Scientific Research Publishing

Open Journal of Obstetrics and Gynecology, 2023, 13, 737-744
https://www.scirp.org/journal/ojog
ISSN Online: 2160-8806
ISSN Print: 2160-8792

Serum Vitamin E Reference Intervals in a Black Congolese Population of Kinshasa

Monzango Sibo[1,2], Mboloko Esimo[1], Mputu Lobota[1], Mbemba Fundu[3], Nganga Mireille[4], Itewa Monka[1,2], Mayoka Mamoi[1,2], Ntanga Kabuya[1,2], Amba Naomie[2], Lezaka Ensemeya[2], Bola Jonita[2], Maxime Fastrez[5], Lebwaze Bienvenu[6], Balthazar Phoba[7], Destin Mbongi[7], Tshilolo Léon[7], Kahindo P. Muyalalo[1], Mbanzulu Pita[1], Emile Darai[8]

[1]Department of Gynecology and Obstetrics, Faculty of Medicine, University Clinics of Kinshasa, Kinshasa, Democratic Republic of the Congo
[2]Department of Gynecology, Service of Laparoscopic Surgery, Onyx Medical Center, Kinshasa, Democratic Republic of the Congo
[3]Department of Biology, Faculty of Science, University of Kinshasa, Kinshasa, Democratic Republic of the Congo
[4]Department of Medical Biology, Faculty of Medicine, University Clinics of Kinshasa, Kinshasa, Democratic Republic of the Congo
[5]Department of Gynecology and Obstetrics, Erasme Endometriosis Clinic, Free University of Brussels, Brussels, Kingdom of Belgium
[6]Department of Histopathology, Faculty of Medicine, University Clinics of Kinshasa, Kinshasa, Democratic Republic of the Congo
[7]Institute of Biomedical Research (IRB), Health Training and Support Center (CEFA) Monkole, Kinshasa, Democratic Republic of the Congo
[8]Department of Gynecology and Obstetrics, Tenon Hospital, Sorbonne University, Paris, French Republic
Email: drguymonzango@gmail.com

How to cite this paper: Sibo, M., Esimo, M., Lobota, M., Fundu, M., Mireille, N., Monka, I., Mamoi, M., Kabuya, N., Naomie, A., Ensemeya, L., Jonita, B., Fastrez, M., Bienvenu, L., Phoba, B., Mbongi, D., Léon, T., Muyalalo, K.P., Pita, M. and Darai, E. (2023) Serum Vitamin E Reference Intervals in a Black Congolese Population of Kinshasa. *Open Journal of Obstetrics and Gynecology*, **13**, 737-744.
https://doi.org/10.4236/ojog.2023.134062

Received: February 15, 2023
Accepted: April 24, 2023
Published: April 27, 2023

Abstract

Context: Vitamin E is a powerful antioxidant and plays an important role in human reproduction. However, micronutrient deficiency is a major public health problem, particularly in developing countries. This study aimed to establish reference intervals (RIs) for vitamin E in black Congolese people of childbearing age using an ELISA method to provide a reference for clinically assessing vitamin E status. **Methods:** A total of 127 healthy people between the ages of 20 and 42 who underwent check-ups were randomly selected for the study. ELISA method measured the level of vitamin E. The effect of gender on vitamin E level was assessed, and RI was established using a parametric approach. **Results:** Women showed significantly higher levels of vitamin E than men ($p = 0.01$). The RI of vitamin E in people of childbearing age was 3.71 to 13.72, 4.52 to 14.64, and 4.17 to 13.52 mg/L, respectively, for the whole population, women and men. **Conclusion:** Using an ELISA method, this study established RI for vitamin E in the black Congolese population of childbearing age. We also found that women had significantly higher vitamin E levels than men. The results could provide a scientific basis for interpreting vitamin status in people of childbearing age in our setting.

Keywords

ELISA, Reference Interval, People of Childbearing Age, Vitamin E

1. Introduction

Vitamin E was first discovered in 1922 as a substance necessary for reproduction [1] [2] [3]. This fat-soluble vitamin has four forms, α-, β-, γ- and δ-tocopherol. Among them, α-tocopherol is the most abundant and has the highest biological activity in humans. This form is often used to assess vitamin E levels [1]. There are various reports on the benefits of vitamin E on general health [2]. Following this discovery, vitamin E was extensively studied and became widely known as a powerful antioxidant. Indeed, vitamin E functions as an antioxidant and free radical scavenger, protecting the integrity of unsaturated lipids in the biomembranes of all cells and preserving vitamin A from oxidative destruction [2] [3]. In addition, vitamin E has been found to lower body cholesterol levels and act as an anti-cancer agent [3]. Numerous studies have reported that vitamin E exhibits anti-proliferative, anti-survival, pro-apoptotic, and anti-angiogenic effects in cancer and anti-inflammatory activities.

Vitamin E has received a lot of attention in recent years due to its ability to improve reproductive health. It exerts beneficial effects as an antioxidant against reproductive disorders. Therefore, it is strongly recommended that women consume vitamin E regularly, especially those who are of childbearing age. [1] [3] [4]

In addition to its role as a powerful antioxidant, vitamin E is involved in a wide range of physiological processes, from immune function and inflammation control to the regulation of gene expression and cognitive performance [4]

Vitamin E deficiency represents a global public health problem [5] [6]. It is estimated that 2 billion people in the world suffer from micronutrient deficiencies, and this problem is more accentuated in Africa [7]-[12]. Inadequate micronutrient intake is also a problem in people of childbearing age [13] [14] [15].

Results from several studies suggest that poor nutritional status and higher prevalence of other oxidative stressors such as malaria and HIV infection predispose populations in developing countries to vitamin E deficiency [4]

Vitamin E deficiency caused by a diet low in vitamin E is common in developing countries because of its denaturation by cooking [9] [10] [11]. This deficiency is associated with increased infections, anemia, and stunted growth. [5] [6]. However, a well-established RI for vitamin E in the Congolese population is still lacking in our setting. Therefore, establishing RIs in Congolese people is essential for a proper vitamin E status assessment and adequate interpretation of the laboratory results.

Therefore, estimating the distribution of vitamin E levels in healthy individuals is crucial to assess whether deficiencies exist. Such estimation is possible through the use of a reference interval (RI), which is defined as the central dis-

tribution of values for a certain percentage (usually 95%) of apparently healthy individuals, according to guideline C28 A3 [12]. By fitting the distribution of the original data and fitting the distribution of the data through the use of a data transformation algorithm, we can establish the distribution range for healthy individuals using a parametric or non-parametric method to provide a basis for clinical decision-making [12].

This study aimed to establish RI for vitamin E in people of childbearing age in a black Congolese population using an ELISA method and provide a baseline for assessing vitamin E status.

2. Material and Methods

2.1. Selection of Participant

We randomly selected people of childbearing age 20 - 42 years old who underwent testing at Onyx Medical Center in the Democratic Republic of the Congo from January 2021 to October 2022. Participants on long-term treatment or taking health supplements, those with systemic disease or diagnosed with cardiovascular, kidney, gastrointestinal, lung disease, or cancer were excluded. Using a statistical method, we also excluded those with outliers or incomplete information. Finally, we recruited 127 people of childbearing age for the analysis. Residual Serum samples were collected and stored at −20˚C until analysis.

2.2. Ethics Approval

This study was approved by the ethics committee of the School of Public Health of the University of Kinshasa under number ESP/CE/187/2021. Since this study used serum remaining from the subjects after examination and all data is privatized, it will pose no risk to the subjects.

2.3. Laboratory Measurements

Venous blood was collected from the participants' antecubital vein early in the morning after 8 - 12 h of overnight fasting. Collected blood was centrifuged, and the serum separated from the blood cells after 4 to 6 hours of collection, aliquoted, and then frozen below −20˚C. A set of samples was also transported under standard conditions to the Molecular Medicine Laboratory of the Biomedical Research Institute (IRB) of the Center for Training and Health Support (CEFA) MONKOLE /DR Congo, where serum vitamin E was analyzed.

Serum vitamin E was analyzed using an enzyme-linked immunosorbent (ELISA) sandwich method (Mybiosource Ltd.USA) and an Elisa Micro Plate Reader plate analyzer from Inqaba Biotech, Inqaba Biotechnical Industries (Pty Ltd Pretoria, South Africa) in accordance with the manufacturer's instructions. A microelisa strip plate provided in the kit was pre-coated with an antibody specific to vitamin E standards. A horseradish peroxidase (HRP)-specific vitamin E conjugate was added to each microelisa strip plate well and incubated. The 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution was added to each well.

Only wells that contain vitamin E and HRP-conjugated anti-vitamin E antibodies appeared blue in color, which then turned yellow after the addition of Stop Solution. Spectrophotometric optical density (OD) was measured at a wavelength of 450 nm. The OD value is proportional to the concentration of vitamin E. The concentration of vitamin E in the samples was calculated with respect to the OD of the samples at the curve.

2.4. Statistical Analyzes

We entered data using Microsoft Excel 2016 software and analyzed using SPSS 25.0 software. Data normality was assessed using the Shapiro-Wilk test. Normally distributed data are expressed as means and standard deviations, while no normal data are presented as medians and interquartile ranges. The Mann-Whitney U test was used to compare differences between men and women. The estimation of the distribution of vitamin E levels in healthy individuals was obtained through the use of a reference interval (RI), which is defined as the central distribution of values for a certain percentage (usually 95%) of apparently healthy individuals, according to guideline C28 A3 [11]. By fitting the distribution of the original data and the distribution of the data through a data transformation algorithm, we can establish the distribution range for healthy individuals using a parametric or non-parametric method to provide a basis for clinical decision-making [12]. Tukey's method was used to identify outliers before establishing the RI for vitamin E. The RI was also established to observe the significant sex-specific difference. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. The Basic Characteristics

The basic characteristics of 127 participants are presented in **Table 1**. Among the participants, 65 were females (55.4%) and 62 were males (44.6%). The mean age was 32.4 years ± 5.8. Median vitamin E levels were 3.924 mg/L for both sexes, in men at 2.943 mg/L and women at 6.867 mg/L.

3.2. Effects of Sex on Vitamin E Levels

A significant difference in vitamin E levels was found between men (median [P25 - P75]: 2.943 [1.953 - 4.905] mg/L) and women (6.867 [2.943 - 11.772] mg/L) ($p < 0.01$). In the multiple linear regression model, we found a significant sex-specific difference in vitamin E levels ($p = 0.002$).

3.3. RI Established for Vitamin E

Using Tukey's method, we identified the lower limit of vitamin E levels at 0.806 mg/L, and the upper limit at 17.116 mg/L. Thanks to a Box-Cox conversion, the normality of the data was improved and the RIs for vitamin E were established based on the parametric method. Sex-specific RIs have also been established in **Table 2**.

Table 1. Basic characteristics of participants.

Characteristics	Total (n = 127)	Females (n = 65)	Males (n = 62)	Mean ± SD	p
Age (years) (%)				32.4 ± 5.8	0.173
≤24	8.66	4.62	12.90		
25 - 30	25.98	32.31	19.35		
31 - 35	30.71	27.69	33.87		
≥36	34.65	35.38	33.87		
Marital status (%)					0.137
Married	59.84	53.23	66.15		
Singles	40.16	46.77	33.85		
BMI (%)					
<18.5	25.98	30.77	20.97		0.086
18.5 - 24.9	56.69	60.00	53.23		
25 - 29.9	13.39	7.69	19.35		
≥30	3.94	1.54	6.45		
Median vitamin E (mg/L)	3.924	6.867	2.943		0.002

Table 2. Sex-specific reference ranges for vitamin E.

	Total				Females				Males				p
	LL	IC à 95%	UL	IC à 95%	LL	IC à 95%	UL	IC à 95%	LL	IC à 95%	UL	IC à 95%	
Vitamin E	3.71	3.61 - 4.26	13.72	12.99 - 14.63	4.52	4.03 - 5.24	14.64	13.91 - 15.33	4.17	3.74 - 4.92	13.52	12.83 - 14.64	0.001

Value of Vitamin E is in milligrams per liter. Abbreviations: CI: confidence interval; LL: lower limit; UL: upper limit.

4. Discussion

Vitamin E is a fat-soluble chemical playing a crucial role in maintaining reproductive life activities [1]. Thus, the assessment of vitamin E status requires precise quantification. The gold standard for measuring vitamin E levels is ELISA and reverse-phase high-performance liquid chromatography (HPLC) on plasma, which has high specificity and sensitivity and prevents interference from non-specific reactions and cross-reactivity, leading to greater accuracy and reliability [16] [17] [18].

In our laboratory, we used the ELISA method, which is precise and reliable to establish the RI of vitamin E that can be used in a clinical setting. We established RI of 3.71 to 13.73 mg/L for vitamin E, in people of childbearing age and with a significant difference in favor of women.

The Biomnis laboratory has proposed an RI for serum vitamin E of 4.29 to 13.30 mg/l for adult men [8] [9] [10]. In Algeria, S. Hamma found an RI of 6.75

to 15.73 mg/l, and in women, the average concentration of vitamin E is higher than that of men (13.18 ± 6.25 mg/l versus 10. 7 ± 3.85, $p < 5\%$) [18]. Veres in a Hungarian population reported an RI for serum vitamin E of 5.50 to 17.00 mg/L, for adults over the age of 18 years [19]. Using a high-performance liquid chromatography method, Johnson-Davis showed that the RI for vitamin E was 4.80 - 12.77 mg/L in children. Meanwhile, other studies have found no statistical sex-specific differences in vitamin E levels in children [20] [21] [22].

These results indicate that the RI for vitamin E varies among different populations. To our knowledge, using the ELISA method, this study is the first to establish the IR for serum vitamin E levels in people of childbearing age in Kinshasa.

This study has certain limitations, including the limited sample size and the failure to take into account the eating habits of our participants.

5. Conclusion

Using the ELISA method, this study established RI for serum vitamin E in black Congolese people of childbearing age. We also found that women had significantly higher vitamin E levels than men. Our results can contribute to assessing micronutrient status in people of childbearing age.

Authors' Contributions

All authors have accepted responsibility for the entire content of this submitted manuscript and have approved its submission.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Hacquebard, M. and Carpentier, Y.A. (2005) Vitamin E: Absorption, Plasma Transport and Cell Uptake. *Current Opinion in Clinical Nutrition and Metabolic Care*, **8**, 133-138. https://doi.org/10.1097/00075197-200503000-00005

[2] Wiseman, E.M., Bar-El, D.S. and Reifen R. (2017) The Vicious Cycle of Vitamin a Deficiency: A Review. *Critical Reviews in Food Science and Nutrition*, **57**, 3703-3714. https://doi.org/10.1080/10408398.2016.1160362

[3] Bar-El Dadon, S. and Reifen, R. (2017) Vitamin A and the Epigenome. *Critical Reviews in Food Science and Nutrition*, **57**, 2404-2411. https://doi.org/10.1080/10408398.2015.1060940

[4] Daphna, K.D. and Lindsay, H.A. (2011) Vitamin E Deficiency in Developing Countries. *Food and Nutrition Bulletin*, **32**, 124-143. https://doi.org/10.1177/156482651103200206

[5] Miyazawa, T., Burdeos, G.C., Itaya, M., Nakagawa, K. and Miyazawa, T. (2019) Vitamin E: Regulatory Redox Interactions. *IUBMB Life*, **71**, 430-441. https://doi.org/10.1002/iub.2008

[6] Traber, M.G. (2014) Vitamin E Inadequacy in Humans: Causes and Consequences. *Advances in Nutrition*, **5**, 503-514. https://doi.org/10.3945/an.114.006254

[7] Bailey, R.L., West, K.P. and Black, R.E. (2015) The Epidemiology of Global Micronutrient Deficiencies. *Annals of Nutrition and Metabolism*, **66**, 22-33. https://doi.org/10.1159/000371618

[8] Liu, Z., Zhao, L., Man, Q., Wang, J., Zhao, W. and Zhang, J. (2019) Dietary Micronutrients Intake Status among Chinese Elderly People Living at Home: Data from CNNHS 2010-2012. *Nutrients*, **11**, Article 1787. https://doi.org/10.3390/nu11081787

[9] De Bandt, J.P. and Vitamine, E., (1996) Cahier de formation Biochimie, Tome III. Bioforma, Paris, 207-216.

[10] Leboulanger, J. (1984) Les vitamines. Biochimie-Mode d'action-Intérêt thérapeutique. F. Hoffmann-La Roche et Cie Ed, Neuilly-sur-Seine, 57-64.

[11] Léger, C.L., Carbonneau, M.A. and Fouret, G. (2007) Vitamine E. *Cahier de Formation en Biologie Médicale Bioforma*, **38**, 49-63.

[12] Clinical Laboratory Standards Institute (2008) Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, 3rd Edition. CLSI Document EP28-A3c. Clinical Laboratory Standards Institute, Wayne, PA.

[13] Quann, E.E., Fulgoni, V.L. and Auestad, N. (2015) Consuming the Daily Recommended Amounts of Dairy Products Would Reduce the Prevalence of Inadequate Micronutrient Intakes in the United States: Diet Modeling Study Based on NHANES 2007-2010. *Nutrition Journal*, **14**, Article No. 90. https://doi.org/10.1186/s12937-015-0057-5

[14] Mensink, G.B., Fletcher, R., Gurinovic, M., *et al.* (2013) Mapping Low Intake of Micronutrients across Europe. *British Journal of Nutrition*, **110**, 755-773. https://doi.org/10.1017/S000711451200565X

[15] ter Borg, S., Verlaan, S., Hemsworth, J., *et al.* (2015) Micronutrient Intakes and Potential Inadequacies of Community-Dwelling Older Adults: A Systematic Review. *British Journal of Nutrition*, **113**, 1195-1206. https://doi.org/10.1017/S0007114515000203

[16] Yu, S., Yin, Y., Wang, D., *et al.* (2019) Development and Validation of a Simple Isotope Dilution-Liquid Chromatography-Tandem Mass Spectrometry Method for Detecting Vitamins A and E in Serum and Amniotic Fluid. *International Journal of Mass Spectrometry*, **435**, 118-123. https://doi.org/10.1016/j.ijms.2018.10.024

[17] Tsou, P. and Chang-Jiun, W.-U. (2019) Serum Vitamin E Levels of Adults with Nonalcoholic Fatty Liver Disease: An Inverse Relationship with All-Cause Mortality in Non-Diabetic but Not in Pre-Diabetic or Diabetic Subjects. *Journal of Clinical Medicine*, **8**, Article 1057. https://doi.org/10.3390/jcm8071057

[18] Jungert, A., Neuhäuser B.M. (2020) Interrelation entre les concentrations plasmatiques de vitamines C et E le long de la trajectoire du vieillissement en tenant compte du mode de vie et de la composition corporelle : une étude longitudinale sur deux décennies. *Nutriments*, **12**, Article 2944. https://doi.org/10.3390/nu12102944

[19] Hamma, S., Benlatrech, M., Abadi, N. and Benlatrech, C. (2007) P055 Détermination des valeurs usuelles de la vitamine E dans la population de Constantine. *Nutrition Clinique et Métabolisme*, **21**(Supplement 2), 72-73. https://doi.org/10.1016/S0985-0562(07)78857-X

[20] Veres, G., Szpisjak, L., Bajtai, A., *et al.* (2017) L'établissement d'intervalles de référence de tocophérol pour la population adulte hongroise à l'aide d'une méthode HPLC validée. *Biomedical Chromatography*, **31**, e3953. https://doi.org/10.1002/bmc.3953

[21] Johnson-Davis, K.L., Moore, S.J., Owen, W.E., Cutler, J.M. and Frank, E.L. (2009) Une méthode HPLC rapide utilisée pour établir des intervalles de référence pédiatriques pour les vitamines A et E. *Clinica Chimica Acta*, **405**, 35-38. https://doi.org/10.1016/j.cca.2009.03.058

[22] Raizman, J.E., Cohen, A.H., Teodoro-Morrison, T., *et al.* (2014) Pediatric Reference Value Distributions for Vitamins a and E in the CALIPER Cohort and Establishment of Age-Stratified Reference Intervals. *Clinical Biochemistry*, **47**, 812-815. https://doi.org/10.1016/j.clinbiochem.2014.03.025