ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Role of Zinc in immune modulation in Egyptian children.

Azza Abdel Shaheed#, Nermine N. Mahfouz#*, Salwa Refat El-Zayat†, Hiba Sibaii†, Sara F. Sallam#, Reham F. Fahmy#, Amany El-Wakkad†

#Department of Child Health, National Research Centre (33rd El Bohouth st, former El Tahrir st, Dokki, Giza, Egypt. PO 12622) [Affiliation ID: 60014618].

† Department of medical physiology, National Research Centre (33rd El Bohouth st, former El Tahrir st, Dokki, Giza, Egypt. PO 12622) [Affiliation ID: 60014618].

ABSTRACT

Nutrition has a great influence on the functions of the immune system. Zinc is one of the essential trace elements, and has a broad impact on key immunity mediators and on the regulation of lymphoid cells. Aim of was to evaluate Zinc status and its impact on immunity. Forty eight Egyptian children were enrolled in our study. Anthropometry and nutritional status were assessed. Zinc level, Thymosin ß4 level and CD4 level were measured. Serum levels of $CD4^+$and Thymosin beta-4 were measured by using a commercial ELISA kit. Serum level of Zinc, serum albumin and total protein were measured by colorimetric method. A highly significant positive correlation was found between the levels of Thymosin ß4 and CD4 and that of Zinc. ***Conclusion:*** Zinc is an essential micronutrient for proper immune system functioning.

Keywords: *CD4, malnutrition, Thymosin* ß4*, Zinc*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Identification of the link between communicable and non communicable diseases is increasing. The dual impact of over- and undernutrition has clarified the bidirectional relation between nutritional status and the development and function of the immune and inflammatory reactions.5

Zinc is an essential micronutrient and its deficiency is a crucial public health problem in low- and middle-income countries. Zinc deficiency has a deleterious effect on growth and has a major contribution in child deaths per year due to infectious diseases.19

There is sufficient data to putting forward for consideration that Zinc deficiency is widely distributed in many low socioeconomic countries. Animal foods that are abundant in Zinc are difficult to obtain in many of the world's poorer population. Diets based on cereals and legumes and low in animal products present an obstacle in fulfillment of the Zinc requirements because they contain high Phytate that lessens the bioavailability of Zinc.20

Approximately 165 million children under five years of age are stunted (height-for-age < -2 SD below the WHO Child Growth Standards median), with the great majority living in Africa and Asia. Zinc is fundamental for cellular growth, cellular differentiation and metabolism and deficiency impairs childhood growth and reduces resistance to infections. Although severe Zinc deficiency is rare in humans, mild to moderate deficiency may be pervading worldwide.28

Zinc deficiency primarily affects epithelial barrier, macrophage, and neutrophil function.13

Zinc deficiency alters the cells of the immune system. It causes a decrease in the count of B lymphocytes and T lymphocytes (CD4+ lymphocytes in particular) through more apoptosis and also lessens their functional capacity. The functions of the macrophage, another key immunological cell that engulfs and destroys bacteria, are also impaired. The production and potency of several cytokines, the central messengers of the immune system, are also compromised by Zinc deficiency.1

So the aim of this study was to find out the influence of nutritional status and Zinc level on immunological statuses as indicated by Thymosin ß4 and CD4 levels

MATERIALS AND METHODS

Our study is a cross sectional one done in the outpatient center of research clinics of excellence at National Research Centre Institute. The study included 48 children of both sexes, within the age range of 2 to 17 years old. By screening for symptoms and signs of malnutrition, our study group was further subdivided into two subgroups. One subgroup consisted of 38 malnourished children; and the second subgroup consisted of 10 children showing no signs of malnutrition.

We excluded any child suffering from chronic disease, infants and adults were out of our scope and we also excluded any child on supplements (vitamins and/or minerals and/or trace elements).

All cases were subjected to the following:

I) Careful history taking and examination including-:

- Demographic characteristics (Age, sex, socio-economic status)
- Anthropometric measurements were taken (weight, height, body mass index BMI calculation) and plotted on Centers of Disease Control and prevention (CDC) growth charts 2000.3
- General examination included vital signs (blood pressure, Radial pulse, respiratory rate and temperature).
- Systematic screening for signs of infections and inquiry about repeated attacks of upper respiratory tract or gastrointestinal infections.

- Cases of malnutrition were depicted through the presence of symptoms and or signs suggestive of vitamins deficiency, protein deficiency and the presence of anemia, hypoproteinemia and hypoalbuminemia in laboratory investigations.
- Screening for symptoms and signs of vitamins deficiency included night blindness, muscle cramps, cheilosis, dermatitis, diarrhea, poor memory, abdominal pain, numbness in the extremities, bleeding tendency, previous bone fracture and disturbed gait

Sample collections:

Five millimeters of blood samples were drawn from the antecubital vein of the participants, CBC was done immediately for all cases, sera were separated by centrifugation and kept frozen at -80^{o}C until laboratory analysis.

II) Laboratory investigations:-

1. Complete blood Count
2. Serum total proteins and serum albumin
3. Serum CD4+ level
4. Serum Thymosin beta-4 (Tβ4)
5. Serum Zinc level

Methodology

Serum levels of CD4+ and Thymosin beta-4 (Tβ4) were measured by using a commercial enzyme linked immunosorbent assay ELISA kit, produced by Glory Science Co., Ltd. 2400 Veterans Blvd. Suite 16-101, DelRio,TX78840,USA.Tel:001-830-734-0090 www.glorybioscience.com. The detection range of CD4+ kit is 0.7 U/ml – 20 U/ml, Catalog Number #:C2328 and the detection range of Tβ4 kit is 180 ng /ml – 2000 ng /ml, Catalog Number #: A0981. Serum level of Zinc was measured by colorimetric method with 5-Brom-PAPS, produced by Salucea Co., Haansberg 19, 4874 NJ Etten Leur, The Netherlands, Tel: +31-76-5032797, Fax: +31-76-5032540, www.Salucea.com. according to the method of Johnsen and Eliasson.11 Serum levels of Albumin and Total protein were measured by colorimetric method produced by Biodiagnostic Co., Tel: 02-33385184, Mobil: 0109-3492077, Fax: 02-33385184 (102), info@bio-diagnostic.com, Catalog Number: AB 10 10 and TP 20 20 respectively according to the method of Doumas et al., and Gornal et al., respectively.6,8

A written informed consent was obtained from the legal guardian of each child before enrollment.

Statistical Analysis:

Data were collected, checked, revised and entered the computer. Data analyzed by SPSS statistical package version 16. Excel computer program was used to tabulate the results, and represent it graphically. Quantitative variables were expressed as mean and standard error. Qualitative variables were expressed as count and percent.

One Way ANOVA used to declare the significant difference between groups at $p<0.05$. Duncan multiple comparison test at $p<0.05$ was used to declare the significant between each two groups. Independent sample student's t test was used to determine the difference in means between the two groups .Pearson correlations were used to analyse the association between zinc with CD4 and thymosin ß4 levels.

Chi square test used to declare the significant difference in the distribution between groups at $p<0.05$.

RESULTS

Our study group included 48 children. Their age ranges from 2 to 17 years old. Twenty eight were males (58%) and twenty were females (42%). We searched for symptoms and signs of malnutrition in all

candidates. Our results revealed a high prevalence of malnutrition among the study group, affecting 38 out of 48 children, representing 79% versus 21%.

The results revealed that there was no statistical significant difference between the age range of well-nourished (8.25±0.91 y) and malnourished subgroups (9.83±0.67 y). Regarding sex distribution 75% of the males were malnourished versus 85% of the females.

Anthropometric measurements were significantly affected in malnourished subgroup compared to well nourished subgroup. Stunting was detected in 14 children out of 48. Three of them were well nourished and eleven were malnourished (P0.001). Two exceptions were found as regards the height of two children who were tall although malnourished as shown in table (1). This is probably due to affection of weight prior to height in malnutrition.

Table (1) height distribution according to CDC growth charts 2000

Height	Well nourished	Malnourished	P value
Tall $> 95^{th}$	Zero	2 (5%)	0.001
Normal 5^{th} to 95^{th}	7 (70%)	25 (66%)	0.001
Stunted $< 5^{th}$ centile	3 (30%)	11 (29%)	0.001

Body mass index (BMI) was calculated by using the following formula: BMI= weight in kg / height in m2

According to BMI, three children (one well nourished + 2 malnourished) were underweight. Thirty one children (7 well nourished +24 malnourished) were of normal BMI. Forteen children (2 well nourished + 12 malnourished) were overweight or obese as shown in table (3). As malnutrition is sometimes associated with obesity due to unhealthy eating habits.

Table (2) Body Mass Index distribution according to CDC Growth charts 2000

BMI	Well nourished	Malnourished	P value
Underweight $<5^{th}$ centile	1(10%)	2(5%)	0.001
Normal 5^{th} to 85^{th}	7 (70%)	24 (63%)	0.001
Overweight/Obese $>85^{th}$	2 (20%)	12 (32%)	0.001

Laboratory investigations showed no statistically significant difference between well nourished and malnourished children regarding complete blood count, serum protein and albumin as shown in table (4).

Table (3) laboratory parameters

Parameters	Mean±SE Well nourished children N=10	Mean±SE Malnourished Children N=38	Significance
Serum Albumin	5.0410±.40584	4.9616±.11205	.791 NS
Serum Protein	4.5900±.19969	4.7550±.10662	.480 NS
Hb	12.2400±.16746	12.3832±.14949	.641 NS
RBC	4.5670±.11738	4.7037±.07460	.391 NS
WBC	7.7600±.27333	7.1184±.32160	.326 NS
Neutrophils	45.4000±1.83908	46.0789±2.53493	.894 NS
Lymphocytes	49.6000±2.42762	44.1579±2.22891	.237 NS
Monocytes	4.9000±.17951	5.5000±.36518	.411 NS
Eosinophils	2.3000±.36667	2.2368±.22451	.895 NS
Basophils	.2000±.13333	.1053±.05045	.431 NS
Platelets	2.9270E2±20.81989	2.9284E2±12.11406	.996 NS

ISSN: 0975-8585

A statistically significant higher value of CD4 levels was found in well nourished children in comparison to the malnourished ones. And so for the Thymosin ß4 level, it was significantly higher in well nourished children whereas the level of Zinc showed no statistically significant difference according to nutritional status of children as shown in table (5)

Table (4) CD4, Thymosin ß4 and Zinc levels

parameters	Mean±SE Well nourished children N=10	Mean±SE Malnourished Children N=38	Significance
CD4 pg/ml	2.3000±.50089	.7295±.07885	0.001
Thymosin ß4 ng/ml	3.2950E2±63.00992	1.4479E2±19.61801	0.001
Zinc µg/dl	3.4210E2±67.81682	2.6658E2±28.43889	0.253 NS

P < 0.001 = very highly significant difference. NS = Non Significant.

Table (5): Correlations between CD4, Thymosin and Zinc

Parameters	Zinc µg/dl
CD4 pg/ml	r=0.463 P=0.001
Thymosin ß4 ng/ml	r=0.459 P=0.001

* Correlation is significant at the 0.05 level (2- tailed).
**Correlation is significant at the 0.01 level (2- tailed).

A highly significant positive correlation was found between Zinc and both CD4 and Thymosin beta-4 as illustrated in diagrams 1 and 2.

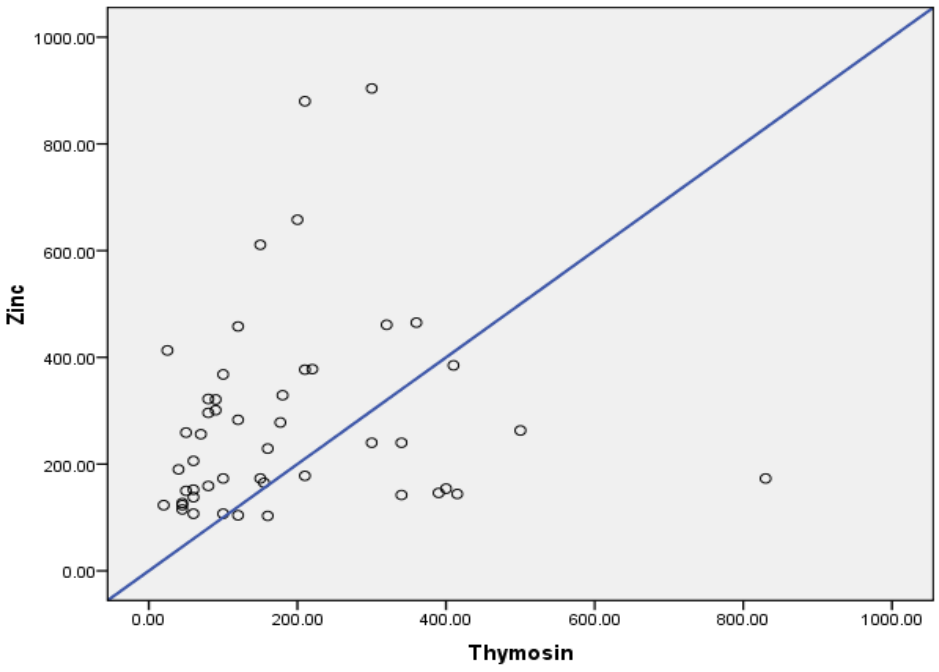

Diagram 1: positive correlation between Zinc and Thymosin β4

ISSN: 0975-8585

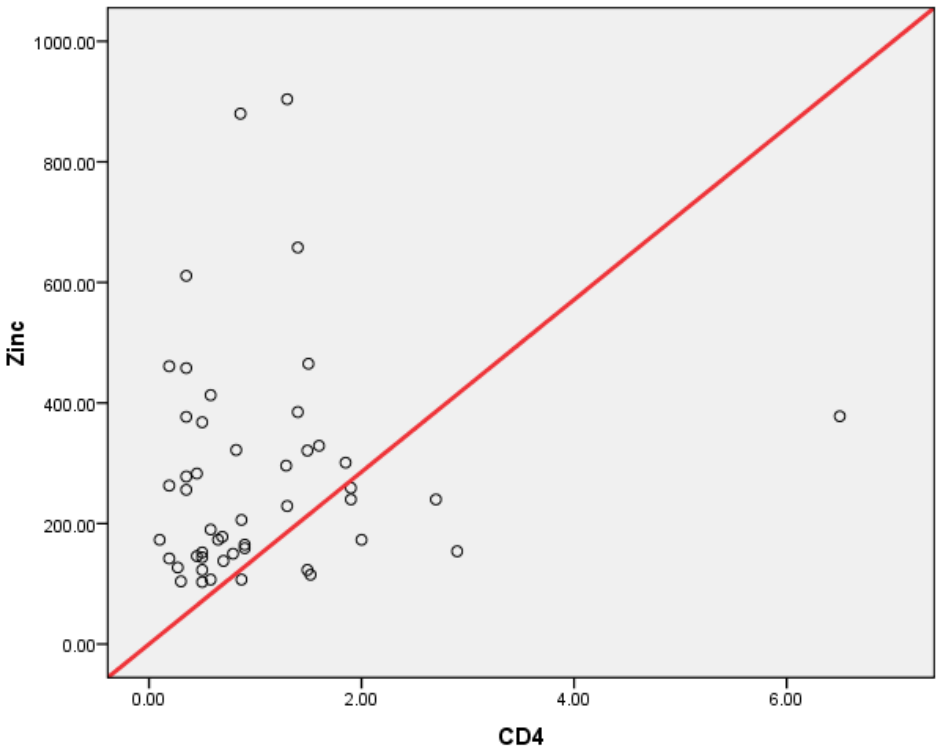

Diagram 2: Positive correlation between Zinc and CD4

DISCUSSION

Diet and nutrition are essential in the promotion and maintenance of healthful life and play a prominent role in prevention measures27

Zinc is fundamental in maintenance of the structural integrity of proteins such as zinc-finger containing transcription factors15

The transcription factors are necessary for leukocyte lineage differentiation (T cell versus B cell differentiation, CD4 versus CD8 lineage differentiation, T regulatory differentiation) and effector function.23

Zinc is crucial for cell-mediated immunity; it is also an efficient antioxidant and anti-inflammatory agent 5.

Zinc is needed for the activation of more than 300 enzymes and more than 1000 transcription factors. In addition, Zinc is a second messenger of immune cells, and intracellular free Zinc in these cells shares in signaling events. The major clinical perturbations of Zinc deficiency include stunted growth; cell-mediated immune dysfunction, and cognitive impairment.2

In this study, the nutritional deficiency of Zinc was assessed in 48 Egyptian children of both sexes with an age ranging from 2 to 17 years old. An evaluation of the nutritional parameters was done and its relation to the level of Zinc was studied. Also the immunity status of the study group was estimated and was correlated with Zinc level.

This age range was our target because they are considered a high risk group for Zinc deficiency. This goes in harmony with the study of King and Cousins who declared that compared to adults; infants; children and adolescents have higher requirements of Zinc and thus, are at a greater risk of Zinc deficiency.12 Also, Maret et al., found that young children have elevated Zinc demands during growth. The highest physiological demands for Zinc are required during adolescence at the time of the pubertal growth spurt, which generally takes place in girls between 10 years and 15 years and in boys between 12 years and 15 years. Even after the

growth spurt has come to an end, adolescents may need an extra Zinc intake to replenish depleted tissue Zinc pools.18

We detected malnutrition in 79% of children which was a real reflection of the Unicef declaration that malnutrition is affecting close to 800 million people, 20% of all people in the developing world.26

Fourteen children were stunted (29%) according to height for age CDC growth charts and 10 (21%) were underweight according to weight for age CDC growth chart. The data obtained by the Egyptian Demographic Health Survey (EDHS) indicated that there is considerable chronic malnutrition among Egyptian children.7

CD4 levels were found significantly higher in well nourished children in comparison to the malnourished ones. This goes in agreement with the study of Chandra who found that Protein-energy malnutrition (PEM) is associated with a significant defect of cell-mediated immunity and cytokine production. Deficiency of single micronutrient also leads to deranged immune response: this is observed even in relatively mild state of deficiency. Of the micronutrients, Zinc, has important impact on immune responses. Overnutrition and obesity also decrease immunity.4 Another study conducted by Daniel et al., confirmed that PEM and vitamin A, Zinc, and Iron deficiencies perform a greatest effect on diminution of CD4+ T cell function. Proliferation is often decreased, and polarization to a particular antigen is disrupted.5

Thymosin β4 level was also significantly higher in well nourished children. Similar to the study of Maren et al., who reported autopsy studies of the thymo-lymphatic system in malnourished children. All detected thymus atrophy in malnourished children, to a severe degree termed “nutritional thymectomy”. Histology revealed depleted thymocytes, substitution with connective tissue, and reduced cortico-medullar differentiation.17

A highly significant positive correlation was noticed between Zinc and CD4 lymphocytes. Hajo et al. explained this positive correlation by the necessity of Zinc as cofactor for Thymulin activation. Thymulin is the hormone secreted by thymic epithelial cells and circulates in the plasma in two forms, a zinc-bound active one, and a zinc-free, inactive form. Thymulin hormone is indispensible for differentiation and function of T cells.9 Another two studies clarified that Zinc finger protein (Zbtb7b) in differentiating thymocytes, both favors CD4-helper and suppresses CD8-cytotoxic differentiation.10, 25 In addition, Lie et al., demonstrated that Zbtb7b is a key CD4-committing factor in the thymus, mandatory in peripheral CD4 T cells to prevent the inappropriate expression of CD8 lineage genes. These findings point to the importance of direct transcription factor control in the maintenance of lineage commitments in the mature T cell compartment.16

A highly significant correlation was also detected between Zinc and Thymosin β4. This is rendered obvious by the evidence that Zinc deficiency leads to thymus involution and Zinc is needed for the activity of thymulin.22

CONCLUSION

Malnutrition was highly encountered in our study group of Egyptian children. Zinc was proved to be an essential micronutrient for proper immune system functioning; although its level is comparable in both well and malnourished groups but it has a great influence on cell-mediated immune response through its positive effects on Thymosin β4 and CD4 levels.

RECOMMENDATIONS

Zinc level should be assessed in all cases of lowered immune status. Correction of Zinc deficiency even its mild form is mandatory for proper cell-mediated immune response. Moreover, knowing that the immune function is an important link between malnutrition, infections and increased mortality, we recommend further studies involving the common factors affecting both nutrition and immunity. To enable the specification of culprits involved in nutritional immunodeficiency. Thus interrupting that threatening vicious circle in developing countries.

ACKNOWLEDGMENTS

We thank all the patients participated in the study and their parents.

REFERENCES

[1] Abi B. What does zinc do? BMJ 2002; 325:1062.
[2] Ananda SP. Discovery of human Zinc Deficiency: Its Impact on Human Health and Disease. Adv. Nutr. 2013; 4: 176–190.
[3] Centers for Disease Control and prevention (CDC), 2000 http://www.cdc.gov/growthcharts/clinical_charts.htm
[4] Chandra RK. Nutrition and the immune system from birth to old age. Eur J Clin Nutr. 2002; 56 Suppl 3:S73-6.
[5] Daniel JR, Fayrouz AS, Catharine R, Simin NM, Harry DD, Charles B S, Bernard JB, Parminder SS, Ben VO, and the INSPIRE Consultative Group. Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence (INSPIRE). J Nutr 2015; 145:1039S–108S.
[6] Doumas B.T. Watson WA, Biggs HG. Clin. Chim. Acta, 31-87. Albumin standards and the measurement of serum albumin with bromcresol greenClin Chim Acta. 1971, 31(1):87-96.
[7] El-Zanaty F. Ministry of Health and Population (Egypt), National Population Council (Egypt) (Cairo), and ORC Macro. http://www.dhsprogram.com/pubs/pdf/FR176/FR176.pdf. Egypt demographic and health survey 2005.
[8] Gornal A.C., Bardawill C.J. and David M.M. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 1949, 177 (2):751-66.
[9] Hajo Haase and Lothar Rink. The immune system and the impact of Zinc during aging. Immun Ageing. 2009; 6: 9.
[10] He X, Dave VP, Zhang Y, Hua X, Nicolas E, Xu W, Roe BA, Kappes DJ. The zinc finger transcription factor Th-POK regulates CD4 versus CD8 T-cell lineage commitment. Nature 2005; 433 (7028):826-33.
[11] Johnson E and Eliasson R. Evaluation of a commerically available kit for the colorimetric determination of zinc. Inter J Andrology 1987, 10(2): 435-440.
[12] King JC, Cousins RJ. Zinc. Modern Nutrition in Health and Disease. 10th edition. Baltimore: Lippincott Williams and Wilkins 2006: 271–85.
[13] Krebs NF. Update on zinc deficiency and excess in clinical pediatric practice. Ann Nutr Metab 2013; 62(Suppl 1):19–29.
[14] Kuczmarski RJ, Ogden C, Grummer-Strawn L, et al. CDC Growth Charts: United States. Advance Data Report No. 314. NCHS, 2000.
[15] Laity JH, Lee BM, Wright PE. Zinc finger proteins: new insights into structural and functional diversity. Curr Opin Struct Biol 2001; 11:39–46.
[16] Lie W, Kathryn F , Wildt KF, Ehydel C, Yumei X, Lionel F, Lino T, and Rémy B. The zinc finger transcription factor Zbtb7b (Thpok, cKrox) represses CD8-lineage gene expression in peripheral CD4 T cells. Immunity 2008; 29(6): 876–887.
[17] Maren J, Heilskov R, Lilian K, André B, Henrik F, and Vibeke BC. The Immune System in Children with Malnutrition—A Systematic Review. PLoS One. 2014; 9(8)
[18] Maret W, Sandstead HH. Zinc requirements and the risks and benefits of zinc supplementation. J Trace Elem Med Biol. 2006; 20:3–18.
[19] Mayo-Wilson E, Junior JA, Imdad A, Dean S, Chan XHS, Chan ES, et al. Zinc supplementation for preventing mortality, morbidity, and growth failure in children aged 6 months to 12 years of age Cochrane Database of Systematic Reviews 2014; Issue 5. Art. No.: CD009384
[20] Nazanin R, Richard H, Roya K, and Rainer S. Zinc and its importance for human health: An integrative review. Journal of Research in Medical Sciences 2013; 18(2): 144–157.
[21] Prasad AS. Effects of zinc deficiency on immune functions.J. Trace Elements Exp. Med. 2002; 13, 1-20.
[22] Maggini S, Wenzlaff S and Hornig D. Essential Role of Vitamin C and Zinc in Child Immunity and Health. The Journal of International Medical Research 2010; 38: 386 – 414.

[23] Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 2004; 22:531–62.

[24] Stanford B and Charles B. Pharmaceutical statistics: practical and clinical applications. 4th ed. Taylor and Francis. 2003.

[25] Sun G, Liu X, Mercado P, Jenkinson SR, Kypriotou M, Feigenbaum L, Galera P, Bosselut R. The zinc finger protein cKrox directs CD4 lineage differentiation during intrathymic T cell positive selection. Nat Immunol. 2005; 6:373–381.

[26] UNICEF: The United Nations Children's Fund. UNICEF's priorities for children 2002-2005. Adapted from UNICEF'S medium-term strategic plan (MTSP) for the period 2002-2005. The full text is available as UNICEF Executive Board document ICEF/2001/13 and Corr. 1, New York: Published by UNICEF, Division of Communication, 3United Nations Plaza, H-9F, NewYork, NY10017, USA. 2002.

[27] World Health Organization (WHO)/Food and Agriculture Organization: *Diet, Nutrition and the Prevention of Chronic Diseases.* WHO Technical Report Series, No. 916. Geneva: World Health Organization, 2003

[28] World Health Organization (WHO), Zinc supplementation and growth in children. E-Library of Evidence for Nutrition Actions (eLENA). 22 July 2015; 18:41