REVIEW ARTICLE

WHETHER WESTERN NORMATIVE LABORATORY VALUES USED FOR CLINICAL DIAGNOSIS ARE APPLICABLE TO INDIAN POPULATION? AN OVERVIEW ON REFERENCE INTERVAL

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ABSTRACT

Reference Intervals denote normative values related to laboratory parameters/analytes used by diagnostic centers for clinical diagnosis. International guidelines recommend that every country must establish reference intervals for healthy individuals belonging to a group of homogeneous population. Considering enormous racial and ethnic diversity of Indian population, it is mandatory to establish reference intervals specific to Indian population. The overview on reference interval describes why the national organizations in India need to initiate nationwide efforts to establish its own laboratory standards for apparently healthy reference individuals belonging to our polygenetic, polyethnic, polyracial, multilinguistic and multicultural predominantly rural and appreciable urban Indian population with varied dietary habits.

KEY WORDS

Reference Intervals, Normative Value, Indian Population.

The question whether clinical laboratory standards, in particular the reference intervals practiced in Western, European and other Asia Pacific countries are applicable to Indian population needs to be evaluated as so far no reference data is yet available for Indian population. Almost all laboratories in India use the reference values published either from available textbooks or from diagnostic kit inserts without giving details of the original source of the data. It is therefore necessary to define specific reference intervals for Indian population in view of our country's enormous ethnic and racial diversity. India's polygenetic 1.13 billion populations comprise six major ethnic groups (the Dravidians, Nordics or the Indo-Aryans, Mongoloids, Negritos, Pro-Australoids or Austrics and Western Brachycephals) and more than two thousand minor ethnic groups. The linguistic demographics describe four main family of languages (the Indo-European, Dravidian, Austro-Asiatic,

and Tibeto-Burman) and 1652 spoken languages and dialects. Out of major 216, Hindi is national language and others are recognized as official languages (Bengali, Telugu, Marathi, Tamil, Urdu, Gujarati, Kannada, Malayalam, Oriya, Punjabi, Assamese, Maithilli, Santhali, Kashmiri, Nepali, Sindhi, Konkani, Dongri, Manipuri (Meitei), Bodo and Sanskrit. According to 2001 census, the heterogeneous Indian population consists approximately of 80.4% Hindus, 13.4% Muslims, and 2.3%, Christians, 1.9% Sikhs, 1.1% Buddhists, 0.4% Jains and 0.5% others. The 2001 census documented 16.2% Scheduled Castes and 8.2% Scheduled Tribes. The age description of population shows that 31.5% are upto 14 years, 63.3% are between 15-64 years and 5.2% are 65 years or more. In the year 2006, the male to female sex ratio was 1.12 at birth, 1.098 under 15 years, 1.061 between 15-64 years, 0.908 at 65 years and above. On an average for total Indian population, the sex ratio is reported to be 1.064 males/females. According to 2007 estimates the life expectancy for males is 66.28 years, for females 71.17 years and for both gender 68.59 years. As per 2001 census 72.22 % population live in about 550,000 villages and remaining 22.78% in about 2000 cities and town (1, 2). The 22% of total population remained below poverty line.

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The normative values in the diagnostic laboratories used for clinical diagnosis were earlier termed as Normal Range, Normal Values, Expected Values or Reference Range. Later the term normal value was discarded as it was difficult to find completely normal individuals without any associated problem. National Committee for Clinical Laboratory Standards (NCCLS) recommended that frequently used terms like normal range and "reference range" or other terms must not be used as range describes minimum and maximum values of entire set of observations.

Based on definitions proposed jointly by International Federation of Clinical Chemistry (IFCC) and International Council for Standardization in Hematology (ICSH), the Clinical Laboratory Standard Institute (CLSI) formerly known as National Committee for Clinical Laboratory Standards (NCCLS) have described following new terminologies(3, 4).

Reference Individuals are the men or women selected carefully for the study based on well defined criteria.

Reference Population is the selected population the reference individuals belonged to

Reference Value is the observed value obtained on a reference individual for specific laboratory parameter/analyte.

Reference Sample Group is a group of selected reference individuals from a reference population on which the reference intervals are determined.

Reference Distribution is the distribution of all reference values of specific laboratory parameter / analyte from which a statistical calculation is made.

Reference Limit is the limit derived from reference distribution. It defines reference intervals and used for descriptive purposes.

Reference Interval is the interval between two reference limits of specific laboratory parameter /analyte i.e from the lower reference limit to the upper reference limit.

Observed Value denotes test results of a patient obtained in laboratory. The reference intervals are the health associated reference values which help in distinguishing healthy from the diseased state. It is important to mention that reference interval can also be determined for other physiological conditions e.g. pregnancy associated reference intervals. An excellent description of reference interval is available in various journals and text books (5-13).

NEED FOR ESTABLISHMENT OF APPROPRIATE REFERENCE INTERVALS

The laboratory results from a patient are compared with the health associated reference intervals for 1) interpreting clinical

laboratory data 2) supporting correct medical diagnosis and 3) decision on therapeutic management and other physiological assessment.

In summary, the carefully determined health associated reference intervals along with patient's clinical data help in determining the clinical status of a patient (14). Very sound understanding of reference intervals is therefore vital as diagnosis of disease frequently depends on results of laboratory analytes measured from blood, serum, urine and other biological fluids viz. Cerebro Spinal Fluid (CSF), ascitic fluid, synovial fluid, gastric juice and saliva etc. The poorly defined reference intervals due to improper basic and uniform process do not serve the medical needs of patients. The lack of direct comparability of results from different laboratories poses further problems to patients as well as to the treating physician (15). The patients do move from one place to another, one clinic / hospital to another, refer to different doctors, some times in between treated by alternate systems of medicine and investigated at different laboratories belonging to different geographical and cultural locations throughout India. The difference in laboratory results and the associated reference intervals impairs the clinical assessment and monitoring of patients. This leads to utter confusion in the minds of patients as well as treating doctor. The difficulty arises in the interpretation of results either due to unanticipated differences in standardization between laboratories or application of wrong reference intervals. It is known that differences in reference intervals between laboratories are not always related to assay standardization (16 – 18). On the other hand, it becomes a difficult task for diagnostic laboratories to establish and maintain reference Intervals for all the tests in parallel with ever changing methodologies and instrumentation over time. Very genuine difficulties with laboratory based reference intervals are 1) difficulty to support combine results of different laboratories from available electronic data bases 2) Prohibitive cost involved in performing reference interval studies in all laboratories for all analytes 3) Difficulty in developing reference intervals for few specialized and costly parameters such as hormones, vitamins, tumor markers and other antigen / antibody based serological markers 4) Problem due to delay in finding suitable healthy reference individual and 5) Lack of willingness amongst laboratories to work together.

Hence, the paradigm that all diagnostic laboratories must determine and maintain their own reference interval for each laboratory parameter offered by them is impractical and also did not meet the need of patients and doctors. In terms of the resources required, it was important to use less expensive

transference method. It was also recommended that if due to some reason the laboratory fails to establish the detailed reference studies, they are required to validate reference intervals published elsewhere using their own methodology for the population served by them. Boyd has recently addressed several issues regarding biological, environmental variability, transferability, lack of established reference measurement systems, lack of traceability of field methods to the reference system and lot to lot variability in reagents and calibrators (19). One of the possible causes of difference in local reference intervals may be due to racial makeup of the local population which may give difference in concentration of some common analytes; the inclusion of these differences in local reference intervals requires very careful attention (20-24).

One of the suggestions was that for determination of reference intervals, the full scale studies may be carried out by larger central clinical laboratories in collaboration with diagnostic kit manufacturers. It is important that collaboration should be established between professional organizations, industry and health authorities to achieve common reference intervals for homogeneous population group within a geographic area. The provision of reliable reference intervals is an important task for both the clinical laboratories as well as for the diagnostic kit manufacturers (25-27). Somehow the current dogma that every lab should establish its own reference intervals was not very successful and soon the concept became out dated. The complexity of project, impact on health, diagnosis and economics has led to a practical approach of sharing reference intervals between laboratories belonging to a same homogeneous population.

GUIDELINES AND RECOMMENDATIONS OF GLOBAL EXPERT ORGANIZATIONS

In Australia the guidelines presented in national association of testing authority (NATA) summary of ISO / IEC 17025 documents suggest that laboratories either establish their own detailed reference intervals or must validate reference intervals published elsewhere for their own methods and population (28). Usually, the recommendation for validation of reference intervals locally is mentioned in product information sheet of all suppliers of in-vitro diagnostic equipment and reagents. Method differences as well as population differences between laboratories should be mentioned in the locally determined reference intervals. The expert committees in USA formulated some set of rules which stated that each manufacturer of diagnostic kit must provide reference intervals and the laboratories are instructed to validate the same and prove

that they are applicable for their environment and also for their population. To be precise, it was suggested that reference intervals need to be locally modified by clinical laboratories. The recommendation of IFCC (9) also provided a list of factors to be controlled while planning to determine the reference intervals for particular lab parameter. The NCCLS and CLSI documents described detailed sample questionnaire and proper selection of reference individuals (4, 27). The expert committee did consider sources due to biological (metabolic, homodynamic, enzyme induction, cell damage) methodological factors (specimen collection, specimen transport, specimen handling), sources of variations and standardization (specific factors: supine vs. upright, prior diet, environmental conditions), pre analytical variation, subject preparation (prior diet, fasting vs non fasting, abstinence from pharmacological agents, drug regimen, sample time in relation to biological rhythms, physical activity, rest period before collection and stress), specimen collection (environmental condition during collection, time, temperature, body posture, specimen type, blood: arterial, venous, capillary, body fluids, collection site, site preparation, blood flow, equipment, technique), specimen handling (transport, clotting, separation of serum / plasma, storage, preparation for analysis), sample storage, analysis of reference values (minimum number of reference values, distribution pattern of reference values, frequency distribution of biochemical analyte, confidence intervals for reference limits, treatment of outlier observation, partitioning of reference values), consolidation of reference intervals, transference validation, presentation of reference values (laboratory presentation, manufacturers presentation) and other issues like qualitative analysis, therapeutic drug levels, time dependent / challenge tests, individual variation, critical values / medical decision limits, and manufacturer's data. The important issues to reconsider are 1) Selection of reference populations and definition of its size 2) Criteria for homogeneity / partitioning of the reference population 3) Definition of pre-analytical conditions 4) Definition of quantity 5) Selection of method of measurement including calibrators, reference materials, control procedures, and analytical quality specifications 6) Transferability and alignment of results 7) Sustainability of reference intervals and 8) Calculation of presentation of reference intervals.

EXCLUSION CRITERIA

The reference individuals should be selected after excluding following criteria such as:

Severe Alcohol consumption, Recent surgery, Professional Blood donors, Tobacco use, High blood pressure, Heavy smokers, Drugs of abuse, Drugs known to alter test results

(e.g. Statins, Steroids), Recent transfusion, Vitamin abuse, Fasting or non-fasting, Diabetes, Genetic factors, Nephropathy, Hospitalization (current/recent), Nephrotic syndrome, Recent Illness, Stroke, Severe Anemia, Lactation, Acute myocardial Infection, Obesity, Congestive heart failure, Oral contraceptives, Hypothyroidism, Hyperthyroidism, Stroke & other factors.

MODEL QUESTIONNAIRE FOR DOCUMENTATION OF REFERENCE INDIVIDUAL DETAILED INFORMATION

The following details should be documented about the selected reference individual

- Identification code
- Sample ID
- Name
- Telephone No
- Postal Address
- Age
- Sex
- Height
- Weight
- Abdominal circumference
- Hip circumference
- Race
- Ethnicity
- Religion
- Spoken Language
- Occupation
- Name of Team leader at site
- Do you think you are healthy? Yes/No
- Do you Exercise regularly? Yes/No
If yes ? (Hours per week)
- And degree of activity?
(Light 1 2 3 4 5 6 7 8 9 10 Vigorous)
- Any other physical manual labor / cycling type of sport activity
- Surrounding Industries if any, if yes details of Industry.
- Have you been sick recently? Yes/No If yes
When? And What?
- Are you taking any prescribed Medication? Yes/No, If yes
What?
- Do you have high Blood Pressure? Yes / No
- Do you take vitamin supplements? Yes/No
If yes, what?
- Do you use Tobacco? Yes/No
If yes?
- Do you smoke, if yes no. of cigarette per day, No. of Beedi's per day?

- Do you have a habit of reverse smoking?
- Do you eat a special diet?
Yes/No? If yes, please describe.
- Do you drink Alcoholic Beverages? Yes/No
If yes, what form?
- Main staple diet:
Rice/ Wheat / Jawar / Bajra / Any other.
How often you eat?
- Are you Vegetarian / Non Vegetarian?
If non Vegetarian, type of main meat- Beef, goat, sheep, chicken, fish, pork, sea food or any other
- What type of cooking oil consumed by you?
Ghee, Dalda, Postman, Ground nut, Til oil, Rice bran oil, Coconut oil, Mustard oil, Palm oil, Sunflower oil, Any other.
- Are you under a doctor's care presently? Yes/ No?
If yes, details?
- Have you been hospitalized recently? Yes/No. If yes, diagnosis?
- Are there any inherited health disorders in your family? Yes/No, if yes details?
- Have you taken Aspirin or any pain relievers recently? Yes/No. If yes
What? When?
- Have you taken any cold or allergy medicine recently? Yes/no.
If yes, what? When?
- Have you taken any antacids or stomach medicine recently? Yes/No. If yes, what? When?
- Are you taking diet pills? Yes/ No

FOR WOMEN

- Presently are you menstruating? Yes/No
If yes, date of last period?
- Are you breast feeding? Yes/No
Are you pregnant? Yes/No
If yes, details of gestational age, due date?
- Are you using oral or implant contraceptives? Yes/No If yes, details?

SELECTION AND PREPARATION OF REFERENCE INDIVIDUALS

The critical factors to be considered regarding subject preparation (29, 30) are a) the individual should fast overnight for 12 hours. Prolonged fasting for more than 12 hours should be avoided. The reference value for glucose, cholesterol, triglycerides, iron, inorganic phosphate, amino acids,

homocysteine, thyroid hormones, insulin, C-peptide etc are known to alter depending on fasting and non fasting prior to blood collection. b) The food containing moderate amount of carbohydrate should be taken one day prior to sample drawing c) Use of Caffeine, alcohol, tobacco and vitamin C must be avoided prior to sampling. Intake of alcohol should be avoided previous night as it will alter triglycerides, gamma GT, CDT and MCV. etc. d) the exercise should be moderate and not heavy e) any drug should be avoided for few days prior to blood collection. f) The posture should be appropriate during blood collection. g) The sample should be collected between 6:00 am to 9:00 am to avoid changes in analyte concentration due to circadian rhythms.

SAMPLE COLLECTION AND STORAGE

In case of Blood sample: 1) it should be clearly defined whether arterial, venous or capillary blood to be collected. 2) A proper selection of anticoagulant should also be defined. 3) It is advised to store the blood immediately after collection at specified temperature depending upon the stability of analytes for which reference intervals to be determined. 4) The serum should be collected after clot retraction only and plasma should be quickly separated from red cell as early as possible (31, 32).

In case of 24 hours urine collection, it is necessary to instruct the reference individual for complete and proper collection of urine.

The precise and accurate results of laboratory parameters will be achieved by preventing various types of common laboratory errors which are as follows

PREVENTING PRE ANALYTICAL VARIATIONS

The sample should not be collected immediately after strenuous exercise. Selection of correct posture during sampling as posture change does affect the concentration of many analytes for e.g. leucocytes, erythrocytes, hematocrit, total protein, few enzymes, lipoproteins and protein bound ions (calcium and Iron). Blood samples should be collected by trained phlebotomist. The selection of appropriate sample collection devices is equally important. Many pre analytical variations could be minimized by using vacuumed collection devices consisting of correct amount of anticoagulants for fixed amount of blood. Care should be taken to mix well the anticoagulant with collected blood by turning the sample tubes softly up and down at least ten times. The sample to be analyzed for Bilirubin should be placed preferably in dark in

order to avoid exposure to sunlight. The serum or plasma should be separated by centrifuging collected blood for ten minutes at 1500g. Gel based serum separating vacuum tubes also help in completely separating cells in blood from serum. While drawing blood the vein should not be compressed for more than a minute by using tourniquets. It is preferable to collect samples either in sitting or in recumbent position. Care should be taken to avoid diurnal variations. Diurnal variations are observed in case of few analytes such as epinephrine, prolactin, somatotropin, testosterone, bone markers hemoglobin and iron. Technical staff must be extremely careful in labeling of samples at all stages. It is recommended to use bar code for multiple sample aliquots. Serum should be separated only after clot is formed and retracted fully. All care should be taken to avoid hemolysis during sample preparation. Heavily icteric, hemolysed and lipemic samples should be discarded as it gives erroneous results. Sample (plasma, serum) should be prepared within one hour of collection. The blood smear should be prepared within three hours of blood collection. Sample should be transported only as serum or plasma. Transport of blood should be avoided in order to prevent hemolysis due to transport. For long term storage it is essential to freeze samples (serum/plasma) at- 20°C (4, 9).

PREVENTING ANALYTICAL VARIATIONS

It is mandatory to use bar-coded samples as well as reagents to avoid mix up or interchange of samples from two reference individuals. Before analysis, the sample should be thawed at room temperature for one hour and then mixed properly before analysis. The selection of diagnostic kit must ensure traceability of standards and calibrators with certified reference materials (CRM's). All the equipments used for analysis must be calibrated at desired intervals by the service engineers a copy of which calibration should be available for record. Prior to quality control the analyzers should be calibrated by technicians with calibrators provided by diagnostic kit manufactures. Following that quality assessment must be carried by using internal and external quality controls (IQC & EQC). The QC values falling beyond two SD and not following Westgard QC rule should be rejected. The analysis must be carried out in air conditioned room to avoid variation due to temperature fluctuations. For enzyme estimation, it is advisable that routine assay should be performed at 37°C and the methodology should be according to the IFCC reference method. The methodology for analysis of laboratory parameter must be selected as per recommendations of expert international committee guidelines (33-35).

PREVENTING POST ANALYTICAL VARIATIONS

It is preferable to interface analyzer bidirectionally with data management system having an appropriate laboratory information system (LIS). Test reports must be generated directly from the data management system in order to avoid transcriptional errors.

DATA COLLECTION AND MANAGEMENT

Data related to instrument manufacturer, instrument name, method used, units of expressed results must be documented. It is advisable to retain the anonymity of the reference individual. The data on age, gender, height, weight, date of last menstrual (women) and number of years residing in India should be documented (3, 4, 9).

POPULATION DISTRIBUTION FOR ANALYTE VALUES

Before applying any statistical method, it is mandatory to study analyte data distribution pattern for a study population. The variety of distribution patterns are described. 1) The Gaussian distribution or normal or bell shaped distribution is the most common pattern in which the Gaussian probability density functions with mean and standard deviation, 2) The log normal distribution pattern wherein the data do not follow a Gaussian distribution and many analyte values exhibit skewed behavior. In such situations, the log transformation is applied to achieve near Gaussian distribution, 3) Normal mixed distribution depicting departures from the Gaussian model and consists of two Gaussian components (a) the standard Gaussian comprising 90% of the population, and the contaminated normal probability density function comprising mixture of two normal densities with the same mean but different standard deviations, (b) Contaminated normal density function comprising a mixture of two normal densities with two different means and standard deviations and 4) Heavy tailed and skewed distribution indicating possibility of sample data containing spurious or outlier value or underlying population having heavy tails which explains that very extreme values may be due to inherent behaviour of the population. Usually the questionable extreme observations occur on only one side of the data mostly towards the larger values resulting in right side skewness. This type of distribution gives difficulties in analyzing reference intervals data due to normally occurring skewness and hence it must be taken into consideration.

OUTLIERS

On computation of reference intervals there is chance of

detecting extreme or atypical reference values called outliers. Outlier values may occur due to recording errors or laboratory errors or erroneous inclusion of a second group in the test group. Importantly the CLSI (clinical laboratory standard institute) experts recognized that outliers in the data may be a real possibility unless one identifies a mistake in analysis. Guidelines stated that such values should be retained instead of deleting them. It was suggested to follow Dixon's outlier range statistical method for identifying outliers. Once the outliers are identified or removed, the remaining observations are used for subsequent computation of the reference interval. In practice, the outliers should be studied further in order to evaluate their unusual behavior.

POSSIBLE PARTITIONING CRITERIA

It is necessary to select large population size so that partitioning could be carried out into different sub groups according to demographic description and other desired criteria (36-47). This helps having a sufficient number of reference individuals to produce high quality reference interval specific to selected subgroup. However one must be cautious in selecting minimal number of partition criteria to get sufficient number in each subgroup. The reference intervals on small sample size by single laboratory do not give satisfactory scientific data for proper partitioning into subgroups. In contrast, the large sample size makes partitioning into subgroups feasible and useful. It is necessary to select a minimum of 120 subjects of each sex or age or for other sample subgroup. The age, gender and ethnic variations were known to cause differences in reference intervals of a few laboratory parameters. Age must be further subcategorized by decades or by narrower intervals especially in case of paediatric group for e.g. neonates, infant, childhood, pre pubertal, pubertal, adult, pre menopausal, menopausal, geriatric etc (43-47). It is justified to define reference interval for particular subgroup only if it is clinically useful or based on physiological differences. The following list indicates appropriate factors on the basis for which partitioning could be done

- Age (pediatric, adult, geriatric)
- Blood group
- Posture at the time of sampling
- Time of day when sample collected.
- Sex / Gender
- Diet
- Exercise
- Fasting or non-fasting
- Industrial exposure
- Stage of menstrual cycle
- Gestational age of pregnancy

- Geographic location
- Racial and Ethnic Background
- Homogenous population
- Migrant population
- Religion
- Smoking
- Other environmental factors
- Altitude
- Vegetarian diet
- Non vegetarian diet
- Type of oil consumption
- Socioeconomic
- Environmental
- Chronobiological

STATISTICS RELATED TO PARTIONING CRITERIA

Several groups have described partitioning into subgroups based on different statistical models. The partitioning is recommended if the difference between the observed means of two subgroups is statistically significant at the 5% or 1 % probability level. According to initial report, the difference between subgroup mean should exceed 25% of the reference range calculated for the combined distribution if the subgroups are to be considered separately. The partition criteria according to Harris-Boyd model for Gaussian distribution is based on ratio(R) between the SD of subgroups (49, 50). According to their model, the percentage and distance criteria could be applied independently. For application of the distance criteria, both subgroup distributions should be Gaussian or have been converted to Gaussian distributions by a logarithmic transformation. If the mean of two subgroups are identical but the ratios of the standard deviations of the subgroups are more than 1.5 then the partitioning is recommended. The third approach classified three clinical decision limits of reference values as, the optimum, desirable and minimum quality based on different percentages. Lahti group have very well laid down new percentage and distance criteria for partitioning Gaussian distribution reference values. His model of partitioning was slightly different from Harris-Boyd model and suggested that non Gaussian distribution data to be transformed into Gaussian distribution. Their criteria suggested 4.1% as a critical minimum percentage outside limit justified partitioning into subgroups, and 2.3% as the critical maximum percentage outside limit justified combining them. The percentage between these two values was classified as marginal indicating non-statistical considerations to make final decision on partitioning (39-41, 50).

STATISTICS FOR CALCULATION AND PRESENTATION OF REFERENCE INTERVALS

It is necessary to use very good statistical software to calculate reference intervals. The responsibility for this job should be performed by an expert statistician. Few approved and universally accepted statistical programs are available for determining reference intervals (8, 51- 57). International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommend that the establishment of reference intervals requires a minimum of 120 individuals in each subgroup. With nonparametric statistics it is not possible to estimate the confidence limit for sample sizes below 120. With this number of samples, the 2.5th Centile is taken from the 4th lowest sample and the 90% of the confidence limit is defined by the 7th lowest sample.

It is suggested that for an accurate estimation of reference intervals, the larger numbers are required when partitioning is to be carried out on the basis of age, sex and other factors for establishing reference intervals for pediatric, adult and elderly population. Additionally the reference intervals will also be determined for different age groups separately for male and female gender and for each ethnic group. The reference intervals are represented as a percentage of Gaussian distribution. By definition, 2.5% of reference values of a reference distribution lie outside each of its two reference limits. And the reference interval is conventionally defined as the central 95% value of the population. If bias exists, the distribution may move to higher (positive bias) or lower (negative bias) values. Irrespective of the type of bias, more than 2.5% of the individuals will be outside one limit and less than 2.5% will be outside the other. The reference studies also include other statistical cut offs for example 99th Centile of healthy population for Troponin, 80th Centile for Apolipoprotein (a), target analyte concentrations, medical decision cut offs points and therapeutic intervals for few drugs.

TRANSFERENCE, ACCEPTANCE OF COMMON REFERENCE INTERVALS AND REVALIDATION

The difficulty of transferability of results between laboratories arises due to variability in various biological factors like ethnicity, dietary habits of population and the different methodologies used by the individual laboratories (58, 59).

The minimization of result variability between laboratories and the transferability of the results would be possible by using common calibrators (traceable to the same primary standard). Very often, the problem of variability can not be solved due to

inconsistent matrix of external quality control sample. The matrix used by the manufacturers for assayed or unassayed quality control serum could be the bovine or equine or human serum or it may be synthetic in nature. The matrix of quality control should be constant. Transferability would be achieved only if same calibrators are used and the calibrator should be traceable to the primary standard. The reference intervals established for the several labs serving a homogeneous population throughout a geographic area is gaining acceptance (4, 9).

There are genetic, environmental and physiological differences in reference intervals for several proteins in Caucasians and Asian Indians in Leeds. Alpha – I antitrypsin showed phenomenal difference between Tokyo, Hong Kong and Singapore. It is possible to establish reference intervals for relevant subgroups of main population as well as ethnic and racial minorities (60, 61).

Acceptance of common reference intervals by laboratories also needs adoption of common reporting formats and acceptance of assumptions concerning the intervals. While establishing common reference intervals, the first step in this process is willingness among laboratories towards one goal and the development of an organizational structure to provide leadership (62-67). Whenever a lab introduces new methodology for particular analyte it is suggested to review and revalidate the reference interval as uncritical transfer of old reference interval amounts to bad practice.

A CASE FOR COMMON REFERENCE INTERVALS

The prevailing difficulty that individual laboratories were unable to do justice with regard to determination of their own lab standards led to emergence of newer idea i.e. a case for common reference intervals. The determination of a reference interval is complex in terms of the inherent analytical and biological variables but rather simple in concept. The concept of determining common reference interval had a very good impact and it was well accepted by laboratory directors that each country must establish reference intervals. The concept of establishing common reference intervals for multiple labs serving homogenous population from same geographical area gained quick acceptance in the year 2002 (66). The theoretical as well as practical aspects of common reference intervals were discussed positively at length in several international meetings. However, a few difficulties arose regarding 1) True standardization differences 2) True local population differences and 3) Agreement on format of results and reference intervals. Many laboratory directors and expert committees made

recommendations relating to common reference intervals viz. 1) necessity to organize and support an expert body to oversee project 2) application of common statistical approaches for determination of common reference intervals so that it is acceptable to all 3) to obtain quality local data for reference intervals 4) criteria to publish common reference intervals and its use by laboratories and lastly 5) to overcome inertia in adapting common reference intervals as well as to encourage wide spread adoption 6) The selection of homogeneous population by following different criteria.

Various expert panels viz. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (9), Nordic Reference Interval Project 2000 (NORIP) (68) and National Committee for Clinical Laboratory Standards (NCCLS) (3, 4) have released a series of recommendations. The theory of reference values provided definitions, principles and procedures for determination of common reference intervals. Their recommendations and guidelines described the protocols in detail for determining reference intervals that meet minimum and mandatory requirements for adequate reliability, accuracy and usefulness.

DETERMINATION OF COMMON REFERENCE INTERVAL FOR HOMOGENOUS POPULATION IN DIFFERENT COUNTRIES

A very successful Nordic reference interval project on common reference intervals with a team of Nordic countries was initiated in the year 2000 and supported by Scandinavian Society of Clinical Chemistry (NFKK) (69, 70). Its results were published by Rustad et al in 2004. The project involved a total of 102 Clinical Laboratories from five Nordic countries comprising 30 labs from Norway, 26 Finland, 24 Sweden, 21 Denmark and 1 Iceland. Each lab worked with 25 reference individuals evenly distributed for gender and age. Each laboratory was instructed to collect serum, plasma and whole blood samples from a minimum of 25 reference individuals comprising of healthy personnel and their healthy adult family members. Each reference individuals were asked to fill out a questionnaire. Each lab also received 5 controls on dry ice one of them was calibrator containing reference method target value. The other two quality control samples were unmodified fresh frozen serum pools from male donors. The 4th QC material (high) was freeze dried serum pool and 5th QC material (low) was a diluted serum pool. Each laboratory was instructed to measure 10 replicates of calibrator and 3 of other controls. The data from each lab were submitted to a central data base. Reference intervals were computed centrally. The informed consent was obtained

from each reference individual and the study was approved by ethics committees in all the Nordic countries. A few of the inclusion criteria were that the individual has not consumed more than two measures of alcohol (24 g pure alcohol) in the last 24 hrs, has not given blood as a donor in the past 5 months, has not taken prescribed drugs other than oral contraceptives or oestrogens during the past 2 weeks, has not smoked during the hour before blood sampling. The Norip committee recommended reference interval for 25 common biochemical analytes.

Alanine transaminase; Albumin; Alkaline phosphatase; Amylase; Amylase Pancreatic; Asparate transaminase; Bilirubins; Calcium, Carbamide; Cholesterol; Creatinine kinase; Creatinine; Gamma GT; Glucose; HDL-cholesterol; Iron; Iron binding capacity; Lactate dehydrogenase; Magnesium; Phosphate; Potassium; Protein; Sodium; Triglyceride and Uric acid.

During the entire project a total of 42964 reference samples as plasma and 81757 as serum were processed and analyzed using five reference materials. A total of 124744 reference values were obtained. Apart from the main project, the reference interval common hematology analytes were also determined by laboratories in Finland and Sweden (71). A central bio bank was also created by depositing reference sample aliquots for further analysis. Subsequent to establish of Biobank, the Scandinavian society of clinical chemistry (NFKK) made available all reference sample for possible future project on other laboratory parameters NFKK has made expert group responsible for handling all data and sample belonging to Nordic Reference Interval project Bio and data bank (NOBIDA) (72). The reference interval calculation was performed by using RefVal 4.0 software which included the simple non-parametric, the bootstrap, and the parametric method (57).

The parametric method used two step transformation procedures to normalize a distribution. The recommended intervals have generally being adopted by all laboratories with minor exceptions. In the year 2004 all the Nordic countries implemented the common reference intervals for twenty five above mentioned chemistry analytes. The details of projects are available on the project website (72, 73). Later, other multi center projects have been undertaken. Following this many labs serving homogeneous populations have accepted common decision points for many analytes which were not determined or validated by individual laboratories. Enthusiasm for the use of common reference interval was growing after the Nordic reference interval project. In USA, the manufacturer is obliged to provide reference intervals and the laboratories

are obliged to prove that they are applicable in their environment.

In one project by Ichihara group on common reference interval for Japanese population, significant differences between laboratory test results were noted while studying reference population in six cities of Japan. Ichihara team was quite cautious in removing all possible variables. A single central laboratory analyzed deep frozen samples collected by different laboratories in six cities of Japan (74). Such differences were in contrast to the experience observed with Nordic reference intervals.

Another example of shared common reference interval was witnessed in Spain wherein each of thirteen Spanish labs collected eleven to fifteen samples comprising a total of 150 samples. The combined data was used to establish reference intervals.

The reference intervals have to be locally modified by the clinical laboratory due to biological variation for e.g. ethnicity, dietary habits and methodology used.

During the second joint European symposium of the clinical laboratory and in vitro diagnostic industry held in 2003 in Spain, the panelist discussed the theme "physiological reference values: A shared business? One of their recommendations stated that the cost for establishing reference intervals being exceedingly high and hence it should be an obligation of health authorities to finance the same. It would be worthwhile to create a team effort between professional organizations, industry and health authorities together to develop common reference intervals for a homogeneous population by involving multi center studies.

The published international standard used for the accreditation of medical laboratories 151895 stated that Biological reference intervals shall be periodically reviewed. If the laboratory has reason to believe that a particular interval is no longer appropriate for the reference population, then an investigation shall be undertaken, followed, if necessary, by corrective actions. A review of biological reference intervals shall also take place when the laboratory changes methodology of particular analyte procedure or pre-examination procedure, if appropriate.

SEMINARS / WORKSHOPS

In order to increase the awareness and better understanding of reference intervals among laboratory supervisors, seminars

and workshops should be conducted for better understanding in different states involving institutes, private laboratories, commercial hospitals and manufacturers of reagents and equipment.

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