[Downloaded free from http://www.new.asmr.eg.net on Thursday, January 3, 2019, IP: 62.193.78.199]

A new insight into the immune regulatory functions of vitamin A in children and adolescents

Azza Abd El-Shaheed[a], Reham F. Fahmy[a], Salwa Refat El-Zayat[b], Hiba Sibaii[b], Nermine N. Mahfouz[a], Rehab S.I. Moustafa[a]

Departments of [a]Child Health, [b]Medical Physiology, National Research Centre, Dokki, Giza, Egypt

Correspondence to Reham F. Fahmy, PhD of Child Health, Department of Child Health, Medical Division, National Research Center, 33 El Bohouth Street, PO Box 12311, Dokki, Giza, 12622, Egypt. Telephone: 00201001236323; e-mail: reham_dodo2@yahoo.com

Received 28 November 2017
Accepted 8 February 2018

Journal of The Arab Society for Medical Research 2018, 13:1–7

Background/Aim
Vitamin A deficiency (VAD) is a serious and widespread public health problem. Vitamin A has an important role in regulating human immune function. It increases rates and severity of infections in young children mainly in developing countries. The present study aims to assess the effect of vitamin A on cluster of differentiation 4 (CD4) and thymosin β4 (Tβ4) levels as indicators of adaptive immunity. Moreover, we evaluate the association between serum vitamin A concentration and BMI among Egyptian children and adolescents.

Patients and methods
This cross-sectional survey was conducted on 46 apparently healthy participants, including 19 girls and 27 boys aged from 3 to 17 years. We assessed weight and height using standard techniques. Serum vitamin A, CD4, and Tβ4 concentrations were assessed by using enzyme-linked immunosorbent assay kits. We planned to divide the participants into vitamin A-sufficient and vitamin A-deficient groups according to its level.

Results
Cutoff for VAD was 44 μg/dl. It was detected in 56.6% of the enrolled participants. Vitamin A was significantly lower in teenagers comparative with children (P=0.04). Vitamin A and Tβ4 levels were significantly decreased in deficient group in comparison with sufficient one at P values of 0.002 and 0.017, respectively, whereas CD4 level was nonsignificantly decreased in vitamin A-deficient patients compared with the sufficient ones. A significant positive correlation was detected between vitamin A and both of CD4 (r=0.348, P=0.018) and Tβ4 (r=0.392, P=0.007). A significant positive correlation was found between vitamin A and BMI (r=0.311, P=0.035).

Conclusion
Vitamin A may influence Tβ4 and CD4 levels. This study is the first to explore the effect of vitamin A on Tβ4 level in children and adolescents and correlate it with CD4 level. This finding must be verified using large-scale studies.

Keywords:
CD4, children and adolescents, growth, thymosin β4, vitamin A

J Arab Soc Med Res 13:1–7
© 2018 Journal of The Arab Society for Medical Research
1687-4293

Introduction

Vitamin A is an essential micronutrient that is needed in sufficient amounts in our diet to keep a proper physiological well-being. Mammalian living organisms need vitamin A particularly during periods of growth and development. It has important functions in vision, reproduction, and cellular differentiation, and its absence could be life-threatening [1,2]. It includes a set of retinoid compounds with the biologic action of all-trans-retinol [3]. We can get preformed vitamin A from animal sources in the diet (liver, fish liver oils, and dairy products) as retinylpalmitate; on the contrary, carotenoids that are transformed into retinol come from vegetable food sources (dark-green leafy vegetables and deep-orange fruits) [4]. Vitamin A deficiency (VAD) is a main community health quandary in low- and middle-income countries, and at least 250 million children all over the world experience VAD as stated by the WHO [2]. It is the leading cause of preventable blindness in children [5]. Vitamin A is considered one of the broadly studied micronutrients regarding the effect on immune system [6]. It is named anti-infectious vitamin owing to its effect in regulation of human immune function. A relationship between VAD and increased vulnerability to infections has been detected in early studies in animals and humans [7]. Its insufficiency subjects human beings, particularly infants, to diseases of eye, respiratory, and gastrointestinal tract [8]. Vitamin A has significant effects in both cell-mediated and humoral antibody response and supports a

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

[Downloaded free from http://www.new.asmr.eg.net on Thursday, January 3, 2019, IP: 62.193.78.199]

Th2-mediated anti-inflammatory cytokine outline. Lack of vitamin A weakens the response of innate immunity (mucosal epithelial regeneration) and adaptive immunity to infections, leading to an impaired capability to offset extra-cellular pathogens [9]. Vitamin A in diet functions through its active metabolite retinoic acid (RA) [10]. Recently, increasing data inform of a more reflective systemic blow of RA on leukocyte role and commitment. Animal examples using genetic handling of RA signaling help us to learn when and how RA controls T-cell fate [11]. The idea that T-helper (Th) cells stably differentiate into two discrete pathways, resulting in Th1 and Th2 cells, was supposed in the late 1980s as an outline to recognize the distinctive patterns of cytokine secretion noticed in cloned, activated CD4 T cells [12]. The notion that effector Th1 and Th2 cell types undergo stable differentiation provoked large concern in understanding how pathogens and host ecological factors, including micronutrients, act together to control T-cell activation and discrimination. VAD makes the environment favorable for the discrimination of naive precursor CD4 T cells into interferon γ-secreting Th1 cells [13]. Alternatively, vitamin A and RA commonly maintain discrimination in the direction of Th2 cells and the production of interleukin-4 and interleukin-5 [14] or augment the proportion of Th2 cytokines compared with Th1 cytokines by decreasing the Th1 reaction [15]. Thymus gland produces β-thymosin hormones which stimulate the proliferation and differentiation of CD4 T lymphocytes [16]. Thymosin β4 (Tβ4) is the main form in mammalian cells and tissues, representing 70–80% of the total thymosin content [17]. Tβ4 is an intracellular protein with 43 amino acids [18]. It was primarily isolated from thymosin fraction 5, and prepared in five steps from calf thymus [19]. It has a number of biological effects. It is drawn in endothelial cell migration and angiogenesis, and its amount increases at sites of injury signifying a great effect of this biopeptide in wound healing [20]. The significance of vitamin A for host defense is indisputable, and the study of its mechanisms is required. Therefore, we aimed to assess the effect of vitamin A on CD4 and Tβ4 levels as indicators of adaptive immunity and to evaluate also the association between serum vitamin A concentration and BMI.

Patients and methods

Study design

This cross-sectional survey was conducted on 46 apparently healthy participants including 19 girls and 27 boys aged from 3 to 17 years. The participants were recruited from the centre of medical excellence at National Research Center. Children were defined as participants younger than 10 years, and teenagers as participants who were 10 years and older, according to the WHO definition [21]. All participants were subjected to full history taking laying stress on age, sex, and symptoms of VAD such as night blindness and diarrhea, and thorough clinical examination, nutritional questionnaire, and anthropometric measurement. Night blindness was assessed for children younger than 8 years by asking the parents if their child had a problem seeing in low levels of light whereas older children answered by themselves [22]. Weight was measured using electronic scale which was regularly checked for its accuracy. Height was measured using a calibrated scale consisting of a wooden platform with a scale and sliding head piece, with children wearing light clothing and no shoes. BMI was calculated as weight (kg)/height $(m)^2$ [23]. We planned to divide the participants into vitamin A-sufficient and vitamin A-deficient groups according to its level. Blood samples (5 ml) were taken for laboratory assessment of vitamin A, CD4, and Tβ4 using enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (Glory Science Co. Ltd, Del Rio, Texas, USA). Stool samples were collected for detection of parasitic infestations.

Ethical consideration

Informed consent was obtained from all participants, and the study protocol was approved by the ethical committee of National Research Center, and the study was carried on in accordance with the declaration of Helsinki 1964.

Statistical analysis

The collected data were coded, tabulated, and statistically analyzed using SPSS program, version 16 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics were done for numerical parametric data as mean±SE. Inferential analyses were done for quantitative variables using independent *t*-test. However, correlations were done using Pearson's correlation (correlation coefficient) for numerical parametric data. *P* value of less than 0.05 indicated statistical significance. To evaluate the performance of vitamin A, we used receiver operating characteristic curve. The curve was done to illustrate its sensitivity and specificity at different decision cutoff level. In this type of curve, the *x*-axis represents the false positive rate (1–specificity), and the *y*-axis represents the true positive rate (sensitivity). The best cutoff is the nearest point to the upper left corner [24].

Results

The percentage of VAD was 56.6% of the enrolled participants. Vitamin A-sufficient group included 20

[Downloaded free from http://www.new.asmr.eg.net on Thursday, January 3, 2019, IP: 62.193.78.199]

participants, and vitamin A-deficient group included 26 participants. The mean concentration of vitamin A in the sufficient group was significantly higher compared with the deficient group (P=0.002). In addition, the mean of Tβ4 was significantly higher in vitamin A-sufficient group compared with vitamin A-deficient group (P=0.017), whereas the mean of CD4 was nonsignificantly higher in vitamin A-sufficient group compared with vitamin A-deficient group at (P=0.276) as shown in Table 1. The cutoff level of vitamin A was 44 μg/dl as determined by the receiver operating characteristic curve and represented in Fig. 1.

Approximately half of the participants were children [23 (50%)], with mean age of 6.37±0.44 years, and the others were teenagers [23 (50%)], with mean age of 12.89±0.44 years. The results showed that the mean concentration of vitamin A was significantly lower in teenagers compared with children (P=0.043).

The present results showed significant positive correlations between vitamin A and both of Tβ4 and CD4 (r=0.392, P=0.007, and r=0.348, P=0.018, respectively) (Fig. 2a and b), as well as a significant positive correlation between vitamin A and BMI at r=0.311, P=0.035 (Fig. 2c). A significant positive correlation between Tβ4 and CD4 was recorded at r=0.308, P=0.037, as shown in Fig. 3.

The results showed in Table 2 demonstrated that the frequency of night blindness, *Entamoeba histolytica* infection, respiratory infections, and diarrhea among vitamin A-deficient group were 11.5, 19.2, 15.4, and 38.5%, respectively, whereas the frequency of night blindness, *E. histolytica* infection, respiratory infections and diarrhea among vitamin A-sufficient group were 5, 30.0, 15.0, and 35.0%, respectively.

Table 1 Serum levels of vitamin A, cluster of differentiation 4 and thymosin β4 in children and adolescents with vitamin A-sufficient and vitamin A-deficient groups

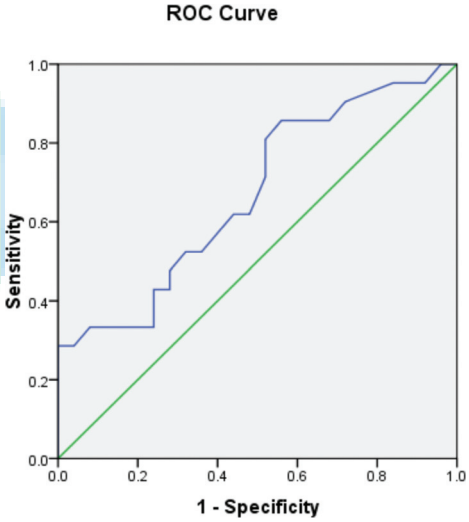
	Vitamin A-sufficient group (N=20)	Vitamin A-deficient group (N=26)
Vitamin A (μg/dl)	79.50±13.48	32.42±1.41*
Tβ4 (ng/ml)	2.30±42.76	1.13±15.86*
CD4 (pg/ml)	0.98±0.16	0.77±0.10

All data are represented as mean±SE. CD4, cluster of differentiation 4; Tβ4, thymosin β4. *Significant than vitamin A-deficient group at P<0.05.

Discussion

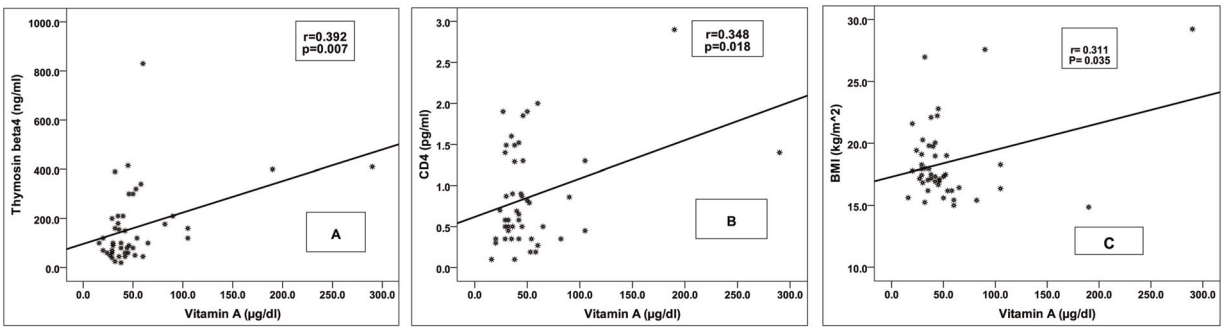
Micronutrient is the name used to represent important vitamins and minerals required from the diet to sustain all normal cellular and molecular functions [5]. Sufficient intakes of vitamins and trace elements are needed for the immune system to act competently [9]. Infection and

Figure 1



Receiver operating characteristic (ROC) curve of vitamin A: cutoff level of vitamin A is 44 (μg/dl).

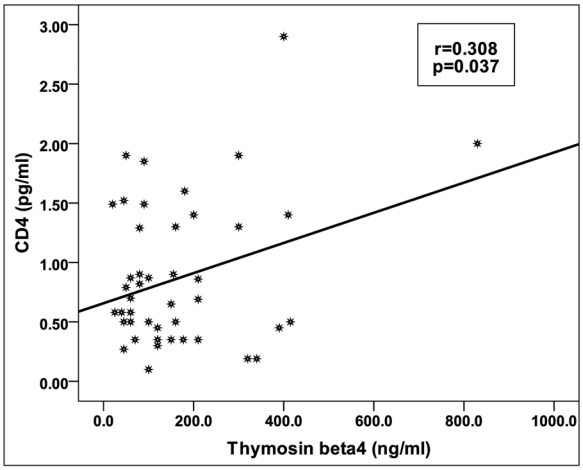
Figure 2



(a) Positive correlation between vitamin A and Tβ4. (b) Positive correlation between vitamin A and CD4. (c) Positive correlation between vitamin A and BMI.

[Downloaded free from http://www.new.asmr.eg.net on Thursday, January 3, 2019, IP: 62.193.78.199]

Figure 3



Pearson's correlation between Tβ4 and CD4.

Table 2 Frequency of night blindness, *Entamoeba histolytica* infection, respiratory infections, and diarrhea among children and adolescents with vitamin A-sufficient and vitamin A-deficient groups

	Vitamin A-sufficient group (*N*=20) [*n* (%)]	Vitamin A-deficient group (*N*=26) [*n* (%)
Night blindness	1 (5)	3 (11.5)
Entamoeba histolytica infection	6 (30)	5 (19.2)
Respiratory infections	3 (15.0)	4 (15.4)
Diarrhea	7 (35.0)	10 (38.5)

undernutrition are widespread in developing countries and show a synergistic relation [25]. Micronutrient deficiency suppresses immune functions leading to dys-regulation of the reasonable host reaction. This increases the propensity to infections, with amplified morbidity and mortality. Sequentially, infections worsen micronutrient deficiencies by decreasing nutrient intake, augmenting losses, and interfering with utilizations by changing metabolic pathways [9].

The present study revealed that 56.6% of the recruited participants had VAD. This is in agreement with most studies which showed that VAD is a serious and widespread public health problem affecting about 190 million children aged less than 5 years [26].

In children, VAD can lead to increased risks of mortality and morbidity associated with infections such as measles and diarrheal infections [27]. Several of these effects can be attributed to the immunological influences of vitamin A [28].

The current study showed that 19.2% of vitamin A-deficient participant had *E. histolytica* infection. The association between parasitic infections and vitamin A is diphasic, in which poor vitamin A status could increase vulnerability to parasitic infections and vice versa [29]. We also found that 38.5% of vitamin A-deficient participants had diarrhea. Vitamin A insufficiency could increase the risk of morbidity and mortality in the course of impaired reaction to diarrheal infections [30]. It is essential for maintaining intestinal integrity [31], and regulating mucin gene expression [32]. It is supposed that vitamin A and its biologically active metabolite 'RA' together with additional local ecological factors have a role in gut-associated immunity by enhancing generation of IgA-producing B cells, gut–tropic $CD4^+$ and $CD8^+$T cells, and innate lymphoid cells (a novel lymphocyte subpopulation) [33]. The new findings that 'RA' marks the homing of leukocytes to the gut and facilitates the generation of regulatory T cells emphasize a possible effect of RA in mucosal tolerance [11]. Examination of the type of T cells in the intestine of vitamin A-insufficient mice reported that the submucosal lamina propria region was almost devoid of $CD4^+$ $CD8^+$ T cells. The immune response of the intestine to pathogens that have breached the epithelium could be affected by lack of lamina propria T cells [34]. It was found that vitamin A supplementation reduced diarrhea-related deaths by 30% in children aged 6–59 months [35].

VAD is the principal cause of blindness worldwide [36]. This study reported that 11.5% of the VAD participants had night blindness. A study in Yemen also stated a very low prevalence of ocular manifestations of VAD among children aged 1–5 years [37]. The present study showed that 15.4% of vitamin A-deficient group had respiratory infections. The effect of vitamin A on respiratory infections is varying. Some of public-based studies showed an obvious increase of respiratory symptoms regarding vitamin A supplementation, especially in children who are not experiencing malnutrition. It is not obvious if this noticeable increase in respiratory symptoms is related to a proinflammatory immune reaction linked to the supplements [38]. It is also observed that children with VAD are exposed to severe pneumococcal infections even after receiving Prevnar-13 vaccine (PCV-13) [39].

The present study revealed that the frequency of night blindness, respiratory infections, and diarrhea was comparable between vitamin A-sufficient group and vitamin A-deficient group, and we attributed this finding to the small number of the recruited participants, and it could be also owing to that VAD was not severe enough to cause symptoms. To

[Downloaded free from http://www.new.asmr.eg.net on Thursday, January 3, 2019, IP: 62.193.78.199]

reduce the risks associated with vitamin A deficiency, WHO continues to advocate giving periodic high-dose vitamin A supplementations to children at the age of 6–59 months who live in low-income countries, because at the period of strategy arrangement, this interference was shown to lessen all-cause deaths by 23–30% in this age group [40].

This study showed a significant increase in serum level of Tβ4 in vitamin A-sufficient group compared with vitamin A-deficient group (P=0.017), whereas CD4 level was nonsignificantly higher in vitamin A-sufficient group compared with vitamin A-deficient group (P=0.276). In addition, a significant positive correlation was found between vitamin A and both of CD4 and Tβ4 (r=0.348, P=0.018 and r=0.392, P=0.007, respectively).

Malnutrition, mainly if occurs earlier in life, could hinder the growth and role of lymphoid tissues. This could result in a wide range of immune insults. The adaptive and innate arms of the immune system are negatively influenced by different kinds of nutritional insufficiencies including VAD [41]. Tβ4 was thought to be specially formed and released by the thymic gland, and it has hormonal effects that modulate the immune reaction [42]. It is found in most tissues and cell lines and is present in high concentrations in blood platelets, neutrophils, macrophages, and other lymphoid tissues [43]. The thymus provides most favorable cellular and humoral microenvironment for the development of immunocompetent T lymphocytes [44]. Animal (*in vivo* and *in vitro*) and human in-vitro studies confirm that vitamin A and its metabolites (mainly 'RA') have a potent effect in the ruling of innate and adaptive immune responses [45]. Regarding innate immune reaction, this includes the integrity of mucosal epithelial [46] and the numbers, discrimination, and cytokine secretion profiles of monocytes, macrophages, natural killer cells, and neutrophils [47]. Regarding adaptive immune response, it is supposed that vitamin A has an influence in thymic development and maturity of thymocytes [48], thus VAD could harm thymic task, leading to effects on the peripheral T-cell pool. VAD has an influence on lymphopoiesis, distribution, and cytokine production [28]. Some pediatric supplementation trials have suggested a possible effect of vitamin A on human lymphopoiesis. In a study performed in South Africa, it was found that the total lymphocyte number considerably increased in infants after 42 days of vitamin A supplementation [49], whereas in another study conducted in Indonesia, vitamin A supplementation resulted in an elevated percentage of CD4-naive T cells ($CD4^+$ $CD45RA^+$) after 5 weeks, compared with controls [50]. Therefore, the effect of vitamin A supplementation on the increase in the number of T cells, mostly CD4 subpopulation, and its direct effect on cytokine production and T-cell activation [51] highlights the importance of sufficient vitamin A condition either obtained from intake of preformed retinol or β-carotene, for keeping a good equilibrium of well-synchronized T-cell functions and for avoiding too much or lengthened inflammatory reactions [52].

Our study showed a significant positive correlation between Tβ4 and CD4 (P=0.037). This is in agreement with Knutsen *et al.* [53]. This finding could be ascribed to the fact that Tβ4 is the predominant form of thymic hormones [54] and that its primary function is to stimulate the production of T cells which are targets of thymosin activity [55].

This study revealed that the mean concentration of serum vitamin A was significantly lower in the teenagers group (38.95±2.77) compared with the children group (66.86±12.77) (P=0.043). This result is in accordance with that observed by de Souza Valente *et al.* [56] who reported that retinol inadequacy was significantly higher in adolescents compared with children. However, Hu *et al.* [57] and Fiorentino *et al.* [58] reported that the mean concentration of serum vitamin A was significantly lower in children compared with teenagers. Our interpretation for this difference in serum vitamin A between children and teenagers is that once a child gets to school age, it is observed that mothers can be less concerned of their children's diet [59]. In addition, eating habits in the teenager group are characterized by a predilection for fast food with a high-fat and carbohydrate contents and low nutritional value [60]. So, a proper guideline is required to better describe vitamin A condition between different age groups, and research on this issue is obviously needed.

The present study revealed a significant positive correlation between serum vitamin A and BMI (P=0.035). This result comes in accordance with the outcomes of many other studies. In a study conducted in Sudan, vitamin A taken in diet related to attaining weight and height following the control of confounding factors. Increased vitamin A ingestion in diet was linked to decreased threat of stunting and wasting [61]. In another study carried out by Donnen *et al.* [62] in Zaire, vitamin A supplementation seemed to enhance growth. It was

[Downloaded free from http://www.new.asmr.eg.net on Thursday, January 3, 2019, IP: 62.193.78.199]

found that weight increases were elevated considerably in vitamin A-supplemented group compared with the control group in a period of follow-up of 6 months [62]. Reduction of gaining weight could be one of the first signs of vitamin A insufficiency in human beings [63]. Nutrient deficiencies have been linked to reduce linear growth [64]. Because of the influence of vitamin A on child's growth, attempts to enhance vitamin A condition starting from an earlier age become essential.

Conclusion

The present study revealed a significant correlation between vitamin A and both of CD4 and Tβ4, suggesting that insufficient intake and state of vitamin A decreases the immunity that could dispose to infections and exaggerates malnutrition.

Recommendation

Further studies are required to better describe the compound effect that vitamin A and retinoic acid encompass on immune system regulation and reaction to infections. A useful public health policy could be achieved by periodic vitamin A supplementation to children of at least 6 months living in low-income countries to enhance child survival and to reduce the hazards of nutritional blindness and of morbidity of infectious basis owing to VAD.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Sommer A. Vitamin A deficiency and clinical disease: an historical overview. J Nutr 2008; 138:1835–1839.

2 World Health Organization. Global prevalence of vitamin A deficiency in populations at risk 1995-2005: WHO Global database on vitamin A deficiency. Geneva: World Health Organization; 2009. 2009.

3 Solomons NW. Vitamin A and carotenoids, P. 127–145. In: Bowman BA, Russell RM, editors. Present knowledge in nutrition. Washington, DC: I LSI press; 2001.

4 Sommer A, West KP Jr. Vitamin A deficiency: health, survival, and vision. New York, NY: Oxford University Press; 1996.

5 West KP Jr, Stewart CP, Caballero B, Black RE. Nutrition. In: Merson MH, Black RE, Mills AJ, editors. Global health: diseases, programs, systems, and policies. 3rd ed. Burlington: Jones and Bartlett Learning; 2012. pp. 271–304.

6 Karter DL, Karter AJ, Yarrish R, Patterson C, Kass PH, Nord J, Kislak JW. Vitamin A deficiency in nonvitamin-supplemented patients with AIDS: a cross – sectional study. J Acquir Immune Defic Syndrome Hum Retrovirol 1995; 8:199–203.

7 Bates CJ. Vitamin A. Lancet 1995; 345:31–35.

8 Underwood BA. Vitamin A deficiency disorders: international efforts to control a preventable 'pox'. J Nutr 2004; 134:2315–2365.

9 Wintergerst ES, Magginis S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab 2007; 51:301–323.

10 Semba RD. The vitamin A and mortality paradigm: past, present, and future. Sc and J Nutr 2001; 45:46–50.

11 Brown CC, Noelle RJ. Seeing through the dark: new insights into the immune regulatory functions of vitamin A. Eur J Immunol 2015; 45:1287–1295.

12 Mosmann TR, Coffman RL. Th 1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989; 17:145–173.

13 Cantorna MT, Nashold FE, Hayes CE. Vitamin A deficiency results in a priming environment conducive for Th1 cell development. Eur J Immunol 1995; 25:1673–1679.

14 Ross AC, Chen QYM. Augmentation of antibody responses by retinoic acid and costimulatory molecules. Semin Immunol 2009; 21:42–50.

15 Ma Y, Chen Q, Ross AC. Retinoic acid and polyribionosinic: polyribocytidylicacid stimulate robust anti-tetanus antibody production while differentially regulating type 1/type 2cytokines and lymphocyte populations. J Immunol 2005; 174:7961–7969.

16 Mizuki N, Simon KA, Olivier NK, Robert LT, Phillip MB, Hiroto H. The Thymus: a comprehensive review radio graphics. RadioGraphics 2006; 26:335–348.

17 Huff T, Müller CS, Otto AM, Netzker R, Hannappel E. Beta thymosins, small acidic peptides with multiple functions. Int J Biochem Cell Biol 2001; 33:205–220.

18 Bubb MR. Thymosin beta 4 interactions. Vitami Horm 2003; 66:297–316.

19 Mannherz HG, Hannappel E. The beta-thymosins: intracellular and extracellular activities of a versatile actin binding protein family. Cell Motil Cytoskeleton 2009; 66:839–851.

20 Kaur H, Mutus B. Platelets function and thymosin ß 4. Biol Chem 2012; 393:595–598.

21 WHO. The second decade. Improving adolescent health and development. Geneva: WHO; 2001.

22 World Health Organization. Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes. Micronutrient Series WHO/NUT/96.10. Geneva: WHO; 1996. 66 pp.

23 Sopher A, Shen W, Pietrobelli A. Pediatric body composition methods. In: Heymsfield SB, Lohman TG, Wang ZM, Going SB, editors. Human body composition. Champaign, IL: Human Kinetics; 2005. 129–140.

24 Gopal K. 100 Statistical tests, 3rd ed. ????: SAGE Publications Ltd; 2006.

25 Bresnahan KA, Tanumihardjo SA. Undernutrition, the acute phase response to infection, and its effects on micronutrient status indicators. Adv Nutr 2014; 5:702–711.

26 Imdad A, Mayo-Wilson E, Herzer K, Bhutta ZA. Vitamin A supplementation for preventing morbidity and mortality in children from six months to five years of age. Cochrane Database Syst Rev 2017; CD008524.

27 Villamor E, Fawzi W. Vitamin A supplementation: implications for morbidity and mortality in children. J Infect Dis 2000; 182:S122–S133.

28 Villamor E, Fawzi W. Effects of vitamin A supplementation on immune responses and correlation with clinical outcomes. Clin Microbiol Rev 2005; 18:446–464.

29 Rabiee F, Geissler C. Causes of malnutrition in young children: Gialan Iran. J Trop Pediatr 1990; 36:165–170.

30 Bhutta ZA, Ahmed T, Black R, Cousens S, Dewey K, Giugliani E, *et al.* Maternal and Child Undernturition Study Group. What works? Interventions for maternal and child undernutrition and survival. Lancet 2008; 371:417–440.

31 Ziegler TR, Evans ME, Fernandez-Estivariz C, Jones DP. Trophic and cytoprotective nutrition for intestinal adaptation, mucosal repair, and barrier function. Annu Rev Nutr 2003; 23:229–261.

32 Gray T, Koo JS, Nettesheim P. Regulation of mucous differentiation and mucin gene expression in the tracheobronchial epithelium. Toxicology 2001; 60:35–46.

33 Sirisinha S. The pleiotropic role of vitamin A in regulating mucosal immunity. Asian Pac J Allergy Immunol 2015; 33:71–89.

34 Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song SY. Retinoic acid imprints gut-homing specificity on T cells. Immunity 2004; 21:527–538.

35 Imdad A, Yakoob MY, Sudfeld C, Haider BA, Black RE, Bhutta ZA. Impact of vitamin A supplementation on infant and childhood mortality. BMC Public Health 2011; 11(Suppl 3):S20.

36 Bailey RL, WestJr KP, Black RE. The epidemiology of global micronutrient deficiencies. Ann Nutr Metab 2015; 66(Suppl 2):22–33.

[Downloaded free from http://www.new.asmr.eg.net on Thursday, January 3, 2019, IP: 62.193.78.199]

37 Rosen DS, Al Sharif Z, Bashir M, Al Shabooti A, Pizzarello LD. Vitamin A deficiency and xerophthlmia in Western Yemen. Eur J Clin Nutrn 1996; 50:54–57.

38 Fawzi W, Mbise R, Spiegelman D, Fataki M, Hertzmark E, Ndossi G. Vitamin A supplements and diarrheal and respiratory infections among children in Dar es Salaam, Tanzania. J Pediatr 2000; 137:660–667.

39 Penkert RR, Iverson A, Rosch JW, Hurwitz JL. Prevnar-13 vaccine failure in a mouse model for vitamin A deficiency. Vaccine 2017; 35:6264–6268.

40 World Health Organization. Guideline: vitamin A supplementation in infants and children 6–59 months of age. Geneva, Switzerland: WHO; 2011.

41 Schaible UE, Kaufmann SHE. Malnutrition and infection: complex mechanisms and global impacts. Plos Med 2007; 4:e115.

42 Mannherz HG, Mazur AJ, Jockusch B. Repolymerization of actin from actin: thymosin beta 4 complex induced by diaphanous related formins and gelsolin. Ann N Y Acad Sci 2010; 1194:36–43.

43 Crockford D. Development of thymosin beta 4 for treatment of patients with ischemic heart disease. Ann NY Acad Sci 2007; 1112:385–395.

44 Bodey B, Bodey BJr, Siegel SE, Kaiser HE. The role of the reticulo-epithelial (RE) cell network in the immuno-neuroendocrine regulation of intrathymiclymphopoiesis. Anticancer Res 2000; 20(3A):1871–1888.

45 Mielke LA, Jones SA, Raverdeau M, Higgs R, Stefanska A, Groom JR, *et al.* Retinoic acid expression associates with enhanced IL-22 production by gammadelta T cells and innate lymphoid cells and attenuation of intestinal inflammation. J Exp Med 2013; 210:1117–1124.

46 Thurnham DI, Northrop-Clewes CA, McCullough FS, Das BS, Lunn PG. Innate immunity, gut integrity, and vitamin A in Gambian and Indian infants. J Infect Dis 2000; 182(Suppl 1):S23–S28.

47 Pino-Lagos K, Guo Y, Noelle RJ. Retinoic acid: a key player in immunity. Biofactors 2010; 36:430–436.

48 Engedal N. Immune regulator vitamin A and T cell death. Vitam Horm 2011; 86:153–178.

49 Coutsoudis A, Kiepiela P, Covadia H, Boughton M. Vitamin A supplementation enhances specific IgG antibody levels and total lymphocyte numbers while improving morbidity in measles. Pediatr Infect Dis J 1992; 11:200–209.

50 Semba RD, Ward MBJ, Griffin DE, Scott AL, Natadisastra G, West KP Jr, Sommer A. Abnormal T-cell subset proportions in vitamin A-deficient children. Lancet 1993; 341:5–8.

51 Pino-Lagos K, Guo Y, Brown C, Alexander MP, Elgueta R, Bennett KA, *et al.* A retinoic acid-dependent checkpoint in the development of $CD4^+$ T cell-mediated immunity. J Exp Med 2011; 208:1767–1775.

52 Ross AC. Vitamin A and retinoic acid in T-cell related immunity. Am J Clin Nutr 2012; 96:1166s–1172ss.

53 Knutsen AP, Freeman JJ, Mueller KR, Roodman ST, Bouhasin JD. Thymosin-α 1 stimulates maturation of CD34+ stem cells into CD3+4+ cells in an in vitro thymic epithelia organ coculture model. Int J Immunopharmacol 1999; 21:15–26.

54 Galy AH, Hadden EM, Touraine JL, Hadden JW. Effects of cytokines on human thymic epithelial cells in culture: IL1 induces thymic epithelial cell proliferation and change in morphology. Cell Immunol 1989; 124:13–27.

55 Kouttab NM, Goldstein A, Lu M, Lu L, Campbell B, Maizel AL. Production of human B and T cell growth factors is enhanced by thymic hormones. Immunopharmacology 1988; 16:97–105.

56 de Souza Valente da Silva L, Valeria da Veiga G, Ramalho RA. Association of serum concentrations of retinol and carotenoids with overweight in children and adolescents. Nutrition 2007; 23:392–397.

57 Hu W, Tong S, Oldenburg B, Feng X. Serumvitamin A concentrations and growth in children and adolescents in Gansu Province, China. Asia Pac J Clin Nutr 2001; 10:63–66.

58 Fiorentino M, Bastard G, Sembene M, Fortin S, Traissac P, Landais E, *et al.* Anthropometric and micronutrient status of school – children in an Urban West Africa Setting: a cross-sectional study in Dakar (Senegal). Plos one 2013; 8:e84328.

59 Benkhala A, Bastard G, Broutin C. Rechercher des reponsesviables aux defis de la nutrition des populations vulnerables, synthese de l'etudeexploratoire. Paris: Danone communities; Gret; Ifan; Enda Graf; 2009.

60 Silva CS, da Silva Junior CT, Ferreira BS, da Silva FD, Silva PS, Xavier AR. Prevalence of underweight, overweight, and obesity among 2,162 Brazilian school adolescents. Indian J Endocrinol Metab 2016; 20:228–232.

61 Fawzi W, Herrera MG, Willett WC, Nestel P, el Amin A, Mohamed KA. The effect of vitamin A supplementation on the growth of preschool children in the Sudan. Am J Public Health 1997; 87:1359–1362.

62 Donnen P, Brasseur D, Dramaix M, Vertongen F, Zihindula M, Muhamirizia M, Hennart P. Vitamin A supplementation but not deworming improves growth of malnourished preschool children in Eastern Zaire. Am J Clin Nutr 1998; 128:1320–1327.

63 Lewis CJ, McDowell MA, Sempos CT, Lewis KC, Yetley EA. Relationship between age and serum vitamin A in children aged 4–11 years. Am J Clin Nutr 1990; 52:353–360.

64 Bellamy C. State of the world's children. New York, NY: UNICEF; 1998.