

EFFECT OF ORAL VITAMIN D3 ON THYMIC INDEX IN MALNOURISHED CHILDREN

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ABSTRACT

Objectives: To compare the thymic Index, vitamin D3 level, and T cell receptor excision circle (TREC) of healthy and malnourished infants. To evaluate the effect of oral vitamin D3 supplementation on the Thymic Index and TREC in malnourished children.

Material and Methods: This quasi-experimental study (non-equivalent control group design) included 44 infants within the age range of 2 to 6 months. The cases were 22 malnourished infants, and 22 were healthy infants (controls) based on WHO Z scores classification (weight for length). Weight, age, thymic Index (TI), Vitamin D levels, and TRECs were recorded for cases and controls (pre-test). The cases were given Vitamin D supplementation for 8 weeks. The weight for length (z-score), thymic Index (TI), vitamin D levels, and TRECs were checked in these malnourished children on follow-up (post-test). Thymic Index (TI) was checked by ultrasonography (length multiplied by breadth). ELISA was performed to check Vitamin D levels and RT-PCR was done to compare TRECs.

Results: The malnourished infants had a mean thymic index of 8.015 ± 3.2192 as compared to controls of 11.667 ± 3.2730 . Post Vit D supplementation TI increased (8.459 ± 3.2191) in cases. Weight, length, and Z scores were found to be directly related to TI values. Pre and Post supplementation TI were strongly correlated to each other ($r = 0.985$, $p < 0.001$). The thymic index increased significantly ($p = 0.001$) after intervention. There was a statistically non-significant rise in TRECS (809 vs 4242) ($p = 0.075$) after vitamin D supplementation. The Pearson Correlation showed no significant correlation of TRECS to any of the variables including the Thymic index.

Conclusion: TI (thymic size) and vitamin D levels increase with vitamin D supplementation in malnourished children. However, Vitamin D supplementation has no significant effect on TRECS.

Keywords: Malnourished, Vitamin D3, T cell receptor excision circles, Thymic Index, TRECs.

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INTRODUCTION

Children under 5 with Malnutrition can be underweight (too thin for their age), stunted (too short for their age), wasted (too thin for their weight), or even obese. Worldwide, 149 million children <5 y of age were reported as stunted and 45 million as wasted¹. More than half of the malnourished children live in South Asia; 25.1 million are wasted and 7.7 million are severely wasted.² The mortality

rate in malnourished children is estimated at 3.1 million and morbidity of 45%.^{1,2} A survey in Pakistan reported that an estimated 38% of children under 5 years were underweight.³

Common infections have higher morbidity and mortality in malnourished children. This is due to decreased immune competence in malnutrition.⁴ The 'vicious cycle of infections leading to malnutrition and vice versa'.⁵

Undernutrition during the first 1000 days of life compromises immune development. Thus the primary and secondary lymphoid organs are influenced and so is the response to pathogens.⁵ The thymus is a primary lymphoid organ producing T lymphocytes. Malnutrition reduces the size of the thymus and thus all the physiolog-

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ical processes crucial for T cell development. Ultrasound is used to measure the size of the thymus; expressed as thymic index. Its size increases from birth till 8 months of age and then decreases. The variations in the thymic index can be explained by breast-feeding status, body size, and to a lesser extent by frequency of illness.⁶

T lymphocytes develop and mature in the thymus. Splicing and rearrangement occur due to recombination of the T lymphocyte receptor gene. Circulating T cell receptor excision circles (TREC) are formed by the excision of DNA segments from the T cell receptor (TCR) gene. Thus measuring TREC reflects the maturation process of lymphocytes in the thymus and thus thymic activity.⁷ TREC concentrations are measured by RT-PCR and PCR-ELISA.

Focusing on nutritional rehabilitation by providing macro and micronutrients should improve nutritional status and in turn thymus structure and function to respond effectively to infections.⁸ results in stunting, wasting and/or underweight, three deleterious forms of malnutrition that affect roughly 200 million children under the age of five years. Undernutrition compromises the immune system with the generation of various degrees of immunodeficiency, which in turn, renders undernourished individuals more sensitive to acute infections.

Vitamin D is vital for the growth and development of children. Six out of ten South Asian children are vitamin D deficient. Besides rickets Vitamin D deficiency is reported in various kinds of chronic diseases like diabetes, cardiovascular disease, cancer, tuberculosis, etc.^{9,10}

MATERIAL AND METHODS

This prospective quasi-experiment study with a control group (non-equivalent control group design) was carried out to assess the effectiveness of Vitamin D supplementation in the improvement of TI (Thymic size) and TRECs (Thymic function) in malnourished infants under 6 months.

A purposive sampling technique was used to select malnourished children based on WHO Z scores classification weight for length in infants under the age of six months. This study was conducted from January 2017 to November 2017. A sample size of 44 has been calculated through STAT UBA (<http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html>) by keeping the power of 0.80 and a value of 0.05. (Sample size <10).

A total of 22 malnourished infants admitted to the Nutritional Rehabilitation Unit (NRU) of the Department of

Child Health KTH were included. 22 healthy infants were selected from the immunization center in KTH Peshawar.

The inclusion criteria for cases were a malnourished infant ≤ 6 months of age (z score -2 to -3) admitted in NRU. Infants with HIV, any acute or chronic medical condition (hepatitis B surface antigen or HCV positive, renal and cardiac diseases), and critically ill were excluded. Informed written consent (Urdu/English) was obtained from guardians/parents of eligible subjects. A proforma with two parts was used for all the participants. Part, one had a detailed history and examination including anthropometric measurements (weight, length, z-score,) and contact number/address for follow-up The other part included laboratory investigations such as complete blood count (CBC), Vitamin D levels, TRECs, and TI using ultrasonography. Samples of blood were obtained by a trained phlebotomist from both healthy and malnourished infants included in our study following a strict aseptic technique. Oral vitamin D3 was given to malnourished children for eight weeks (2000IU loading dose and 400IU per day Or 2000/ week for 8 weeks) along with other micronutrients like zinc, magnesium and folic acid (Nutritional rehabilitation protocol).

There is no drug-to-drug interaction of these micronutrients. Dosage schedule was explained to parents/guardian of patients and a proforma (annexure B) was given to parents/guardian to fill or mark it after giving the dose. It was shown to us after two months on follow-up. TI, TRECs & Vitamin D levels of the malnourished children who received Vitamin D3 supplementation were repeated. Laboratory procedures were done in "The Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU) Peshawar," Physiology Laboratory and Pharmacology Laboratory. Informed consent of all the eligible children was taken from the Guardian/Parents, and approved by the ethical committee of KMC and KMU.

RESULTS

This study included 44 children within the age range of 2 to 6 months. 22 children were malnourished (cases) and 22 were healthy (controls). The cases were given Vitamin D supplementation for 8 weeks.

The data was normally distributed (checked by the Shapiro-Wilk test) and presented as mean \pm SD. TRECs before and after intervention were not normally distributed, and were Log transformed for analysis, and presented as geometric means (confidence interval)

An Independent sample T-Test was used to check

the difference between cases and controls as shown in table 1 below.

Paired sample T-Test was used for comparison between pre- and post-intervention vitamin D supplementation. The thymic index was increased significantly ($p=0.001$) after intervention (fig.2).

Vitamin D levels also increased significantly with intervention as shown in fig. 3. TRECS also increased with vitamin D supplementation the p-value is not statistically significant (paired sample t-test comparison = 0.075) as

shown in Fig. 4

Table 2 shows Pearson Correlation of TRECs, and TI with anthropometric and other variables in cases only. The pre-thymic index was significantly co-related to post post-thymic index, weight, length, and Z score. Post Thymic index was significantly co-related to weight, length, and Z score. TRECs before and after intervention were not related to any of the variables.

Table 1: Comparison of anthropometric measures and TI between cases and controls

Group	Cases	Control	P
	Mean ± SD	Mean ± SD	
Age (m)	5.16 ± 1.340	3.69 ± 1.677	0.003
Weight (kg)	4.191 ± 1.1840	5.910 ± .9412	<0.001
Length (cm)	56.909 ± 6.4469	58.429 ± 5.5549	0.413
Pre Thymic Index (cm ³)	8.015 ± 3.2192	11.667 ± 3.2730	0.001
Z Score (SD)	1.50 ± 0.512	3.00 ± 0.000	<0.001
Hemoglobin (gm/dl)	9.723 ± 1.1322	11.557 ± 1.3185	<0.001

Table 2: Correlation table showing the association of TRECs, and TI with other variables in cases

		TRECs Before ***	TRECs After ***	Pre TI	Post TI	Vit D Before	Vit D After	Wt	L	Z Score	Hb
TRECS Before ***	r	1	.333	-.015	-.044	.129	.147	-.014	-.252	-.184	-.490*
TRECS After ***	r		1	.070	.102	.051	-.150	-.007	-.081	-.017	-.318
Pre Thymic Index	r			1	.986**	-.235	-.205	.938**	.793**	-.544**	.065
Post Thymic Index	r				1	-.216	-.267	.947**	.803**	-.536*	.026
Vit D Before	r					1	.342	-.119	-.189	-.110	.098
Vit D After	r						1	-.238	-.190	.236	-.011
Weight	r							1	.811**	-.621**	.089
Length	r								1	-.346	.116
Z Score	r									1	-.078
Hemoglobin	r										1

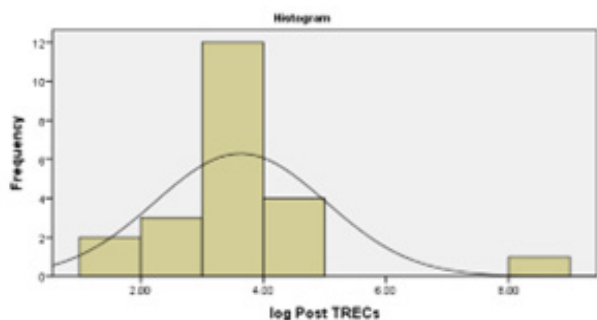


Fig 1: Normality figure Post TRECs after log transformation

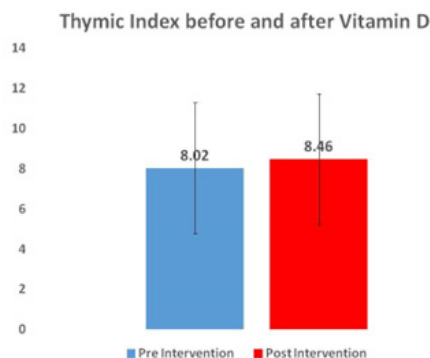


Fig 2: TI before and after Vitamin D in malnourished children

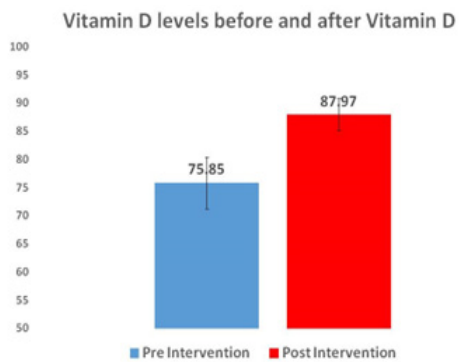


Fig 3: Initial and after intervention Vitamin D levels in malnourished children

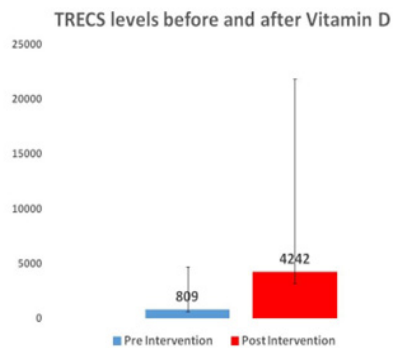


Fig 4: Initial and after intervention TRECs level in malnourished children

DISCUSSION

In this study, the size of the thymus was measured on ultrasound and was found to be directly proportionate to the age, weight, and nutritional status of the infant. The same was reported by many studies.¹¹⁻¹³ Thymus size, assessed as thymus index (TI Prentice AM described the thymus as the “barometer of malnutrition”).¹³ Moore SE measured the thymic index by ultrasound and found it to be affected by both maternal and infant nutrition.¹² Catch-up growths postnatally are associated with an increase in the thymic index.¹² Small thymic size in early infancy as a risk factor for high mortality due to infections was reported in data from Guinea-Bissau, West Africa, Gambia, and Bangladesh. In all these studies interventions to improve nutrition, improved infants’ weight, and thymic size, and reduced infections and mortality.^{12,14-16} prenatal and early postnatal

In our study after vitamin D3 supplementation vitamin D levels, weight and thymic index of malnourished infants improved. Siddiquee et al. reported a systematic review and meta-analysis of Vitamin D deficiency among South Asian children. He found that Vitamin D is vital for

the growth and development of children.⁹ DeLuca et al. reported that Vitamin D has a significant effect on the immune system and therefore can be used for the treatment of a variety of diseases.¹⁷ Saleem et al. conducted a study in Pakistan on uncomplicated severely malnourished children. He reported that a High dose of vitamin D3 improved the “mean weight for height (z-score)” for severely malnourished children in Pakistan comparable to our study.¹⁰

In our study pre-intervention TRECs were low. Wilson Savino et al also reported low T-lymphocyte count in malnutrition. He explained thymic atrophy in undernutrition and severe infection leads to Small thymic size and in turn thymocyte reduction.⁹ results in stunting, wasting and/or underweight, three deleterious forms of malnutrition that affect roughly 200 million children under the age of five years. Undernutrition compromises the immune system with the generation of various degrees of immunodeficiency, which in turn, renders undernourished individuals more sensitive to acute infections. The severity of various infectious diseases including visceral leishmaniasis (VL) The impaired T cell development, has a detrimental effect on the adaptive immune system. Similarly, David Grenadier et al. reported that thymic function is extremely sensitive to deleterious acute insults, such as infection or malnutrition. These stresses lead to thymic involution but thymus also has the capability of remarkable repair and rejuvenation. He used a TREC assay to assess the exact number of lymphocytes produced by the thymus. TRECs strongly correlate with thymic function in backgrounds of age and conditioning.¹⁸ Similarly Raqib et al reported that low birth weight affects immunocompetence and increases vulnerability to infectious diseases. They further explained that low T cell receptor excision circles (TRECs) turnover from the thymus adversely affects the immune functional reserve in preschool-age children born with LBW.¹⁸

In our study, there was a statistically insignificant increase in TRECs when Vitamin D was supplemented in malnourished infants. Similarly mixed results from intervention trials in <6 months of age have been reported.^{19,20} Adrian F. Gombar et al reported that a combination of Vitamin D, C, and Zinc supplementation enhances immune function and decreases the risk of infection. These supplements in higher doses have the potential to reduce the global burden of infection.²¹ integrated immune system needs multiple specific micronutrients, including vitamins A, D, C, E, B6, and B12, folate, zinc, iron, copper, and selenium, which play vital, often synergistic roles at every stage of the immune response. Adequate amounts are

essential to ensure the proper function of physical barriers and immune cells; however, daily micronutrient intakes necessary to support immune function may be higher than current recommended dietary allowances. Certain populations have inadequate dietary micronutrient intakes, and situations with increased requirements (e.g., infection, stress, and pollution Dietary interventions; a combination of macronutrients, micronutrients, and probiotics can recover the structural and functional damage in the thymus and rescue proper immune responses to infection.⁸

This study had a small sample size, a proper standard was not available for TRECs assay to compare our result so we used an internal marker in our study which may also affect our TRECs results.

CONCLUSION

In malnourished children vitamin D supplementation increases vitamin D levels and Thymus size (thymic index) and has an insignificant effect on TRECs.

There is a need for future comparative studies including healthy as well as malnourished children with an increased number of participants to contribute to the findings of the present study.

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Authors Contribution:

Following authors have made substantial contributions to the manuscript as under

Authors	Conceived & designed the analysis	Collected the data	Contributed data or analysis tools	Performed the analysis	Wrote the paper	Other contribution
Bashir H	✓	✗	✓	✗	✓	✗
Malik MO	✓	✓	✗	✓	✓	✗
Amir S	✓	✗	✗	✓	✗	✗
Ahmad H	✓	✗	✓	✗	✓	✗
Rehman SKU	✗	✓	✓	✗	✗	✓

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethical Approval:

This Manuscript was approved by the Ethical Review Board of Khyber Medical College, Peshawar Vide No. 94/DME/KMC.

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