DHR International Journal Of Biomedical and Life Sciences (DHR-IJBLS)
ISSN 2278-8301, Vol. 4(2), 2013
Available online *http://www.doublehelixresearch.com/DHRIJBLS*

©Double Helix Research

Original Article

Reference Intervals for Serum Bicarbonate in Men and Women of Semi-Urban Population of Mangalore

Sarfraz Ahmad [1], Sukanya Shetty [2*], Ashalatha V. Rao [3], Deepti G. Inglesgwar [4]
Roopa Bhandary [5], Tirthal Rai [6], Srinidhi Rai [7], Manisha Ojha [8]

[1,4] Post Graduate, Department of Biochemistry, K S Hegde Medical Academy, Deralakatte, Mangalore
[2] Professor and HOD, Department of Biochemistry, K S Hegde Medical Academy, Deralakatte, Mangalore
[3] Professor, Department of Biochemistry, K S Hegde Medical Academy, Deralakatte, Mangalore
[5] Lecturer, Department of Biochemistry, K S Hegde Medical Academy, Deralakatte, Mangalore
[6,7] Assistant Professor, Department of Biochemistry, K S Hegde Medical Academy, Deralakatte, Mangalore
[8] Post Graduate, Department of Pharmacology, K S Hegde Medical Academy, Deralakatte, Mangalore
[*]Corresponding author: Sukanya Shetty

Abstract: Age, gender, diet, and socio-economic conditions all affect the physiology of a population, and hence, measures of normal physiological functions are expected to differ from population to population. This calls for a need to establish reference values that are applicable to specific population, rather than take a set of reference values determined for one population and apply to another population. The aim of this study was to establish the reference intervals of serum bicarbonate levels in men and women of a semi-urban population. A cross sectional study was conducted over a period of 1 year and 8 months from November 2011 to June 2013. The study comprised 124 subjects of both genders, 19 – 50 years of age. Blood sera for bicarbonate was collected after obtaining informed consent. The results were analyzed by appropriate statistical methods. The median and reference interval (2.5th- 97.5th percentile) for serum bicarbonate for men and women obtained in the present study are 24.6 (19.3 - 29.6) and 24.0 (19.3-29.0) mmol/L respectively. There is statistically not much difference in reference intervals between males and females. The reference interval for the total study population in the present study is 19.4 to 29.4 mmol/L. The reference interval obtained in our study is wider when compared to the reference interval provided by assay manufacturer.

KEYWORDS: Reference interval; serum bicarbonate

Introduction

A major need for laboratory medicine and clinical chemistry personnel in particular, is to provide the clinicians updated and appropriate information on reference values, previously known as normal values. Introduction of the concept of reference values and reference population simplifies the task for laboratories; as long as they define the reference population, the outcome can always be recognized as reference values and reference intervals. Selecting reference individuals is an essential step in the production of reference values throughout world [1]. Reference interval is defined as the range of values obtained by observation or measurement of a particular type of quantity in a reference population.

Reference Intervals are the most common decision support tool used for interpretation of numerical pathology reports. As laboratory results may be interpretated by comparison with these intervals, the quality of the reference intervals can play as large a role in result interpretation as the quality of the result itself. Age, gender, ethnicity, diet, physical environment and socio-economic conditions all affect the physiology of a population, and hence, measures of normal physiological functions are expected to differ from population to population. So there is a need to establish reference values that are applicable to specific population rather than take a set of reference values determined for one population and apply to another population.

So far, few large population based studies have been done on reference limits in Indian population. The reference limits in use are either borrowed from textbooks and articles or insert literature from the kit manufacturers. The upper and lower limits of measurements vary dependent on the source of information as well as the methodology followed.

This study might help in establishing the reference values for serum bicarbonate in the local healthy semi-urban population in the age group of 18-55 years.

Materials and Methods

Study design: Cross-sectional study

Setting: K S Hegde Charitable Hospital

Sample size: 124 subjects (according to CLSI guidelines) [2].

Duration of study: November 2011 to June 2013

Selection of subjects:

The study was conducted in K S Hegde Charitable Hospital. Ethical clearance was obtained from the Institutional Ethical Committee before the start of the study. The informed consent was obtained from the subjects who were willing to participate in the study. Healthy subjects were selected based on the clinical history and clinical examination.

The subjects in the age group between 18 to 55 years of both sexes were randomly selected from the population, which included medical students, health professionals, and a few were also recruited from those attending different OPDs for general health check-ups under health plan scheme. Subjects below 18 years and above 55 years, with diabetes mellitus, hypertension, cardiac disease with hepatic/renal involvement, congenital or acquired diseases, hepatic or renal diseases, alcoholics, women on oral contraceptives, pregnancy, malabsorption syndromes and nutritional anemias were excluded from the study.

Analysis was done in clinical biochemistry laboratory.

Method of data collection

All the pre-analytical factors were standardized [4]. Blood samples were collected randomly, after giving 15mins of physical rest [5]. Blood was collected in sitting posture by venepuncture. 2 ml of blood samples were collected from the antecubital vein in plain red-topped vacutainer tubes containing clot activator. They were left undisturbed for 30 mins and then centrifuged for 5 mins at 3000 rpm. Sera were separated and analyzed in Roche Hitachi 902 autoanalyzer.

All the analyses were carried out on serum samples only. The precision of the instrument was checked on many occasions. All the analytical procedures were standardized, the reagents were calibrated to the instrument before sample analysis was done [6,7] .

METHODS OF ANALYSIS

The separated serum was analyzed for serum bicarbonate by enzymatic method.

Test principle for bicarbonate [8,9] **:**

Bicarbonate reacts with phosphoenolpyruvate (PEP) in the presence of phosphoenolpyruvate carboxylase (PEPC) to produce oxaloacetate and phosphate:

$$PEP + HCO_3^- \xrightarrow{PEPC} Oxaloacatate + H_2PO_4^-$$

The above reaction is coupled with one involving the transfer of a hydrogen ion from NADH analog to oxaloacetate using Malate Dehydrogenase (MDH).

$$Oxaloacetate + NADH\ analog + H^+ \xrightarrow{MDH} Malate + NAD^+\ analog$$

The resultant consumption of NADH analog causes a decrease in absorbance at 415 nm, which is proportional to the concentration of bicarbonate in the sample being assayed.

STATISTICAL ANALYSIS: All the statistical analyses were done using SPSS version 16. Data have been summarized using descriptive statistics (Mean ± SD). Non-parametric method has been used for the determination of Reference Intervals. The reference intervals are defined as the central 95% of the population comprised between the percentiles 2.5 and 97.5, leaving aside 2.5% of the individuals on both sides of the distribution [10]. Mann- Whitney U test has been used for the comparison of reference interval between males and females.

Results

A total of 124 subjects were recruited for the estimation of bicarbonate, out of which 66 (53.2%) were males and 58 (46.8%) were females (Table 1, Figure 1).

Table 1: Gender wise distribution of the age of the subjects for bicarbonate.

Gender	Number (%)	Range (years)	Mean (±SD) years
Females	58 (46.8)	19-45	24.9(±6.1)
Males	66 (53.2)	19-46	24.6(±6.1)
Total	124 (100)	19-46	24.7(±6.1)

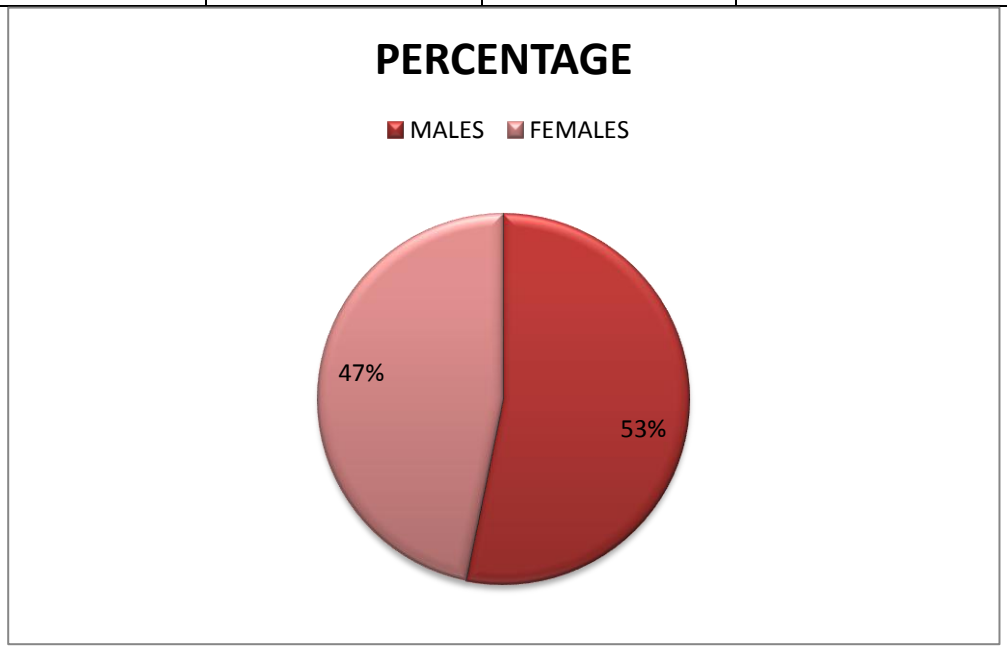

Figure 1: Percentage of subjects according to gender for serum bicarbonate.

Reference intervals for males, females and the total study population are shown in table 2.

Table 2: Reference intervals for serum bicarbonate in the study population

Gender	Number	Bicarbonate (mmol/L)	
		Median	Reference interval (2.5^{th} to 97.5^{th} percentile)
Male	66	24.6	19.3-29.6
Female	58	24.0	19.3-29.0
Total	124	24.3	19.4-29.4

- The reference intervals for serum bicarbonate are similar in males and females (p = 0.457).
- Hence, the reference intervals for serum bicarbonate obtained for the total study population in the present study is 19.4 to 29.4 mmol/L.

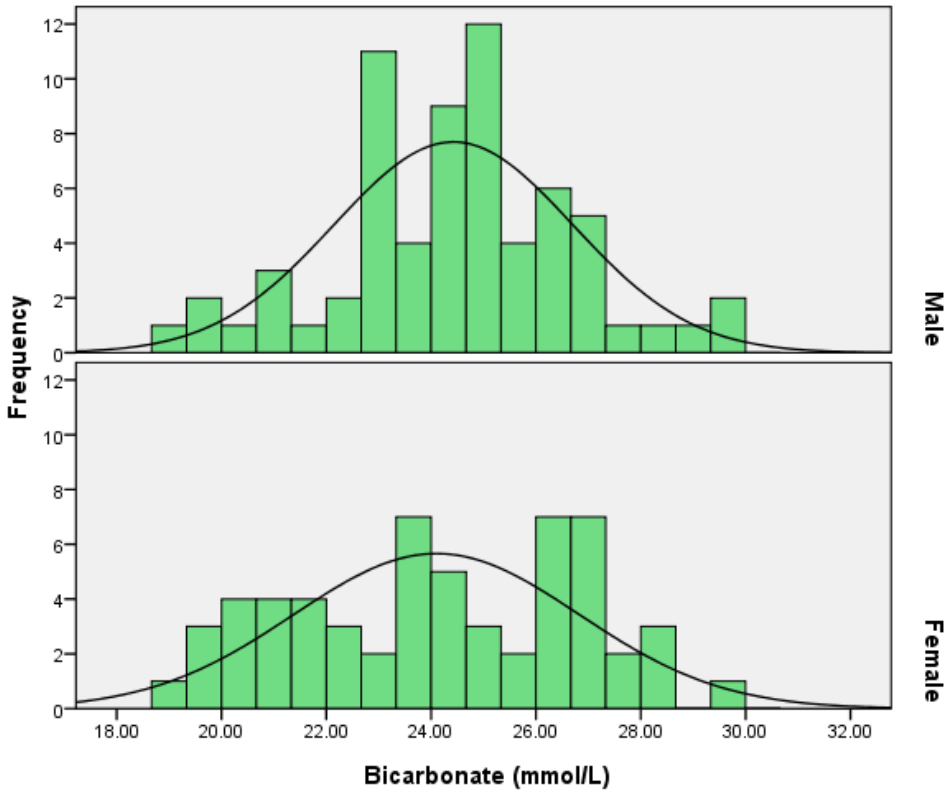

Figure 2: Gender wise distribution of Bicarbonate levels

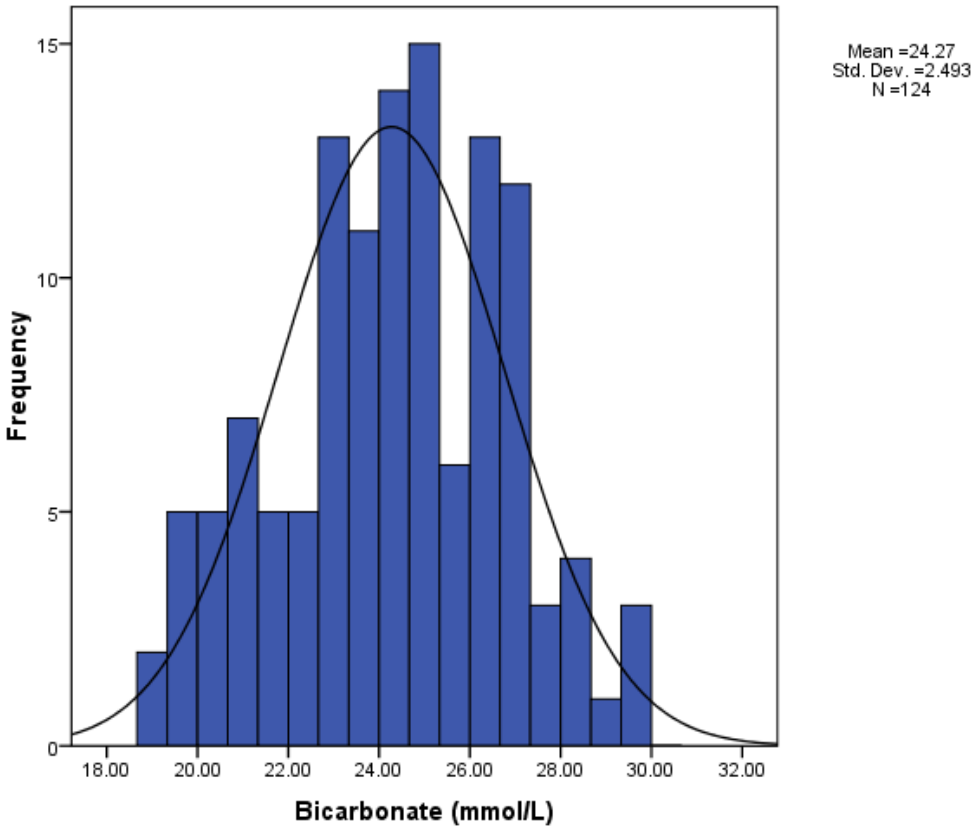

Figure 3: Distribution of Bicarbonate levels for the total study population

COMPARISON OF REFERENCE INTERVALS

Table 3: Comparison of reference intervals with assay manufacturer and standard text books.

Parameters (mmol/L)	**Present study**	**Assay manufacturer** [12]	**Text book** [11]
Bicarbonate	19.4-29.4	22-29	23-29

The reference interval for serum bicarbonate in the present study is wider when compared to the reference intervals provided by the text books and assay manufacturer.

Discussion

Our data collection was in accordance with the CLSI guidelines for determining laboratory ranges which recommends a minimum of 120 subjects for a central 95 percentile (between 2.5th and 97.5th percentiles) clinical reference range determination with 95% confidence interval [2].

The reference interval for serum bicarbonate reported in the present study (19.4-29.4 mmol/L) is wider when compared to the reference intervals provided by the text books [11] and assay manufacturer [12]. Similar variations have been reported by studies in Tanzania [13] and USA [14]. A shift in the reference intervals towards lower side has been reported by another group of Indian [15] researchers and Kenyan [16] researchers.

In the present study there is no difference in reference intervals between males and females and the same observation has been reported in another Indian study [15]. In contrast to this, one of the studies in Kenyan population has reported that males have higher reference intervals when compared to females [16].

A number of factors may be responsible for the variations observed in the reference intervals for the serum bicarbonate. Ethnicity may be one of them as shown by variations in reference intervals obtained in different studies. Such variations have been reported in reference intervals of other parameters in different populations.

In addition, instruments used, sample size and posture all contribute to the variations in the reference intervals.

Conclusion

To conclude, the reference interval obtained in our study is wider compared to those given in the assay kit insert and several documented studies. Although restricted to small sample size, we believe that the observed changes are of significance for the local population. Further revalidation with a large sample size is needed.

The present study highlights the need for laboratories to establish their own reference intervals for the population they serve.

References

1. H.E. Solberg. Establishment and use of Reference Values. Tietz Textbook of Clinical Chemistry. Chapter 14, 3rd edition W.B. Saunders Company, Philadelphia U.S.A. **1999**; 336-356.
2. CLSI. How to define and determine Reference Intervals in the clinical laboratory; approved guidelines. 2nd edition. NCCLS document C 28- A2 **2000**; 20(13)
3. Sultana Furruqh, D. Anitha and T. Venkatesh. Estimation of Reference Values in Liver Function Test in health plan individuals of an urban South Indian population. *Indian Journal of Clinical Biochemistry* **2004**; 19(2): 72-79

4. Solberg HE, PetitClerc C. Approved recommendation (1988) 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* **1988**; 26: 593-598.
5. Solberg HE, Ralph Grasbeck. Reference Values. *Adv Clin Chem* **1989**; 27:1-79.
6. Passey RB, Maluf KC. Linearity and calibration. A clinical laboratory perspective. *Arch Pathol Lab Med.* **1992**; 116(7): 757-760.
7. Gary W. Shires. Correspondence of linearity evaluations to the performance of clinical chemistry instrument and reagent systems, *Arch Pathol Lab Med* **1992**; 116: 761-764.
8. Wilson W, et al. *Clin Chem* **1973**; 19:640.
9. Norris KA, et al 1975; 21:1093.
10. Henny J. Need for Revisiting the Concept of Reference Values. *Clin Chem Lab Med.* **2000**; 38(7):589-595.
11. Burtis, C.A., Ashwood, E.R., Burns D E. Tietz Textbook of Clinical Chemistry. 6rd edition W.B. Saunders Company, Philadelphia U.S.A. **2008.**
12. Kit insert for Roche /Hitachi MODULAR analyzer: CAN 156 (Cat. No 03607402 190).
13. Kratz A, Ferro M, Sluss PM, Lewandrowski KB. Case records of the Massachuetts General Hospital. Weekly clinicopathological exercises. Laboratory reference values. *N Eng. J Med* **2004**; 351: 1548-1563.
14. Elmar Saathoff, Philine Schneider, Vera Kleinfeldt, et al. Laboratory reference values for healthy adults from southern Tanzania. *Tropical Medicine and International Health May* **2008;** 13(5): 612-625.
15. Sundaram M, Mohankrishnan J, Murugavel KG, Shankar EM, et al. Ethnic variation in certain hematological and biochemical reference intervals in a South Indian healthy adult population. *Eur J Intern* **2008**; 19(1): 46-50.
16. Rukia S. Kibaya, Christian T. Butista, Federick K. Sawe, et. al. Reference Ranges for the Clinical Laboratory Derived from a Rural Population in Kericho, Kenya. *PLos ONE* October **2008**; 3 (10): 1-7
17. PetitClerc C, Solberg HE. Approved recommendation (1987) on the theory of reference values. Part 2. Selection of individuals for the production of reference values". *J Clin Chem Clin Biochem* **1987**; 25:639-644.