Original Article

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# Estimation of Coverage Interval of Serum β-Carotene among Bengali Population

Biochemistry Section

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## ABSTRACT

**Introduction:** The reference interval guides the clinician in interpreting the clinical test reports and classifying the subjects as healthy or diseased. The reference values of the common laboratory analytes vary due to immense diversity in race, ethnicity, genetic pattern and food habits. Recommendations from international body's mandates for every laboratory to establish its own reference values for the population it serves for correct interpretation of test results. A series of recent research indicates the protective role of the antioxidant vitamin β-carotene against both exogenous and endogenous free radicals. These free radicals mediated damage to intracellular organelles and cell membrane lead to mutations, coronary artery disease, autoimmune diseases and malignancy.

**Aim:** To determine the coverage interval of serum β-carotene in Bengali population and to compare them with the reference interval of other population.

**Materials and Methods:** The present non-interventional, cross-sectional study was conducted in Medical College and Hospital, Kolkata, from January 2012 to January 2013. Based on IUPAC clinical division technical report with 0.95 confidence interval, 0.95 coverage interval and coverage uncertainty of 0.049, 71 individuals were included in the study. Blood samples were collected from 71 (34 male and 37 female) healthy volunteers (postgraduate trainee students, DMLT students, nursing staff and undergraduate students) at random as per recommendation of IUPAC Clinical Chemistry Division for calculation of coverage interval. The β-carotene levels were measured by Bradley and Hornbeck (1973) method spectrophotometerically. The Kolmogorov-Smirnov test was used to check for normal distribution.

**Results:** The mean age and weight of the study population 28.49±9.49 years and 62.9±11.58 kg respectively. The mean concentration of serum β-carotene was found to be 140.12 µg/dL (2.62 µmol/L) which was much higher than other populations.

**Conclusion:** The study conducted to determine the coverage interval of β-carotene, a replica of reference interval with small number of values, from the general healthy population. The reference values of Bengali population was found to be, much higher than Kuwaiti, European, Chinese, Vietnamese and Thailand populations.

**Keywords:** Free radicals, Reference values, Vitamin A

## INTRODUCTION

In recent times with the advancement of technology, laboratory tests are the torchbearers for the clinical diagnosis of diseases. The reference interval guides the clinician to interpret the results of analytes measured from plasma, serum, body fluids and categorise a person as diseased or healthy. In 1969, Gräsbeck R et al., first introduced the idea of reference values and described them as "normative values of laboratory parameters used by the clinical laboratories for clinical diagnosis" [1]. Due to continuous change of biological data and disease pathology, there is a grey zone between normal and abnormal values. Moreover, in most cases, the biological data from reference individual follows a non-gaussian or non-parametric distribution. Thus, the use of terminology like "normal range" in reports is erroneous. International Federation of Clinical Chemistry (IFCC) mandates the use of at least 120 samples for the establishment of reference interval by non-parametric methods [2]. However, it may not be feasible to analyse 120 samples for each analyte, considering the cost of the methodology and selection of study subjects with stringent inclusion-exclusion criteria. The concept of "coverage interval" was first proposed by International Union of Pure and Applied Chemistry (IUPAC) that defines population-based reference interval obtained from healthy group of few reference individuals and also gives information about the precision of the calculated interval [3,4].

Recent research suggests the role of oxidative stress and antioxidant vitamins in aetiopathogenesis of many diseases. Literature studies since 17th century have shown serious vitamin deficiencies in patients with malabsorption syndrome, haemodialysis, parenteral nutrition or subjects on oral contraceptive pills, antitubercular drugs, antiepileptic drugs and anticancer drugs [5]. β-carotene levels may be altered in hypothyroidism, diabetes, hyperlipidaemia, chronic renal disease, and malnutrition. Many studies indicate that β-carotene supplementation may reduce photosensitivity in patients of erythropoietic protoporphyria and complications of age-related macular degeneration. The literature survey reports that no reference interval of serum β-carotene has been established in Bengali population. Therefore, this study was carried out to establish the coverage interval of β-carotene in 71 individuals in Bengali population and compare them with reference intervals in various other populations.

## MATERIALS AND METHODS

The present non-interventional, cross-sectional study was conducted in Medical College and Hospital from January 2012 to January 2013. The estimation was done in accordance with the Ethical Guidelines of Medical College, Kolkata. This study is part of previously published study of determination of coverage interval of vitamin C and tocopherol [6]. Based on IUPAC clinical division technical report with 0.95 confidence interval, 0.95 coverage interval and coverage uncertainty of 0.049, 71 individuals were included in the study. After obtaining written informed consent, blood samples were collected from 71 (34 male and 37 female) healthy volunteers (postgraduate trainee students, DMLT students, nursing staff and undergraduate students) at random as per recommendation of IUPAC Clinical Chemistry Division for

calculation of coverage interval. Volunteers, belonging to the adult population, who fulfilled the criteria of the questionnaire and were found to be healthy by history, physical examinations, and routine laboratory investigations (fasting plasma glucose, lipid profile, liver function test, urea, creatinine, thyroid stimulating hormone) were selected. Subjects with hypertension, hypercholesterolemia, hypothyroidism, diabetes, any history of drug intake for treatment of chronic disease or vitamin supplementation or conditions like acute thrombocytopenic purpura, antiepileptic or ocular cicatricial pemphigoid were excluded from the study. Pregnant women, smokers, alcoholics or individuals on chemotherapy were also excluded.

The estimation of vitamins was done in the OPD of the Clinical Biochemistry Laboratory, Medical College and Hospital, Kolkata. The estimation of vitamins was done spectrophotometrically by Bradley and Hornbeck (1973) method using Systronics UV-VIS spectrophotometer (Clinicol 631) [7].

After overnight fasting, 3 mL of venous blood was drawn from study participants in serum separation tubes. Serum was stored at -20°C, until further anylysis. The serum was deproteinised with ethanol and carotenes were extracted in light petroleum according to Bradley DW and Hornbeck CL methodology [7]. The intensity of the yellow colour due to carotene was recorded at 440 nm. This spectrophotometric results were compared with interval established by High Performance Liquid Chromatography (HPLC) and validated by determining exactness, bias. Twenty samples were analysed simultaneously. One millilitre of serum was transported to the laboratory maintaining the temperature (2-8°C) and protected from light. The bias was -8.33% after comparing with an established reversed phase High performance liquid chromatography method from an accredited laboratory. The precision, sensitivity, recovery of the method was estimated using commercial preparation of standard material [Table/Fig-1].

| Name of analyte | Performance parameter | Percentage (%) |
|---|---|---|
| Beta-Carotene | Precision | 6.49 |
| | Recovery analysis from Spike sample | 90.75 |
| | Proportional error | 9.25 (<43.1% i.e. total allowable error) [9] |
| | Sensitivity (R2) | 0.9988 |

**[Table/Fig-1]:** In-house performance parameter of the spectrophotometric method.

## STATISTICAL ANALYSIS

The reference values were extrapolated in Microsoft excel were analysed using IBM Statistical Product and Service Solutions (SPSS) version 18. The Kolmogorov-Smirnov test was used to check for normal distribution. For continous variables, Shapiro Wiliks test was used.

## RESULTS

The mean value of serum β-carotene (140.12 µg/dL) was higher than the median value (121.42 µg/dL) indicating a non-central distribution or positively skewed distribution with an extended right tail. Moreover, the Kolmogorov-Smirnov test revealed that the distribution deviated significantly from the normal distribution (p<0.001) and also the Shapiro Wiliks test showed β=0.874 and p<0.001 [Table/Fig-2]. The reference values were arranged in ascending order of magnitude and the number of m values (m=3) was identified according to [Table/Fig-3] (Adapted from Kirkpatrick) [8]. Then the coverage interval was calculated as 46.85 to 289.28 µg/dL in serum. Similarly, the logarithmic transformation of the reference values of the serum β-carotene was done which showed that the distributions no longer deviated significantly from normal distribution p=0.200* (p>0.05). Estimated arithmetic mean of logarithmically transformed data was 4.8057 µg/dL. The parametric coverage interval of serum β-carotene was 41.05 to 363.76 µg/dL in serum.

| | |
|---|---|
| Number of observations (n) | 71 |
| Distribution of reference values (µg/dL) | |
| Mean | 140.12 |
| Median | 121.42 |
| Mode | 103.57 |
| 95% CI | 140+16.95 |
| Test for normal distribution | |
| Kolmogorov smirnov test | ≤0.001 |
| Shapiro Wiliks test | <0.001 |
| Non parametric 0.095 coverage interval (µg/dL) | 46.85-289.28 |
| Parametric 0.095 coverage interval (µg/dL) | 41.05-363.76 |
| Recommended reference interval (µg/dL) | 50-300 |

**[Table/Fig-2]:** Distribution of reference values of β-carotene.
a=Lillieofer's correction

| Confidence (γ)=0.95 | | |
|---|---|---|
| Coverage uncertainity (δ) | Number of study participants (n) | m |
| 0.01 | 1889 | 94 |
| 0.02 | 471 | 23 |
| 0.03 | 210 | 10 |
| 0.04 | 111 | 5 |
| 0.049 | 71 | 3 |

**[Table/Fig-3]:** This table is adapted from Kirkpatrick [8] where n=minimum no. of reference values, m is the number of values outside the coverage interval and γ is the probability that coverage interval (β) will cover between β+δ and β-δ.

## DISCUSSION

With the increase in role of oxidative stress in aetiopathogenesis of several diseases, the measurement of antioxidant vitamins in the serum and plasma is of utmost importance. β-carotene is one of the commonly measured antioxidant vitamins and has been found to be associated with diseases like lung cancer and age-related macular degeneration. The β-carotene concentration is majorly affected by factors like hypothyroidism, serum cholesterol and dietary pattern. In the present study, the mean of serum β-carotene was found to be 140.12 µg/dL (2.62 µmol/L) which was much higher than the Kuwaiti, European, Chinese, Vietnamese, KhonKhen and Spanish populations [Table/Fig-4] [9-14]. The mean value was also found to be considerably higher than that in the Thailand population, which were 0.48 µmol/L in males and 0.60 µmol/L in females [15].

| Sl. No | Authors | Population | Mean value of Beta carotene in males (µmol/L) | Mean value of Beta carotene in females (µmol/L) |
|---|---|---|---|---|
| 1. | Abiaka C et al., | Kuwaitis (Arab) [9] | 0.29 | 0.88 |
| 2. | Olmedilla B et al., | European [10] | 0.40 | 0.47 |
| 3. | Yuan JM et al., | Chinese [11] | 0.17 | - |
| 4. | Kieu NT et al., | Vietnammese [12] | 0.19 | 0.28 |
| 5. | Sripanidkulchai B et al., | Khon Kean (Northeastern Thailand) [13] | 0.61 | 0.70 |
| 6. | | Our study | 2.61 (overall) | |

**[Table/Fig-4]:** Mean values of Beta carotene in serum of different populations [9-13].

The serum concentration of carotenes are largely influenced by dietary intake and excessive consumption may leads to serum concentration as high as >5 mg/L [16]. The dietary pattern of the reference individuals was surveyed and it was found that the staple diets of the study population consisted of pumpkin, spinach, parsley, lettuce and carrots. Since the dietary pattern of the study population was a mixture of both vegetarians and non-vegetarians, so the mean value was influenced substantially.

It is evident from the values that "coverage interval", both parametric and non-parametric, for carotene was 41.05-363.76 µg/dL and 46.85–289.28 µg/dL respectively, which is in close approximation with the values found in literature i.e., 50-300 µg/dL [Table/Fig-2]. The parametric coverage interval was broader and gave a wide range of values than the non-parametric one, and with a "coverage uncertainty" lesser than non-parametric one. It was more precise than the non-parametric interval. Further technical considerations regarding the method of Bradley and Hornbeck documented that light petroleum used in the β-carotene estimation was highly volatile and even a slight delay in absorbance estimation may give erroneously high values [7]. According to the most recent and extensive listing of biological goals provided by Ricós C et al., 18% within subject biological variation, 48% between subject biological variation, 18% imprecision, 13.4% bias and 43.1% allowable total error are permissible for serum β-carotene [17]. The reference method for β-carotene is HPLC and this methodology was adapted for reference interval estimation in Kuwaiti, Swiss, Japanese, American, Italian, and Spanish populations [9-13, 18].

The bias (-8.3%) and proportional error (9.25%) in the present methodology was found to be lower than the recommendations. In literature survey it was found that stringent quality control was followed but in the present study there were no such adaptations. Thus, due to some error of the methodology, different ethnicity, race, genetic make-up, socio-demographic pattern, and dietary patterns of the study population, both parametric and non-parametric coverage interval was found to be different from the established reference interval for western populations [15].

Coverage interval with two confidence (upper and lower) limits gives an idea of the reference values distribution. The coverage interval with a less number of samples can give an estimate of "reference interval". In the present study, the technical considerations of the methodology used for analyte estimation met the pre-defined criteria of acceptability. Yet some errors associated with these crude methods influence the result a lot. From cost-effectiveness point of view the standard method of quantification of these antioxidant analytes i.e., HPLC, is not a feasible approach to calculate the reference interval with 120 individuals. "Coverage interval" with a small number of reference individuals like 71 (as in this study) or even less may purport the goal of estimation of "reference interval".

## LIMITATION

In the present study, sample size was calculated as per IUPAC clinical reports however further studies with sample size 120 (as per IFCC) and stringent quality control measures are required to validate these findings. In addition, studies are required to determine the causes of higher level of β-carotene in Bengali population.

## CONCLUSION

The reference values of β-carotene depend on multiple factors such as food habits, race, ethnicity, socio-demographics, and genetic make-up. The study provides scientifically valid data on the Bengali population, which adds a degree of novelty to the study, and will be a valuable addition to the knowledge base in this area of research.

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## REFERENCES

[1] Grasbeck R, Saris NE. Establishment and use of normal values. Scandinavian Journal of Clinical & Laboratory Investigation. 1962;26(110):62-63.
[2] Ichihara K, Boyd JC, IFCC Committee on Reference and Decision Limits (CRIDL). An appraisal of statistical procedures and in derivation of reference intervals. Clinical Chemistry and LaboratoryMedicine. 2010;48:1537-51.
[3] Solberg HE, Grasbeck R. Reference values. Advances in Clinical Chemistry. 1989;27:1-79.
[4] Poulsen OM, Holst E, Christensen JM. Calculation and application of coverage interval for biological values. Pure and Applied Chemistry. 1997;69(7):1601-11.
[5] Bender DA. Free radical & antioxidants. in Harper's Illustrated Book of Clinical Biochemistry, Murray R, Bender DA, Botham KM, Kennelly PJ, Rodwell VW and PA Weil. Eds., Pp. 564-568, McGraw Hill, 30th edition, 2015.
[6] Chakraborty S, Chakraborty I. Determination of coverage interval of antioxidant vitamins (Vitamin C & Vitamin E) in plasma and serum of Bengali population. Biochemistry Research International. 2013;2013:590791.
[7] Bradley DW, Hornbeck CL. A clinical evaluation of an improved TFA micromethod for plasma and serum vitamin A. Biochem Med. 1973;7:78.
[8] Kirkpatrick RL. Sample sizes to set tolerance limits. J Qual Tech. 1977;9:6-12.
[9] Abiaka C, Olusi S, Simbeye S. Serum concentrations of micronutrient antioxidants in an adult Arab population. Asia Pacific J Clin Nutr. 2002;11:22-27.
[10] Olmedilla B, Granado F, Southon S, Wright AJ, Blanco I, Gil-Martinezz E, et al. Serum concentrations of Carotenoids and Vitamins A, E & C in control subjects from five European countries. Br J of Nutrition. 2001;85:227-38.
[11] Yuan JM, Ross RK, Gao YT, Qu YH, Chu XD, Yu MC. Prediagnostic levels of serum micronutries in relation to gastric cancer in Shanghai, China. Cancer Epidemiol Biomarkers Prev. 2004;13:1772-80.
[12] Kieu NT, Yurie K, Hung NT, Yammato S, Chuyen NV. Simultaneous analysis of retinol, beta-carotene and tocopherol levels in serum Vietnamase populations with different incomes. Asian Pac J Clin Nutr. 2002;11:92-97.
[13] Sripanidkulchai B, Vaikrutta S, Sriamporn S, Vatanasapt P, Sripanidkulchai K. Serum antioxidant vitamin levels of people in KhonKaen, Northeastern Thailand. Asian Ac J Cancer Prev. 2003;4:147-52.
[14] Olmedilla B, Granado F, Gil-Martinez E, Blanco I, Rojas-Hidalgo E. Reference values for retinol, tocopherol, and main carotenoids in serum of control and insulin-dependent diabetic Spanish subjects. Clin Chem. 1997;43:1066-71.
[15] Boonsiri P, Pooart J, Tangrassameeprasert R, Hongsprobhas P. Serum β-carotene, lycopene and α-tocopherol levels of healthy people in northeast Thailand. Asian Pac J Clin Nutr. 2007;16:47-51.
[16] Varley H. Vitamins: Practical Clinical Biochemistry CBS Publishers India 1967 4th ed: pp. 607.
[17] Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, et al. Current databases on biologic variation: pros, cons and progress. Scand J Clin Lab Invest. 1999;59;491-500.
[18] Winklehofer-Roob BM, van't Hof MA, Shmerling DH. Reference values for plasma concentrations of vitamin E and A and carotenoids in a Swiss population from infancy to adulthood, adjusted for seasonal influences. Clinical Chemistry. 1997;43(1):146-53.

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