Journal of Genetic Engineering and Biotechnology xxx (2017) xxx–xxx

HOSTED BY

Contents lists available at ScienceDirect

Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

Original Article

Effect of vitamin A deficiency on thymosin-β4 and CD4 concentrations

Salwa Refat El-Zayat [a,*], Hiba Sibaii [a], Nermine N. Mahfouz [b], Sara F. Sallam [b], Reham F. Fahmy [b], Azza Abd El-Shaheed [b]

[a] Medical Physiology Department, National Research Centre, Medical Division, 33 El-Bohouth Street, Dokki POB:12311, Cairo, Egypt
[b] Child Health Department, National Research Centre, Medical Division, 33 El-Bohouth Street, Dokki, POB:12311, Cairo, Egypt

ARTICLE INFO

Article history:
Received 15 June 2017
Received in revised form 15 September 2017
Accepted 5 October 2017
Available online xxxx

Keywords:
Vitamin A deficiency
Immune function
Thymosin-β4 (Tβ4)
cluster of differentiation (CD) 4

ABSTRACT

Vitamins are evaluated for their role in immunity. Recently, vitamin A received a particular attention as a critical micronutrient for regulating immune system. Therefore, the present study aimed to search for new about vitamin A. Forty-eight Egyptian adults aged from 18 to 42 years old from both sexes were subjected to clinical examination and nutrition questionnaire and were screened for vitamin A by using ELISA method. Forty subjects were selected and subdivided into two groups. Group 1 with vitamin A at level >200 μg/dl consists of 10 healthy subjects. Group 2 with vitamin A deficiency at level <50 μg/dl consists of 30 subjects. Tβ4 and CD4 levels were also determined by a commercial ELISA kit. Results showed a significant decrease in serum levels of Tβ4 and CD4 in group 2 than group 1 at $P < .003$ and $P < .019$ respectively. Both of Tβ4 and CD4 had positive correlation with vitamin A level at $P < .000$ and $P < .003$ respectively as well as with each other at $p < .000$. We concluded that vitamin A deficiency may be influence the levels of Tβ4 and CD4.

1. Introduction

Overall nutritional status is required for the immune system to function efficiently. Deficiency in vitamins can impair phagocytic function in innate immunity [33] and adversely affect several aspects of adaptive immunity [41]. Vitamin A was firstly known as "the anti-infective vitamin" as it is important for immune system to function normally [20]. Vitamin A affected the immune response in both lines of immunity [33]. In innate immunity, it helps to maintain the structural and functional integrity of the skin [51] and mucosal cells of the eye, respiratory, gastrointestinal, and genitourinary tracts [44,45,12]. It is also important for many cells including natural killer (NK) cells, macrophages, and neutrophils to function normally [44]. In adaptive immunity, Vitamin A needed for proper function of T and B lymphocytes [37]. Cluster of differentiation 4 (CD4) is a glycoprotein located on the surface of immune cells especially T-helper cells [3]. They are functioning in signal transduction between the T cell receptor (TCR) and an antigen presenting cell; and in T-cell activation [52]. Vitamin A may affect cell-mediated immunity by decreasing the number or distribute ion of $CD4^+$ T-lymphocytes, altering cytokine production, or by decreasing the expression of cell-surface receptors that mediate T-cell signaling [49]. Vitamin A activates T-cell lymphocytes so they can fight off infection [47], while its deficiency prevents proper lymphocyte function [29]. Thymus gland produces beta thymosin hormones [32]. Thymosin-β4 (Tβ4) is the prevailing form, representing 70–80% of the total thymosin content [23]. It is a protein with 43 amino acids, bind to and sequester G-actin to modulate cell migration [2,8]. It is considered to play a significant role in the cellular metabolism due to its actin-sequestering properties [34]. Tβ4 mRNA has different expression in immune cells suggesting a relationship between Tβ4 and immune response [18]. Several physiological properties of Tβ4 have been reported [14]. It acts as a modulator of wound healing [53] and angiogenesis of heart tissues following injury [25], helping in the development of B cells [19], increasing the efficiency of antigen presentation by macrophages [50], implicated in lymphocyte maturation and differentiation [16], controlling cell morphogenesis and motility [17], regulating immunity [36] and treating liver fibrosis [26]. Vitamin A deficiency (VAD) results in impaired mucosal epithelial regeneration and reductions in the number and killing activity of

Abbreviations: VAD, vitamin A deficiency; Tβ4, Thymosin-β4; CD4, cluster of differentiation 4; BMI, Body mass index; CBC, complete blood picture; NRC, National Research Center.

Peer review under responsibility of National Research Center, Egypt.

* Corresponding author.
E-mail address: salwarefat@gmail.com (S.R. El-Zayat).

https://doi.org/10.1016/j.jgeb.2017.10.007
1687-157X/

NK cells, as well as the function of neutrophils and macrophages [5]. In addition, VAD results in altered cytokine signaling which would affect inflammatory responses of innate immunity [45]. The risks of VAD can be reversed by supplementation [4]. Reports regarding the influence of VAD on thymosin β4 and CD4 levels in adults are rare. Consequently, the present study aimed to explore this relationship in a group of Egyptian adults to search a new insight about vitamin A.

2. Subjects and methods

Our study was held through a project at National Research Center (NRC) and was approved by the Ethical Committee. The Center of Medical Excellence of NRC guaranteed and gave the permission to perform our study at the outpatient clinic. After taking a written informed consent, forty-eight Egyptian adults from both sexes, aged from 18 to 42 years old were subjected to clinical examination and nutrition questionnaire to evaluate their nutrition status and to detect any symptom or sign of vitamin A deficiency that are dry eyes, dry and rough skin, eye inflammation, night blindness, respiratory and urinary infections. They were also screened for vitamin A by using ELISA method. Accordingly, forty subjects were selected and enrolled in this study. They were divided according to the international reference range of vitamin A for adult [9] & [30] into two groups: **Group 1** includes healthy subjects (n = 10) with sufficient vitamin A at level >200 μg/dl. **Group 2** includes subjects (n = 30) with vitamin A deficiency at level <50 μg/dl. Adults who had genetic disorders, chronic or autoimmune diseases, systemic failure or any malignant tumors were excluded. Subjects on daily vitamin A supplement were also excluded from the study. A careful medical history and clinical examination were taken including: demographic data in the form of age and sex. Vital signs including blood pressure, Radial pulse, respiratory rate and body temperature were recorded. Adults were asked about repeated attacks of upper respiratory tract infection and/or gastrointestinal infection. Anthropometric measures regarding height and weight were recorded for each subject. The height was measured to the nearest 0.5 cm on a Holtain portable anthropometer, and the weight was determined to the nearest 0.1 kg on a Seca scale Balance with the subject dressed minimum clothes and no shoes. Body mass index (BMI) was calculated as Weight (kg)/ Height (m^2).

3. Samples collection

Blood samples (5 ml) were drawn from all subjects, a part of blood (2 ml) was taken immediately in EDTA-containing vacutainers to estimate complete blood picture (CBC) and the remaining (3 ml) was centrifuged 3000 rpm for 10 min then sera were isolated and stored at −20 until the determination of vitamin A and other laboratory investigations.

4. Research methods and procedures

4.1. Biochemical assays

Serum levels of vitamin A, Tβ4 and CD4 were measured by using a commercial enzyme linked immunosorbent assay ELISA kit, produced by Glory Science Co., Ltd. 2400 Veterans Blvd. Suite 16 – 101, Del Rio, TX 78840, USA. Tel: 001-830-734-0090 www.gloryb io-science.com., performed at National Research Centre, medical physiology department.

4.2. Statistical analysis

All values are expressed as mean ± SE and the differences between the two groups were calculated by student's t test. The correlation was done between different parameters using Pearson's correlation. A Chi-square (χ^2) test was used to test the significance associations among non-parametric data. All analyses were carried out using Statistical Package for Social Science (SPSS) version 16 (IBM, Chicago IL, USA. Statistical software). The statistical significance was set at $p < .05$.

5. Results

Forty participants were enrolled in our study. Thirty-four were females (85%) and six were males (15%). We screened all the cases for symptoms of vitamin A deficiency (VAD). The results revealed a high rate of vitamin A deficiency 62.5% (group 2 (n = 30)) versus 20.8% (group 1 (n = 10)) with sufficient level of vitamin A. There were 9 cases suffering from night blindness out of 30 adults (group 2) representing 30% of vitamin A deficient subjects.

The results revealed that age was nearly comparable in the two groups at P = .414. In the same time, no significant difference was observed between the two groups for CBC parameters. The results of anthropometric measures were recorded in Table 1 and showed that there was no statically difference between the two groups in height and BMI at p = .3 and p = .89 respectively. While a significant difference between the two groups in weight at p < .017.

There was a high significant decrease in serum levels of vitamin A, Tβ4 and CD4 at P < .001, P < .003, P < .001 in the two groups as shown in Figs. 1–3 respectively.

Figs. 4 and 5 represented a high significant positive correlation between vitamin A and both of Tβ4 and CD4 at (r = .579**, P < .000) and (r = 0.451**, P < .003) respectively, at the same time Fig. 6 demonstrate a high significant positive correlation between Tβ4 and CD4 at (r = 0.567**, P < .000).

The results of Chi-square χ^2 test to examine the relation between non-parametric variables in the studied groups were

Table 1
Anthropometric measures in the two groups.

Mean ± SD	Group I (n = 10)	Group II (n = 30)	Significance
Weight /kg	71.7 ± 5.49	62.88 ± 16.7	P < .017
Height/m^2	1.60 ± 8.5	1.57 ± 8.9	P = .3 NS
BMI kg/m^2	26.4 ± 3.7	26.2 ± 6.37	P = .89 NS

BMI: body mass index P < .01 = significant difference NS = no significant difference.*

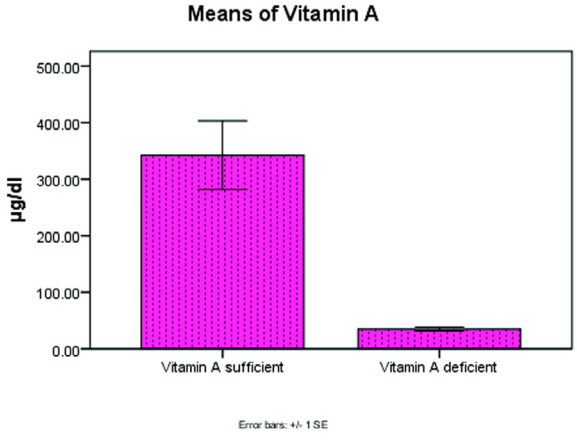

Fig. 1. Significant decrease in vitamin A level at P < .001 in the two groups.

Please cite this article in press as: El-Zayat SR et al. Effect of vitamin A deficiency on thymosin-β4 and CD4 concentrationsPlease check the inserted running head and correct if necessary.[Error /ce:query] –>. Journal of Genetic Engineering and Biotechnology (2017), https://doi.org/10.1016/j.jgeb.2017.10.007