ESTIMATED REFERENCE INTERVAL OF IONIZED (FREE) CALCIUM

Abdus Sattar, Rizwan Hashim*, Syed Mohsin Manzoor, Zeeshan Rana***, Asif Ali*, Muhammad Younas*, Farooq Ahmad Khan***

Combined Military Hospital Lahore, *Armed Forces Institute of Pathology Rawalpindi, **Combined Military Hospital Bahawalnagar, ***Combined Military Hospital Bannu

ABSTRACT

Objective: To determine the reference values of Ca^{++} in whole blood in our setup.

Place and duration of study: The Department of Chemical Pathology & Endocrinology, Armed Forces Institute of Pathology, Rawalpindi from Jan 2008 to June 2008.

Materials and Methods: Three hundred healthy individuals were included in the study after obtaining written consent. Out of these 76 individuals were excluded from the study after clinical assessment and collection of laboratory data. One hundred and fourteen were males with mean age 35±12 years and 110 were females, with mean age 28±9 years of age. Their Ca^{++} was estimated by ion selective electrode (ISE) method in heparinized whole blood (WB).

Results: The mean and SD of whole blood Ca^{++} was calculated separately for the females and the males. The results showed that in our setup males have Ca^{++} levels of 1.12 ± 0.05 (mean ± SD) mmol/l and females have Ca^{++} levels of 1.12 ± 0.04 (mean ± SD) mmol/l.

Conclusion: The study revealed that estimated reference range of Ca^{++} of the studied population was lower than the reference range published for the western population that is used by our physicians for the interpretation and comparison of results.

Keywords: Ionized calcium (Ca^{++}), Reference values.

INTRODUCTION

Calcium is the fifth most common element in the body, and the most prevalent cation[1]. It is found mainly in the skeleton (99%) with small amounts in tissues (1%) and extra cellular fluid (<0.2%)[1]. In blood, approximately 45% of plasma calcium is ionized (free) that is biologically active form, 40% is protein bound (mostly albumin) and 15% is bound to anions such as a bicarbonate, citrate, phosphate and lactate[2]. Binding of calcium ions to protein is pH dependent. Acidic pH increases the ionization of calcium concentration[3]. In healthy individuals, the calcium level is maintained in a very narrow range under strict control of parathyroid hormone (PTH), 1,25-dihydroxyvitamin D and calcitonin[4]. Ionized calcium is vitally important in blood coagulation, nerve conduction, neuromuscular transmission and in muscle contraction through its role in a number of enzymatic reactions and in membrane transport mechanisms.

Correspondence: Dr Syed Mohsin Manzoor, Combined military Hospital Bahawalnagar

Received: 14 Jan 2009; Accepted: 06 Jan 2012

Measurements of ionized calcium have proven of value under the following clinical conditions: transfusion of citrated blood, liver transplantation, open heart surgery, neonatal hypocalcemia, renal disease, hyperparathyroidism, malignancy, hypertension and pancreatitis[5].

The interpretation of medical laboratory data is in fact a special case of decision making by comparison. To follow this decision process, reference values are required[6]. The precise quantitation and interpretation of calcium is important and requires well-established reference values for the given population[7]. The usual practice in Pakistan is to interpret the patient's laboratory results in the light of reference values cited in the standard text books or as mentioned in the literature of manufactured kits which are usually derived from studies based on data collected from the European or American population. These reference ranges might be inappropriate for our given population as none are based on the results of studies conducted or published in this region. Keeping in view the above there is a need to ascertain the reference values of Ca^{++} (WB) in our setup for correct interpretation of

results that could be vital for clinical decision and management.

The reference values are significantly influenced by a number of factors like preanalytical and analytical sources of variation[8]. Different populations have different genetic make up, dietary habits, lifestyle, socio-economic and many other environmental and biological factors which can affect the analytical outcome[9]. Among the dietary factors lactose, amino acids, bile salts and low pH in duodenum favours calcium absorption,[10] while malaborsorption[11] and presence of certain minerals and chelating agents[12] in food decrease calcium absorption.

PATIENTS AND METHODS

The study was carried out in the Department of Chemical Pathology & Endocrinology, Armed Forces Institute of Pathology, Rawalpindi from Jan 2008 to June 2008.

Study design: Descriptive study.

Sample size: Two hundred and twenty-four healthy individuals were included in this study. Out of them 114 (51%) were males and 110 (49%) were females.

Sampling technique: Non probability convenience sampling technique was used.

Sample selection

Inclusion criteria: Following individuals were included.

- Both male and female healthy (nonhospitalized) individuals.
- Individuals having age >18 years and <50 years.

Exclusion criteria: Following individuals were excluded in the study.

- Individuals having age <18 years and >50 years.
- Pregnant and postmenopausal women.
- Any history of tetany, psychiatric manifestations, bone pains, renal disease, anorexia, constipation, malabsorption, endocrine disorders, malignancy or any chronic disorder.
- History of taking calcium supplements or vitamin-D.

All the participants in the study were thoroughly interviewed using a structured proforma including demographic details and clinical history. Informed written consent was obtained from all participants selected.

For Ca^{++} estimation 5 ml of fasting whole blood (WB) samples were collected anaerobically in a lithium-heparin tube from each subject, keeping in mind the precautions to avoid pre-analytical errors, e.g. samples were collected and transported on ice to prevent anaerobic metabolism.

Ca^{++} was estimated in heparinized whole blood (WB) by ion selective electrode (ISE) method on Easylite® autoanalyzer, Medica USA.

Data was analyzed using Statistical Package for Special Sciences (SPSS) version-11. Mean and Standard Deviation (SD) were calculated. The reference range was obtained by adding ± 2SD in the mean value that constituted the middle 95%, leaving out 2.5% of the values on either side[13].

RESULTS

Three hundred healthy individuals were included in the study after obtaining written consent. Seventy six individuals were excluded from the study after clinical assessment and collection of laboratory data. Out of 224 healthy individuals 114 (51%), with mean age of 35±12 years and 110 (49%) were female with mean age of 28±9 years of age were females. The mean and SD of WB Ca++ were calculated separately for the female and the male subjects. The results showed males have Ca^{++} levels of 1.12 ± 0.05 mmol/l and females have Ca^{++} levels of 1.12±0.04 mmol/l (Table). The density curve of frequency distribution of the Ca^{++} concentrations in the healthy population (males and females), shows skewness towards left i.e. towards the low Ca^{++} concentration (Figure).

DISCUSSION

Reference range of various analytes form an essential component of any laboratory

Table: The ionized calcium (WB) Values.

n	Gender	Age (Mean ± SD) (Years)	Ca^{++} levels (WB) (mmol/l)			Ca^{++} Estimated Reference Interval (mmol/l)
			Minimum	Maximum	(Mean ± SD)	
114	Males	35 ± 12	1.00	1.24	1.12 ± 0.05	1.02 – 1.22
110	Females	28 ± 09	1.03	1.22	1.12 ± 0.04	1.04 – 1.20

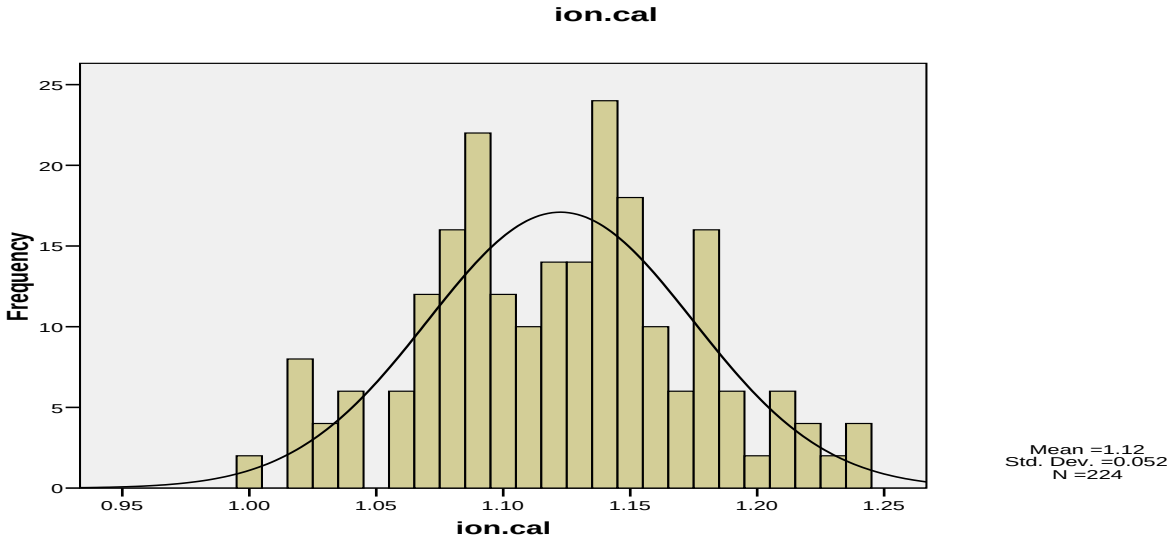

Figure: Frequency distribution of Ca^{++} levels in male & female (combined).

report, the same should be established for given population. The precise quantitation and interpretation of Ca++ (WB) is important and requires well established reference values for the desired population.[7] Significant variation between mean value and reference range of various populations have been noted. After a thorough search of medical literature, no local study was published that established the reference range of Ca^{++} in our population. Moreover the reference range of Ca^{++}, used by the labs and published in the western literature were not separately calculated for males and females, while values estimated for different samples, as in whole blood Ca^{++} range: 1.15 – 1.27 mmol/l, in heparinized plasma range: 1.03 – 1.23 mmol/l and in serum the range: 1.11 – 1.32 mmol/l are used[13]. In this study Ca^{++} in heparinized WB for males and females was estimated separately. The results showed males having Ca^{++} range: 1.02 – 1.22 mmol/l and females having Ca^{++} range: 1.04 – 1.20 mmol/l. The comparison of these results with the reference range published in the literature[13] revealed that calcium levels estimated in this study were lower than the published reference range. The low Ca^{++} level in this initial study could be due to variation in dietary, socioeconomic and genetic factors[14]. These results indicate that more studies with larger sample size are needed to determine the factors and causes in our setup for relatively low calcium levels as compared to the western population and further recommending the changes in the values of reference range being used in pathology laboratories.

CONCLUSION

Estimated reference range of Ca^{++} of the studied population was lower than the reference range published for the western population that is used by our physicians for the interpretation and comparison of results.

Recommendations:

Further studies with large sample size are needed to determine the factors and causes in our setup for relatively low Ca^{++} levels (WB) as compared to the western population.

REFERENCES

1. David BE, Robert KR. Mineral and bone metabolism. In: Burtis CA, Edward RA. Tietz fundamentals of Clinical Chemistry. 5th ed. New Delhi: Elsevier: 2006; p.795 – 821.
2. Toffaletti J, Gitelman HJ, Savory J. Separation and quantitation of serum constituents associated with calcium by gel filtration. Clin Chem 1976;22: 1968-72.
3. Toffaletti J. Calcium, Magnesium and Phosphate. In: Kent lewandrowski. clinical chemistry laboratory management and clinical correlation. 1st ed, Philadelphia (USA): Lippincott williams and wilkins 2002; 710 –24.
4. Rosenblatt M, Kronenberg HM, Potts JT. Parathyroid hormone: Physiology, chemistry, biosynthesis, secretion, metabolism and mode of action. In: Degroot LT eds. Endocrinology. 3rd ed. Philadelphia: WB Saunders: 1995; 848 – 858.
5. Boden SD, Kaplan FS. Calcium homeostasis. Orthop Clin North Am 1990; 21: 31– 42.
6. Solberg HE, Establishment and use of reference values. In: Burtis CA, Ashwood ER eds. Tietz fundamentals of Clinical Chemistry. 5th ed. New Delhi (India): Elsevier 2006; 795- 821.
7. Solberg HE. Approved recommendations (1986) on the theory of reference values. Part 1. The concept of references values. Clin Chem Acta 1987; 165: 111-8.
8. Darfous DR. Sources and control of preanalytical variation: In: Kaplan LA, Pesce AJ, Kazmierczale SC editors. Clinical Chemistry: theory, analysis and correlation. 3rd ed. Philadelphia: Mosby 1996; 365-81.
9. International Federation of Clinical Chemistry (IFCC) Expert Panel on Theory of Reference Values (EPTRV). Pt – 1. Concept of references values. Clin Chem Clin Biochem 1979; 17: 337-9.
10. Mars SJ, Spiegal AM. Parathyroid hormone, calcitonin and the calciferols. In: William RH editors. Text book of Endocrinology. 6th ed. Philadelphia: WB Saunders: 1981; 457–72.
11. Walmsley RN, White GH. Calcium, Magnesium and Phosphate. In: A guide to diagnostic Clinical Chemistry. 3rd ed. London: Black well scientific: 1994; 31: p 178 -221.
12. Crook M, Swami Nathan R. Disorders of plasma phosphate and indications for its measurements. Ann Clin Biochem 1996; 33:376 – 96.
13. Painter PC, Cope JY and Smith JL. Reference information for the clinical laboratory. In: Burtis CA, Ashwood ER editors. Tietz fundamentals of Clinical Chemistry. 5th ed. New Delhi: Elsevier: 2006: 955–1028.
14. Philip J Garry, William C Hunt, Dorothy J, Vander Jagt, Robert Rhyne. Clinical Chemistry: Reference Intervals for healthy subjects. Am J Clin Nutr: 1989; 50: 1219 – 30.