	8.0	- 10.4	8.6 (8.57-8.63)	-	10.2 (10.17-10.23)
Females (n = 2294)	8.0	- 10.4	8.5 (8.48-8.52)	-	10.2 (10.18-10.22)
WBC (cells x10³/ml)					
Males (n = 2172)	4	- 10	4.2 (4.12-4.28)	-	9.3 (9.22-9.38)
Females (n = 2294)	4	- 10	4.2 (4.11-4.29)	-	9.8 (9.71-9.89)
Platelet (cellsx10⁵/ml)					
Males (n = 2172)	1.5	- 4.5	1.6 (1.61-1.68)	-	3.9 (3.87-3.94)
Females (n = 2294)	1.5	- 4.5	1.7 (1.68-1.75)	-	4.1 (4.07-4.14)
Fasting Plasma Glucose (mg/dl)					
Males (n = 2172)	70	- 115	80 (79.5-80.5)	-	116(115.5-116.54)
Females (n = 2294)	70	- 115	79 (78.5-79.5)	-	115(114.5-115.5)
Post Prandial Plasma Glucose (mg/dl)					
Males (n = 2172)	70	- 140	70 (68.9-71.1)	-	141 (139.6-141.7)
Females (n = 2294)	70	- 140	71 (69.9-72.1)	-	142 (140.6-142.7)
Creatinine (mg/dl)					
Males (n = 2172)	0.6	- 1.1	0.7 (0.69-0.71)	-	1.3 (1.29-1.31)
Females (n = 2294)	0.5	- 0.9	0.6 (0.59-0.61)	-	1.0 (0.99-1.01)
Uric Acid (mg/dl)					
Males (n = 2172)	3.4	- 7.0	3.8 (3.74-3.86)	-	7.8 (7.74-7.86)
Females (n = 2294)	2.4	- 5.7	2.6 (2.55-2.65)	-	6.3 (6.25-6.35)
Hemoglobin (g/dl)					
Males (n = 2172)	14	- 16	12.3 (12.2-12.4)	-	16.4 (16.3-16.5)
Females (n = 2294)	12	- 15	11.0 (10.9-11.1)	-	14.4 (14.3-14.5)
Bilirubin (mg/dl)					
Males (n = 2172)	0.2	- 1.0	0.2 (0.18-0.22)	-	1.5 (1.45-1.49)
Females (n = 2294)	0.2	- 1.0	0.1 (0.08-0.12)	-	1.1 (1.08-1.12)
SGPT (U/L)					
Males (n = 2172)	10 - 60		12 (11.07-12.93)	-	72 (71.07-72.93)
Females (n = 2294)	10 - 60		9 (8.38-9.62)	-	50 (49.01-50.25)

Table 1 contd.

Reported Interval			Consolidated Interval LL (90%CI) - UL (90%CI) (LL-lower limit UL-upper limit)		
Alkaline Phosphatase (U/L)					
Males (n = 2172)	40	- 120	38 (36.7-39.3)	-	123 (121.7-124.3)
Females (n = 2294)	40	- 120	35 (33.7-36.3)	-	120 (118.7-121.3)
GGT (U/L)					
Males (n = 2172)	0	- 60	8 (7.35-8.65)	-	49 (48.35-49.65)
Females (n = 2294)	0	- 60	6 (5.53-6.47)	-	35 (34.53-35.47)
Total cholesterol (mg/dl)					
Males (n = 2172)	130	- 220	126(123.8-128.7)	-	267(264.5-268.8)
Females (n = 2294)	130	- 220	123(120.9-125.1)	-	263(260.9-265.1)
LDL Cholesterol (mg/dl)					
Males (n = 2172)	110	- 140	67 (65.5-69.2)	-	192 (190.1-193.9)
Females (n = 2294)	110	- 140	65 (63.2-66.8)	-	185 (183.2-186.8)
Triglycerides (mg/dl)					
Males (n = 2172)	50	- 200	41(37.7-44.3)	-	256 (252.4-258.9)
Females (n = 2294)	50	- 200	35(32.6-38.2)	-	218 (215.2-220.8)
HDL Cholesterol (mg/dl)					
Males (n = 2172)	35	- 55	29 (28.48-29.52)	-	62 (61.48-62.52)
Females (n = 2294)	45	- 65	32 (31.41-32.59)	-	69 (68.41-69.59)

Graph 1. Postprandial plasma glucose mg/dl

Graph 2. Serum creatinine mg/dl

in saturated fats (22). In contrast, HDL cholesterol levels showed a downward swing with even the median levels being on the lower side of our reporting values. This observation correlates with the Coronary Artery Disease Among Indians study, wherein it was found that only 14% of the Asian Indian men and 5% of Asian Indian women had optimal HDL cholesterol levels (23). According to the National Cholesterol Education Program (NCEP) guidelines, in individuals free of CHD, total cholesterol levels of < 200 mg/dl, LDL cholesterol levels <130 mg/dl are classified as "desirable". HDL levels $\geq$ 60 mg/dl are considered as "negative" risk factor for CHD (24). Thus, if we were to revise the reference values for lipids and lipoproteins we would probably be putting the people at greater risk

for CAD events. As abnormal levels of lipids and lipoproteins can also pose a risk for the development of CAD, we further checked these 4466 subjects for hyperlipidemia using the current reporting intervals of our laboratory. Hypercholesterolemia (total cholesterol > 220mg/dl) was present in 18.4% of the subjects (Fig. 2). Elevated LDL cholesterol (LDL-C) [>140 mg/dl], and elevated total cholesterol: HDL cholesterol (HDL-C) levels (>4.5) were also included in the hypercholesterolemic group as total cholesterol levels influenced the levels of these analytes. In our population low HDL-C levels (males < 35 mg/dL and females <45 mg/dL) were present in 22.68% of the population (Fig. 2). This group also contained combinations of (a) low HDL-C and increased ratio, (b) low HDL-C, elevated LDL-C, with and without elevated ratio. Hypertriglyceridemia (>200 mg/dL) (Fig. 2) in our study was present in 1.52% of the subjects. Hypertriglyceridemia in combination with low HDL-C levels was present in 1.46% of the subjects. Finally there were subjects with combined hyperlipidemia. This group of 2.35% consisted of subjects with elevated levels of both total cholesterol and triglycerides with or without increased ratio, increased LDL-C, and low HDL-C. Only 42.18% of the population from 4466 subjects was found to be normolipemic.

Thus, from our study it appears that there are some analytes like cholesterol, triglycerides, HDL and LDL-C whose values need not be revised and some whose values may have to be revised and presented according to age and gender. With lipids it was felt that revising the reference values would no doubt result in a larger percent being classified as normolipemics but these would be unhealthy for the Indian population as they would be more prone to CAD. Under these circumstances it would therefore be advisable to advocate the implementation of recent NCEP Adult Treatment Panel (ATP) III guidelines (24). Also more such studies need to be conducted in laboratories across the country. The pattern emerging from these studies would be a deciding factor for revising the reference values. This would be of great help to the clinicians.

FUTURE ASPECT

Apart from establishing the reference values in healthy subjects in our population, it would be of interest to establish reference values in smokers and alcohol consumers. Smoking through the action of nicotine, is known to affect several laboratory tests. Cholesterol, triglyceride concentrations are higher and HDL cholesterol concentrations are lower in smokers. Other analytes that may get affected are albumin, glucose, and urea nitrogen. Similarly alcohol ingestion is known

to have a considerable influence on HDL and total cholesterol and GGT levels. The establishment of reference levels in these subjects would give an idea of the influence of nicotine and alcohol on the various analytes, as compared with reference values of our healthy population.

REFERENCES

1. Solberg H. (1987) International Federation of Clinical Chemistry, Expert Panel on Theory of Reference Values: Approved Recommendation on the theory of reference values. Part 1. The Concept of Reference Values. *J. Clin. Chem. Clin. Biochem.* 25, 337-342.
2. Solberg, H. (1987) International Federation of Clinical Chemistry, Expert Panel on Theory of Reference Values: Approved Recommendation on the theory of reference values. Part 2. Selection of individuals for the production of reference values. *J. Clin. Chem. Clin. Biochem.* 25, 639-644.
3. Grasbeck, R. and Alstom, T. (1981) Eds: Reference Values in Laboratory Medicine: The Current State of the Art. Chichester, England, John Wiley & Sons, Ltd.
4. Bray, G.A. (1989) Obesity: An endocrine perspective In: DeGroot LJ ed. *Endocrinology*, vol 3, 2nd edn. W.B. Saunders Co. (Publ), 2303-2337.
5. Solberg, H. (1988) International Federation of Clinical Chemistry, Expert Panel on Theory of Reference Values: Approved Recommendation on the theory of reference values. Part 3. Preparation of individuals and collection of specimens for the production of reference values. *J. Clin. Chem. Clin. Biochem.* 26, 593-598.
6. Felding, P., Tryding, N., Hyltoft, P. *et al.* (1980) Effects of posture on concentrations of blood constituents in healthy adults: Practical application of blood specimen collection procedures recommended by the Scandinavian Committee on Reference Values. *Scand. J. Clin. Lab. Invest.* 40, 615-621.
7. Solberg, H. (1991) International Federation of Clinical Chemistry, Expert Panel on Theory of Reference Values: Approved Recommendation on the theory of reference values. Part 4. Control of analytical variation in the production, transfer and application of reference values. *Eur. J. Clin. Chem. Clin. Biochem.* 29, 531-535.
8. Stamm, D. A. (1982) new concept for quality control of clinical laboratory investigations in the light of clinical requirements and based on reference method values. *J. Clin. Chem. Clin. Biochem.* 20, 817-824.
9. Solberg, H.E. (1994) Establishment and Use of Reference Values in Teitz Textbook of Clinical Chemistry. Burtis, C.A. and Ashwood, E.R. Editors, 2nd Edition, W.B. Saunders Co. USA, 471.
10. Solberg, H. (1987) International Federation of Clinical Chemistry, Expert Panel on Theory of Reference Values: Approved Recommendation on the theory of reference values. Part 5. Statistical treatment of collected reference values: Determination of reference limits. *J. Clin. Chem. Clin. Biochem.* 25, 645-656.
11. Solberg, H. (1987) International Federation of Clinical Chemistry, Expert Panel on Theory of Reference Values: Approved Recommendation on the theory of reference values. Part 6. Presentation of observed values related to reference values. *J. Clin. Chem. Clin. Biochem.* 25, 657-662.
12. Whitehead, T.P., Robinson, D., Hale, A.C. and Bailey A.R. (1994) *Clinical Chemistry and Haematology: Adult reference Values*. London: BUPA Medical Research and Development Ltd.
13. Gupta, R., Gupta, S., Gupta, V.P. and Prakash, H. (1995) The prevalence and determinants of hypertension in the urban population of Jaipur in western India. *J. Hypertens.* 13, 1193-1200.
14. Shanthirani, C.S., Pradeepa, R., Deepa, R., Premalatha, G., Saroja, R. and Mohan, V. (2003) Prevalence and risk factors of hypertension in a selected South Indian population-The Chennai Urban Population Study. *J. Assoc. Physicians India* 51, 20-27.
15. Singh, R.B., Bajaj, S., Niaz, M.A., Rastogi, S.S. and Moshiri, M. (1998) Prevalence of type 2 diabetes and risk of hypertension and coronary artery disease in rural and urban population with low rate of obesity. *Int. J. Cardiol.* 68, 65-72.
16. Ramachandran, A., Snehlatha, C., Kapur, A., Vijay, V., Mohan, V., Das, A.K., Rao, P.V., Yajnik, C.S., Prassana, K.K.M. and Nair, J.D. (2001) Diabetes Epidemiology Study Group in India (DESI). High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. *Diabetologia* 44 (9), 1094-1101.
17. Gupta, R. (1996) Lifestyle risk factors and coronary heart disease prevalence in Indian men. *J. Assoc. Physicians India* 44 (10), 689-693.

18. Solberg, H.E. (1994) Establishment and use of reference values In: Burtis CA Ashwood ER (eds). Tietz Textbook of Clinical Chemistry, 2nd edn, W.B. Saunders Co. (Publ), 454-484.
19. Whelton, A., Watson, A.J. and Rock, R.L. (1994) Nitrogen metabolites and renal function In: Burtis, C.A. and Ashwood, E.R. (eds). Tietz Textbook of Clinical Chemistry, 2nd edn, W.B. Saunders Co. (Publ), 1513-1575.
20. Gupta, R. and Singhal, S. (1997) Epidemiological evolution, fat intake, cholesterol levels and increasing coronary heart disease in India: paper presented at National Symposium on Hyperlipidaemia, March 21, New Delhi: NHS.
21. Friedwald, W.T., Levy, R.I. and Fredrickson, D.S. (1972) Estimation of the concentration of LDL cholesterol in plasma, without the use of the preparative ultracentrifuge. Clin. Chem. 18, 499.
22. Enas, E.A. (1996) Cooking oil, cholesterol and coronary artery disease. Indian Heart J. 48, 423-428.
23. 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.
24. Expert Panel on Detection (2001) Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA, 285, 2486-2497.