Reference values of serum osteocalcin in the healthy population: A potential biomarker for bone turnover

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ABSTRACT

Objective: To analyze the serum concentrations of Osteocalcin in healthy subjects to establish the reference intervals in the Pakistani population.

Material and Methods: This Cross-sectional, observational study was conducted at the Department of Clinical Chemistry and Immunology, Chughtai Lab Lahore from October 2022 to March 2023. Serum samples from 240 healthy subjects (120 males and 120 premenopausal females) were collected according to CLSI recommendations after taking informed consent. The samples were analyzed for the quantitative determination of Osteocalcin by sandwich electrochemiluminescence immunoassay. Shapiro Wilk test was applied to check normality. A P-value of < 0.05 was considered significant. The formulas used for calculating the 2.5th and 97.5th percentiles were 0.025 (n+1) and 0.0975 (n+1) respectively.

Results: The histogram revealed a non-parametric distribution of the data. The established reference intervals by the rank-based method for males were 10.16 ng/mL and 43.33 ng/mL and for females were 5.25 ng/mL and 33.25 ng/mL corresponding to 2.5th and 97.5th percentiles respectively.

Conclusion: Ethnic and geographic variation affects the trends of reference intervals of every parameter. This is the need of the hour each laboratory should establish its assay and population-specific reference intervals for accurate clinical decisions.

Keywords: Osteocalcin, Reference values, Bone density, Osteoporosis

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INTRODUCTION

Bone is a dynamic tissue that undergoes constant remodeling. Bone mass in healthy adults is maintained by the coordination between bone formation and resorption [1,2]. Bone turnover markers are the biochemical products that indicate bone metabolic activity and are classified into two major groups: Bone formation and resorption [3,4]. Osteocalcin (OC), also known as bone gamma-carboxy glutamic acid-containing protein (BGLAP), is a non-collagenous vitamin K-dependent bone-specific protein produced primarily during bone formation predominantly by osteoblasts [1]. It binds to hydroxyapatite and accumulates in the bone matrix [5].

Measurement of Serum OC is a convenient way to study bone metabolism, as the levels represent the turnover rate of bone metabolism; therefore, it can be used in monitoring disorders that affect bone health, such as osteoporosis, hyperparathyroidism, renal osteodystrophy, Paget's disease, and bone metastasis [6]. The concentration of circulating OC varies according to age and gender [7]. Serum OC levels in young males are higher than in females of the same age group because they have longer and broader bones and reach peak bone mineral density (BMD) later in life. After peak BMD is achieved a drop in concentration of OC is observed in both males and females. In women transitioning to menopause, serum OC levels increase significantly [1].

Reference values of biological parameters significantly from one laboratory to another based on the population, methodology, and selection criteria for the reference group. (8). Under ideal conditions, a laboratory should conduct its reference intervals (RIs) study to

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determine the RIs specific to its method and population. However, establishing RIs is frequently beyond the capacity of an individual laboratory because it is a complex, costly, and time-consuming procedure [9].

To our knowledge, no study has been conducted to establish the RIs of serum OC in the Pakistani population. The RIs for biochemical parameters in developing nations are obtained from textbooks containing data from developed countries or the literature inserts of reagent kits [10]. To establish RIs, the reference population is sampled according to predefined criteria, and then reference ranges are computed using a direct approach. The majority of RIs in use are described by the central 95% of the reference population used in the study [11]. The Clinical and Laboratory Standards Institute (CLSI) guidelines recommend selecting a statistically significant group with at least 120 healthy reference subjects to establish RIs [12]. According to the standard guidelines, if a laboratory cannot perform its RI study, due to financial constraints or some other reasons, the guidelines emphasize on at least verifying the transferred RIs, which requires samples from as few as 20 reference samples [13]. To establish the reference intervals of serum Osteocalcin in healthy Pakistani population.

MATERIAL AND METHODS

A cross-sectional study was carried out at the Department of Chemical Pathology, Chughtai Laboratory Lahore, from October 2022 to March 2023. Serum samples from 240 healthy subjects (120 males and 120 premenopausal females) were collected according to CLSI recommendations [14]. The CLSI-recommended number of subjects for the establishment of RIs is 120 healthy subjects. As we have established the RIs for both males and females we took a total of 240 individuals. Subjects with underlying bone disorders, fractures, diabetes mellitus, and other chronic diseases like thyroid disorders, malignancies, etc. were excluded from the study. Subjects with a history of drug intake such as Vitamin D, multivitamins, and steroids were also excluded from the study. A purposive, non-probability sampling technique was used. Informed consent was taken from study participants. The samples were analyzed for the quantitative determination of OC by sandwich electrochemiluminescence immunoassay (ECLIA) on a fully automated chemistry analyzer [Cobas 6000 (e601)]. Hemolyzed, lipemic, and icteric samples were rejected. Two levels of quality control were run with each batch and validated by Westgard rules. The Shapiro-Wilk test was applied to assess the distribution of osteocalcin values. Values were arranged in ascending order, followed by a ranking of the data so the corresponding value of Osteocalcin can be taken. The rank numbers were calculated using the formulas 0.025 (n+1) and 0.0975 (n+1) for the percentile values that correspond to the rank no 3 and 118 respectively.

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 27:00. The data was assessed for normality by applying the Shapiro-Wilk test. A CI of 90% was used to establish RIs using non-parametric statistical methodology. A P-value of < 0.05 was considered significant. The lower and upper reference limits correspond to the 2.5th percentile and 97.5th percentile of the distribution of test results for the reference population, respectively (15,16). Values (2.5th and 97.5th percentiles) were computed using the formula 0.025 (n+1) and 0.0975 (n+1) respectively.

RESULTS

Of the 240 subjects, 120 were males and 120 were females. The mean age of male subjects was 26.65 years, and female subjects was 32.09 years. The mean serum OC levels in males and females were 20.85 ng/mL and 16.49 ng/mL, respectively (Table-I). The histogram revealed a non-Gaussian distribution for both males and females. (Figure-I & II respectively). Non-parametric statistics were applied, and the reference intervals based on the 2.5th and 97.5th percentiles (corresponding to rank number 3 and 118, respectively) established were 10.16 ng/mL and 43.33 ng/mL for males and 5.25 ng/mL and 33.25 ng/mL for females (Table-II).

Table-I: Descriptive statistics of Serum OC (ng/mL) in healthy Pakistani population (n=240).

	Minimum	Maximum	Median	Mean	Standard Deviation
Male (n=120)	8.54	45.80	18.78	20.85	7.83
Female (n=120)	4.31	38.87	15.13	16.49	7.11

Table-II: Percentile Details of Serum Osteocalcin in healthy Pakistani population (n=240).

Percentile	Corresponding OC Levels in Males (ng/mL)	Corresponding OC Levels in Females (ng/mL)
2.5	**10.16**	**5.25**
5	11.46	6.55
10	12.58	8.63
25	15.30	11.82
50	18.78	15.13
75	24.45	20.52
90	33.46	27.47
95	35.92	31.06
97.5	**43.62**	**33.20**

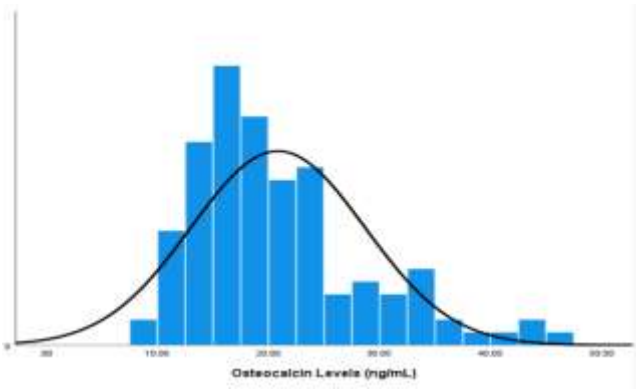

Figure-I: Histogram showing the non-parametric distribution of OC Levels (ng/mL) in males (n=120).

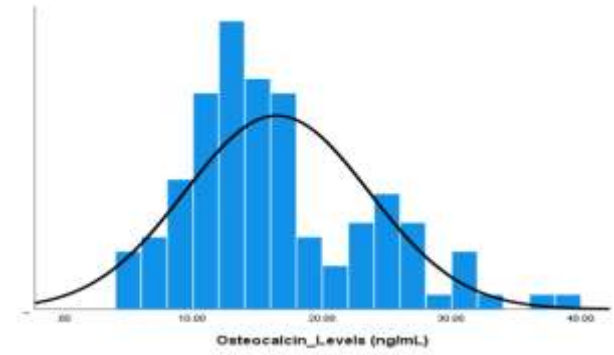

Figure-II: Histogram showing the non-parametric distribution of OC Levels (ng/mL) in females (n=120).

DISCUSSION

The diagnostic utility of the laboratory results depends on their interpretation, which helps clinicians to differentiate between health and disease states [17]. Each laboratory should establish its RIs for each parameter according to the standard recommendations specific to the testing methodology used and the population covered by that particular laboratory [18]. Establishing the RIs is expensive, complex, and time-consuming, and many laboratories cannot determine their RIs due to these constraints [17,18].

This study establishes the RIs of Serum OC in the healthy adult Pakistani population. The RIs are established as the difference between two threshold values, the 2.5th and 97.5th percentiles of the distribution of the data, which account for 95% of observations from healthy subjects [1,2]. The idea of establishing RIs and their application seems relatively simple, but the accurate and reliable procedure for their determination is somewhat complex. Problems are frequently encountered by a lack of samples from healthy populations, moral issues, and disparities such as age- and sex-specific variations in physical characteristics, immunological response, and metabolism [10].

For quite some time, there has been an increased interest in quantifying markers of bone metabolism in clinical practices. They might offer a dynamic, momentary assessment of skeletal health that is not just reflected in the physical characteristics of bones [2]. Thus far, no single parameter has matched all the requirements needed to be the perfect indicator of bone turnover [13]. High intraindividual variation, lack of specificity for bone tissue, release during distinct anabolic and catabolic processes, and the effect of non-skeletal activities on circulating

levels all pose challenges to the therapeutic efficacy of bone markers. Markers such as OC indicate both bone formation and resorption simultaneously and can be used to assess bone turnover [3].

A study was conducted by Hannemann A *et al*. in Pomerania in 2013 to establish the RIs for OC. The established RIs for adult males using 2.5th and 97.5th percentiles were 6.5 and 36.2 ng/mL, and for adult premenopausal females, the established reference values were 7.6 and 39.5 ng/mL, respectively [1]. These values differ slightly from the RIs established in our study. The testing methodology in our research study was electrochemiluminescence immunoassay, and the instrument used was Cobas 6000. While in the Pomeranian study, the analytical technique used was chemilumine-scence, and the instrument used for the OC analysis was the ids-Immunodiagnostic system.

The comparison with other studies shows that RIs are affected by factors such as analytical assays, ethnic origins, living styles, population, and geographic differences [19]. It is the need of the hour each laboratory should establish its RIs for the population being covered based on the specific testing method used at that particular laboratory.

CONCLUSION

The established reference intervals by the rank-based method for males were 10.16 ng/mL and 43.33 ng/mL, and for females, they were 5.25 ng/mL and 33.25 ng/mL, corresponding to 2.5th and 97.5th percentiles, respectively. It was concluded that RIs are affected by ethnic and geographic variation. It is recommended that every laboratory should establish its reference intervals.

LIMITATIONS OF THE STUDY

Our study covered a small population; there is a dire need for more extensive studies to establish RIs for the effective and timely management of patients.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none

AUTHORS CONTRIBUTION

Tayyaba Rashid: Paper write-up, literature search, data collection and analysis
Muhammad Dilawar Khan: Study design, proofreading, finalization of study
Hijab Batool: Statistical analysis, paper write-up, proofreading
Masood Afzal: Data analysis, discussion
Muhammad Hashir Nazir and Muhammad Ahmad: Sample collection, paper write-up

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