ESTABLISHMENT OF A LOCAL BIOCHEMICAL REFERENCE RANGE: JOS UNIVERSITY TEACHING HOSPITAL EXPERIENCE

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SUMMARY

Objective:

The objective is to establish a local biochemical reference range for neonates in Jos environment.

Materials and Methods:

Three hundred and fifty normal full term babies were studied at birth for the purpose of establishing reference values for calcium, inorganic phosphate, Alkaline phosphatase, total protein and Albumin. The babies were selected from major delivery centres in Jos and the collection span for fifteen months. Exclusion criteria for the selection includes apgar score of less than 6/10, birth weight of less than 2.6kg and obvious signs of distress at birth.

Findings:

The result of the analysis of the cord blood samples were as follows:- Calcium (mean = 2.4mmol/L and range 2.0 = 2.8 mmol/L) inorganic phosphate, (mean 1.6mmol/L and range 1.1 = 2.1mmol/L) Alkaline phosphates (mean 52 IU/L and range 30 = 74 IU/L) total protein (mean 67 g/L and range 59 - 76g/L) albumin (mean 33 g/L and range 28 - 38g/L).

Conclusion:

There has been no previous studies on these variables in our environment, hence these figures can be taken as the local biochemical reference range for this age group.

This is to enable us interprete our laboratory data more meaningfully. This is vital for us to be able to manage effectively the metabolic disorders involving these variables in neonatal period in our environment.

Key Words: Reference value, neonate, environment.

INTRODUCTION, AIMS AND OBJECTIVES

The purpose of laboratory result is to aid the physician in making decision in the diagnosis and treatment of patients. It is therefore important that laboratory data be interpreted professionally by taking into consideration pre-analytical, analytical and post-analytical components.

In the early days of laboratory investigations, laboratory tests were qualitative tests which were read as negative or positive meaning normal or abnormal. Although it was recognized that borderline results occur occasionally and that different degrees of positivity were obtained with some test procedures. Even with quantitative tests introduced later, borderlines cases exisits making it difficult for straightforward interpretation.

The results obtained from these tests are used in screening for diseases, diagnosis of diseases and monitoring the response to treatment in established cases of disease. It is important therefore to consider biological variation between healthy individuals, inherent variations in laboratory methods, specificity and sensitivity of a test as well as errors of sampling and hospital practice all of which can influence the result of the laboratory test.

It is important to provide relevant sets of reliable reference data because laboratory results are interpreted by comparison with "Normal" (reference) values for a defined population. Recognizing difficulty with the term : "Normal," Clinicians have attempted to avoid it by recommending substitution of the term with "Reference" values.

Murphy as quoted by Henry [1], took an extreme position when he said "Normalcy is a vestigial concept left in medicine from its unscientific era. It is properly a subject for the philosopher to explore and not one to be settled by experimentation and observation which are the methods of science. We cannot come up with anything like an absolute definition of 'normal' from a scientific view point."

The population for which the reference values are to be obtained must be properly defined to avoid skewed distribution of laboratory data. This is to avoid major differences which can occur due to differences in age, sex, race and geography (environment). The methodology used for the assay should be clearly stated as variations in the results can be attributable to different methods used [2].

Reference values are a set of values of a measured quantity obtained from a group of individuals (or a single individual) in a defined state of health[3].

It is important to specify the level of health based on the criteria for inclusion or exclusion of subjects from whom reference values are obtained.

Paediatric clinical biochemistry is a relatively new area in chemical pathology but its relevance to the practice of clinical medicine cannot be overemphasized. This study is done on full term babies born in Jos located on a high plateau about 1400 metres above sea level in Nigeria. Convulsions and tetany are common presentations in neonatal life[4], and proper assessment of calcium status in this period is important in the differential diagnosis of these disorders.

The other parameters measured are equally important first in establishing a baseline figure for comparison and in the differential diagnosis of many metabolic disorders.

MATERIALS, METHODS AND RESULTS

The subjects used in this study were full term babies delivered vaginally between 37 and 42 weeks of gestation following uncomplicated labour in the hospital. Ethical considerations was observed by obtaining an informed consent from the mothers after explanation of the procedure. The subjects included were those whose mothers were not on any drugs such as calcium tablets or severe oedema following nephrotic syndrome that will significantly affect serum proteins and calcium levels. The babies included were those whose apgar score were not less than 6/10, and birth weight not less than 2.6kg and without obvious sings of distress at birth[5].

Three hundred and fifty (350) babies were included in the collection which span over fifteen months (January 1994 - March 1995). The babies included were delivered at the labour wards of both Our Lady of Apostle (OLA) Hospital and Jos University Teaching Hospital (JUTH) all in Jos Metropolis. One hundred and eighty-eight (188) of the babies were females representing 53.7%, while one hundred and sixty-two (162) were males, representing 46.3% of the total.

SAMPLE COLLECTION

The umbilical cord was clamped immediately after birth to avoid changes in umbilical vessel PH which might significantly affect the level of calcium [6,7]. The first set of clamp was placed proximally to the foetus and the other distally and 3 - 5 mls of blood taken from the umbilical vessel using needle and syringes (aseptically) into a dry plain specimen bottles and allowed to clot after 30 minutes. The samples were then spun immediately using Heraeus - Christ minifuge 2 centrifuge at 5000 rpm for 10 minutes. The supernatant (serum) was obtained using a Pasteur pipette and the analysis done in batches at a convenient time as the samples were stored deep frozen at (-30°C).

The total calcium was determined using the spectrophotometric determination of calcium in serum with O-cresolpthelein complexone[8]. Inorganic phosphorus was determined by the method of (Gomorri 1942)[9]. The enzyme alkaline phosphatase is expressed in international units (U) defined as the amount of enzyme that would convert 1 micromole of substrate per minute under standard conditions.

The total protein was measured by the biuret method while albumin was measured by the modification of (Bartholomew and Delaney Method - 1966).

The results obtained from the analysis of the serum samples of the newborn babies using EPI info ver. 5.0 and Harvard graphics ver. 2.12 computer programme (IBM PS2 Model 50Z) are shown in figures 1 - 5 below.

From the analysis of variance (ANOVA) done to compare the differences between the mean values of the two sexes, the P - values of all the variables for the test of significance between males and females were greater than 0.05 meaning that there were no significant differences between the two sexes for all the variables and this is however not unexpected.

The total sum, mean and standard deviations of each of the variables are shown in table 1. The reference range for each of the variables were calculated using the 2.5 percentile and 97.5 percentile formula given by range = mean $\pm$ 1.96 SD where SD =confidence interval= standard deviation.

The above formula was used to calculate the reference range because the frequency distribution of the variables follow a Gaussian distribution pattern as can be seen from the histogram presented in the figure (1-5).

Table 1 Means, Standard Deviation and Confidence Interval of Study Variables.

	Means $\pm$ (SD)	Range (95% CI)
Calcium (mmol/L	2.4 $\pm$ (0.2)	2.0 - 2.8
Inorganic Phosphorous (mmol/L	1.6 $\pm$ 0.3)	1.1 - 2.1
Alkaline Phosphates (IU/L)	52 $\pm$ (11.1)	30 - 74
Total Protein g/L	67.2 $\pm$ (4.3)	59 - 76
Albumin g/L	32.7 $\pm$ (2.6)	28 - 38

DISCUSSION AND CONCLUSION

The practice of laboratory medicine involves collection of empirical data and interpreting the data using scientific knowledge and professional experience in making decisions concerning diagnosis, prevention and therapeutic actions.

Absolute health does not exist, hence health is a relative term and it implies that it has to be related to something (i.e. comparison with reference data for the individual)[10]. It is usually better for each laboratory to have its own reference range values. Reference ranges are affected by many factors including the population from which they are obtained, the conditions under which the specimen were collected, actual handling of the sample and the analytical methods used. Because of the effects of geography (environment), age, sex, race, socio-economic factors and even abnormal births[11], reference range is best determined for the same population from which patients will be taken. The need for a reliable and simple method for the determination of a reference range cannot therefore be overemphasized[12]. The determination of sample size was done by empirical approach and certain basic requisites of a reliable sampling methods were undertaken[13]. These includes size, coverage, goal orientation, feasibility and cost efficiency without undermining the objectives of the study.The sampling procedure used was probability sampling and the approach is cluster sampling[14], where representative clusters (in this case the major centres of delivery in Jos) were selected.

Figure 1
Frequency distribution of serum calcium in newborn babies

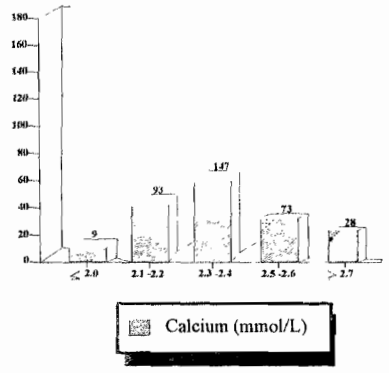


Figure 2
Frequency distribution of serum inorganic phosphorus in newborn babies

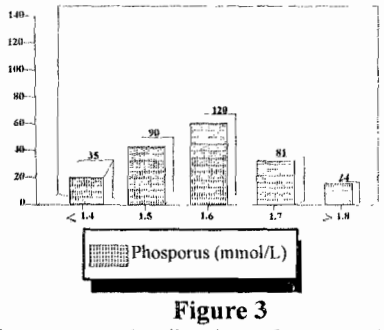


Figure 3
Frequency distribution of serum alkaline phosphorus in newborn babies

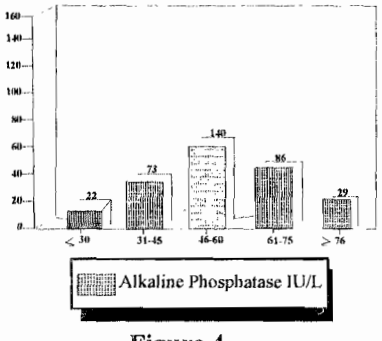


Figure 4
Frequency distribution of serum total protein in newborn babies

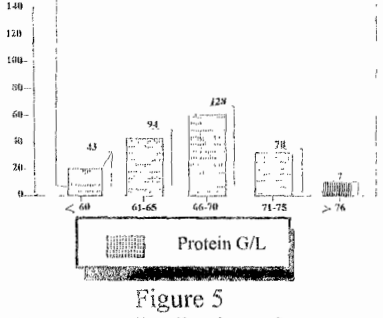
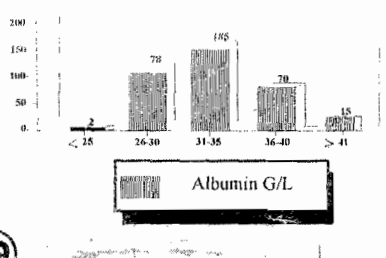


Figure 5
Frequency distribution of serum albumin in newborn babies



This was combined with stratification (clusters are stratified) to arrive at a properly defined population to avoid undue skewness in the distribution of the parameters under investigation. Methods available for determination of reference ranges includes parametric and non-parametric tests[15]. Parametric tests assume that a population has a symmetrical distribution (such as Gaussian or Log-normal) and this is the approach used in this study.

Determination of reference ranges by probit analysis and the use of probability paper has been beautifully described but these methods are too mathematical and cumbersome[16]. When it became the vogue to apply statistical techniques to the field of medicine, it was only natural that a simple statistical concept be applied to the reference range. This concept was the Gaussian curve, referred to as "normal" curve. Some statisticians believe that any defined population will Not have Gaussian distribution if a sufficiently large sample is obtained[17]. Yet others believe that the Gaussian curve should apply not only to repeated measurement of the same thing but to normal value too, and that any deviation from Gaussian distribution is due to failure to disentangle major sources of variations such as age, sex, season, socio-economic factors, and environment. The crucial point really is whether the distribution we have, for which we wish to calculate the reference range is or is not Gaussian, distributed. For non-Gaussian distribution, the curve can either be made to conform with Gaussian distribution by the use of mathematical transformation such as log-transformation or root transformation. If the curve cannot be transformed to conform with Gaussian distribution (negatively skewed curve) then non-parametric approach should be applied to calculate the reference range. Examples of non parametric tests includes chi square test, friedman's two way analysis of variance, Wilcon's matched pair test, and spear man's rank correlation test. The mean and range values obtained for the various variables studied in this work show some differences compared with published figures for Caucasians in this age group [18,19]. The paper by Adewoye and Bello [20], from Ilorin studied pre-school children. It has been reported by some workers [21], that the ratio of some analysis in the blood of neonates to those of adults are (Alkaline phosphatase = 2.5), (Inorganic phosphorus = 1.9), (Choesterol = 0.5).

In conclusion, there is a need for a local biochemical reference values for a more meaningful interpretation of laboratory data in our own environment for this age group.

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