



ISSN NO. 2320-5407

Journal Homepage: -[www.journalijar.com](http://www.journalijar.com)

## INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/7796  
DOI URL: <http://dx.doi.org/10.21474/IJAR01/7796>



INTERNATIONAL JOURNAL OF  
ADVANCED RESEARCH (IJAR)  
ISSN 2320-5407  
Journal Homepage: <http://www.journalijar.com>  
Journal DOI:10.21474/IJAR01

### RESEARCH ARTICLE

#### STATUS OF VITAMIN A, D, E IN CHILDREN OF JIAMUSI AREA; A CROSS SECTIONAL STUDY.

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#### Manuscript Info

##### Manuscript History

Received: 2 August 2018

Final Accepted: 4 September 2018

Published: October 2018

##### Keywords:

Vitamin A, Vitamin D, Vitamin E, Deficiency.

#### Abstract

**Background:** Vitamin A, D and E are very essential to normal growth, development and health of children. Deficiencies are being global problem. The objective of this study is to find out the status of serum vitamin A, D, E level in Children below 14 years in Jiamusi city area (latitude 46°48' N longitude 130°19'E) of Heilongjiang province in northeast of China.

**Methods:** Children from birth to 14 years of age who came for normal health check up in First affiliated hospital of Jiamusi University were used to collect blood samples for vitamin A, D, E. Vitamin A and E were measured by High Performance Liquid Chromatography (HPLC) and vitamin D was measured by liquid chromatography-tandem mass spectrometry from sigma company (USA).

**Results:** A total of 3365 children were participated in a year time. These samples were categorized according to age, gender and season groups. The mean levels of serum vitamin A, vitamin D and vitamin E was 0.06mg/L, 24.24ng/ml and 8.56mg/L respectively. There was vitamin A deficiency (VAD) (n=1658, 57.6%), more in 6-14 year age group (n=708, 24.6%) and more in summer season (n=552, 19.2%). Similarly vitamin D deficiency (VDD) (n=1203, 36.9%) more in 6-14 years age group (n=756, 23.2%) and in winter season (n=453, 13.9%) was found. There was significant differences on gender, age group and season for analysis of VAD,  $p < 0.05$ . Vitamin E deficiency (VED) was significantly more frequent in autumn samples (OR=3.34, 95% CI 1.20, 9.28;  $p = 0.01$ ). The study showed no significant differences with age group and gender for vitamin E levels.

**Conclusion:** The prevalence of VAD, VDD was more in Jiamusi city area children with significant difference of vitamin E in autumn season. Vitamin deficiencies are more with increasing age group suggesting the timely adjustment of vitamin and need of supplementation to elder children to have a healthy youth in future.

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## **Introduction:-**

### **Brief points**

1. There are researches showing high prevalence of vitamin D deficiency in northeast china but Jiamusi area has less number of data in research so this will be helping to add one more research and find real scenario of the vitamin levels.
2. There are researches done about vitamins A, D, E in children with different diseases and their relation. This study is aimed to study in normal healthy children and find the status. This will help to adjust the vitamin supplement to the children.
3. Very less number of researches have been done to find the seasonal aspect of vitamins in children. This study will find out the relation of vitamins in different seasons and try to help to find the adjustment of vitamin supplement to the children.

### **Background**

Vitamins are essential chemically unrelated to the organic compounds which cannot be synthesized in adequate amount in human body. These are essential for normal growth and development. Vitamin A, D, E are fat soluble vitamin as they are released, absorbed and transported with help of dietary fat.

Vitamin A deficiency (VAD) is a major nutritional problem found in developing, economically poor and illiterate society. The less supply, poor feeding habit, periodic illness and infections are main cause leading to deficiencies of vitamins for the children. This deficiency will fail to meet the physiologic need of our body. This deficiency will impair normal growth and development, normal metabolism, development of immune system and causes many disorders related to vitamin A deficiency like xerophthalmia, anemia, and chances of getting chronic illness [1].

The deficiency of vitamin D (VDD) is more common throughout the pediatric age group which present as signs of rickets including tremor, carpal pedal spasm, failure to thrive, frontal bossing, rachitic rosary, large anterior fontanel, craniootabias, Harrison's groove, widened wrists and ankle, bowing of leg. It is also supposed to be associated in autoimmune disease, cancer, endocrinal diseases, high blood pressure and infectious disease [2]. They may also have the chances of having hyper-reflex, developmental delay, delayed dentition and increased chances of having decayed teeth. Also children may present with multiple fractures and bone fragility. Severe deficiency of vitamin D can present with seizures and tetany from profound hypocalcaemia. Older children may present with short stature and loss of appetite in VDD [3].

Vitamin E is the other essential fat soluble vitamin which was not much known vitamin needed to our body in past. This vitamin is having a great role in providing immune function, preventing inflammation, regulation of gene expression and cognitive performance [4]. It is a potent antioxidant. Vitamin E deficiency (VED) is common in population who don't consume the food rich in vitamin E like almonds, germinating seeds, mustard green, spinach, plants oil, avocado, papaya, and broccoli. VED is associated with motor and sensory neuropathy, intestinal fat malabsorption which includes bowel disease, pancreatic disease, chronic cholestasis, celiac disease, cystic fibrosis, short bowel syndrome, intestinal lymphangiectasis where as excess of vitamin E may be associated with heart failure, coagulopathy, impaired immunity. But it is said that it's difficult to achieve the vitamin E toxicity [5].

Deficiency of any one of these micronutrients is now becoming a global problem. More over this is mostly common in the population of low socioeconomic status, undeveloped parts of world and place having different climatic state and cultural belief. Heilongjiang province lies in northeast part of china which is having less sunshine, heavy snow fall, low socioeconomic status, backward, remote and less developed than other parts of China. Some of study has shown high prevalence of Vitamin D deficiency. At present there lacks large samples data of vitamin A, D, E in eastern Heilongjiang. This study is aimed to see the prevalence of vitamin A, D and E in Jiamusi and differences with age, gender and season. It plays a guiding role for children's health in Jiamusi area.

## **Method and Materials:-**

### **Study participants**

This study was done in first affiliated hospital of Jiamusi University which lies in north east of China. The children from the nearby cities and villages of Jiamusi city were included. There were 3365 children who participated in the analysis of vitamin A, D, E who came for regular health check up from September 1, 2016 to August 30, 2017.

Children with known diseases of rickets, calcium metabolism, renal and liver diseases were excluded. Ethics approval was obtained from the first affiliated hospital of Jiamusi University.

### Methods

The study was done to find the levels of vitamins. We categorized the samples taken according to age where children were divided into four sub classes; 0~1year, 1~<3years, 3~<6years, 6~14years of age, according to the season into four subgroups; autumn (September to end of November), winter (December to end of February), spring (March to end of May) and summer (June to the end of August), according to gender into two subgroups; male and female child. Those children who came for normal health check up were used for our study. The children who did not follow the inclusion criteria were not used in this study.

### Blood samples and biochemical measurements

Samples were collected from the children visiting first affiliated hospital of Jiamusi University. 2 ml of venous blood was collected for the test and collected in the test tube. The blood was collected in the heparin added test tube and kept away from the light. The test tube was marked with the name of the children and the special code and stored in ice bag. The levels of vitamin were measured by reverse phase high performance liquid chromatography (FT-HPLC) and liquid chromatography-tandem mass spectrometry from sigma company (USA).

### Statistical Methods

Statistical computations were performed using SPSS for windows statistical software package version 23 (SPSS Inc., Chicago, IL, USA). Mean serum levels of vitamin A, D, E were compared between groups by using the t test or analysis of variance where appropriate. Difference between proportions was elevated by Pearson's  $X^2$  test.

### Results:-

#### General characteristics

The study was done in First affiliated hospital of Jiamusi University (JUH) from September 2016 to end of August, 2017. The frequency of distribution of vitamin A, D, E is shown in the figure no. 1. The total children (0 to 14 years of old) were 3365. The mean serum vitamin A was 0.06mg/L (ranging from 0.06-0.60), serum vitamin D was 24.24ng/ml (ranging from 1.90-99.90) and serum vitamin E was 8.56mg/L (ranging from 2.00-22.10). There were n=1999(59.40%) and n=1366(40.60%) female children. There were n=755(22.44%) in spring, n=1266(37.62%) in summer, n=610(18.13%) in autumn, n=734(21.81%) in winter. Among these, there were n=307(9.10%) children 0~<1 year of age, n=651(19.3%) children 1~<3 year of age, n= 899(26.70%) children 3~<6 of age, 1508(44.80%) children 6~14 years of age in Jiamusi City area. See Table No.1 and Table no.2 for detail general information and the status of vitamin levels in different groups.

#### Serum vitamin A levels and prevalence of low vitamin A status

There were 2876 cases (male 1701 and female 1175) for vitamin A sampled in Jiamusi city area. There was prevalence of vitamin A deficiency (n=1658, 57.6%) with Male (n=1004, 34.9%) and female (n=654, 22.7%). The study showed that there was no significant difference in male and female children being  $p>0.05$ . There was more prevalence of vitamin A deficiency with increasing of age; maximum deficiency was found in 6-14 years child (n=708, 24.6%) least deficiency was found in 0-1 year (n=180, 6.3%). There was more prevalence of vitamin A deficiency in summer season (n=552, 19.2%). The study showed that difference according to Age group and season being  $p<0.05$ . This result was made with the cutoff limit of 0.3mg/L for vitamin A deficiency.

#### Serum vitamin D and prevalence of low vitamin D status

There were 3258 children (male 1932 and female 1326) for vitamin D sampled. There was prevalence of vitamin D deficiency (n=1203, 36.9%) with male children (n=683, 21%) and female children (n=520, 16%). The study showed significant differences on gender, age group and season with  $p<0.05$ . Prevalence of vitamin D increased with increase of age. Children of 0-1 year age group were having least prevalence of vitamin D deficiency (n=37, 1.1%) whereas more prevalence of vitamin D deficiency (n=756, 23.2%) in 6-14 years age group. Most vitamin D deficiency was found in winter (n=453, 13.9%) whereas least in summer (n=156, 4.8%). This was done with cutoff limit of 20ng/ml for vitamin D deficiency. In logistic regression, Vitamin D deficiency was more prevalent in female (OR=1.31, 95% CI 1.10,1.56;  $p=0.00$ ), 6-14 years age group children and winter season (OR=16.66, 95% CI 12.96, 21.41;  $p=0.00$ ).

**Serum vitamin E levels and prevalence of low vitamin E status**

There was 1785 children (male 1052 and female 733) for vitamin E analysis. It was found that the prevalence of vitamin E deficiency (n=30, 1.7%) with male children (n=21, 1.2%) and female children (n=9, 0.5%). There was more vitamin E deficiency in autumn season (n=16, 0.9%) whereas less in spring season (n=3, 0.2%). This study showed that it was significant with season variation being  $p < 0.05$ . The study showed no significant differences with age group and gender. In logistic regression, Vitamin E deficiency was significantly more frequent in autumn samples (OR=3.34, 95% CI 1.20, 9.28;  $p = 0.01$ ). (See Table no. 3)

**Table no.1:-**Overall information of samples of vitamin A, D, E in Jiamusi City area

Variable	Vitamin A					Vitamin D					Vitamin E				
	n	%	mean	min	max	n	%	mean	min	max	n	%	mean	min	max
Gender	2876		0.29	0.06	0.60	3258		24.42	1.90	99.90	1785		8.56	2.00	22.10
Male	1701	59.1	0.29	0.06	0.60	1932	59.3	23.08	1.90	71.40	1052	58.9	8.37	3.20	22.10
Female	1175	40.9	0.29	0.07	0.58	1326	40.7	22.54	3.40	99.90	733	41.1	8.75	2.00	21.80
Age group															
0-<1 y	243	8.4	0.26	0.09	0.51	304	9.3	31.25	4.40	57.20	118	6.6	10.54	3.20	22.10
1-<3 y	506	17.6	0.28	0.09	0.57	635	19.5	29.37	1.90	99.90	289	16.2	9.61	4.50	19.30
3-<6 y	783	27.2	0.28	0.06	0.60	874	26.8	22.93	3.50	76.40	505	28.3	8.32	2.00	18.00
6- 14 y	1344	46.7	0.30	0.06	0.58	1445	44.4	19.51	3.40	71.40	873	48.9	8.02	3.90	21.80
Season															
Autumn	572	19.9	0.27	0.06	0.52	593	18.2	23.97	1.90	99.90	461	25.8	8.30	2.00	21.80
Winter	663	23.1	0.29	0.07	0.57	704	21.6	17.78	3.40	57.20	418	23.4	8.57	3.50	18.60
Spring	616	21.4	0.29	0.06	0.53	730	22.4	19.66	4.20	68.90	402	22.5	8.54	3.20	22.10
Summer	1025	35.6	0.37	0.09	0.60	1231	37.8	28.50	3.60	71.40	504	28.2	8.68	4.00	20.70
Age(year)			5.61	0.08	14			5.18	0.08	14			5.80	0.08	14

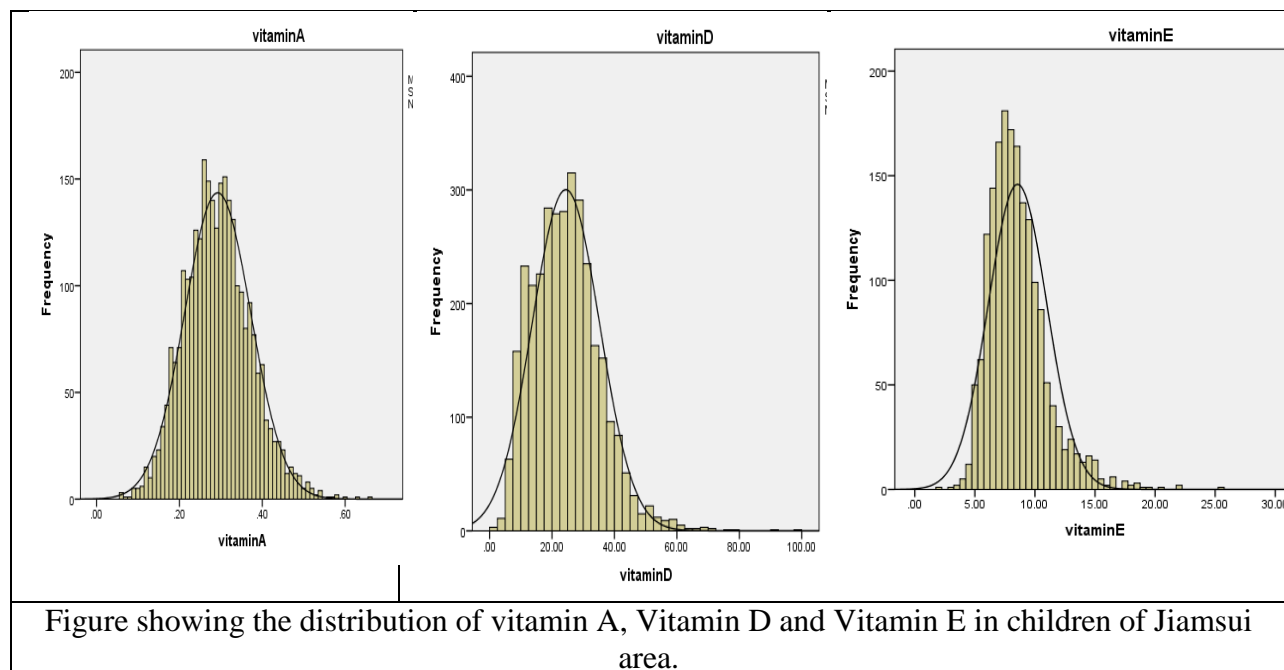
**Table no. 2:-**Status of vitamins in children 0-14 years of children in Jiamusi City area

Status	vitamin A			Vitamin D			Vitamin E		
	Definition	cases	%	Definition	cases	%	Definition	cases	%
Severe Deficiency	<0.1mg/L	10	0.3	<5ng/ml	14	0.4	N/A		
Deficiency	0.1-<0.2mg/L	292	10.2	5-<15ng/ml	670	20.6	<5mg/L	30	1.7
Insufficiency	0.2-<0.3mg/L	1356	47.1	15-<20ng/L	519	15.9	N/A		
Sufficiency	>=0.3mg/L	1218	42.4	20-100ng/L	2055	63.1	5-20mg/L	1751	98.1
Excess	>1.3mg/L			>100ng/L			>20mg/L	4	0.2
Total		2876	100.0		3258	100.0		1785	100.0

**Table no. 3:-Prevalence and unadjusted OR with 95%CI for vitamin A, D, E deficiencies in Jiamusi city area**

Variable	Vitamin A deficiency					Vitamin D deficiency					Vitamin E deficiency				
	n	%	OR	95% CI	p	n	%	OR	95% CI	p	n	%	OR	95% CI	p
Gender	1658	57.6			0.06	1203	36.9			0.00	30	1.7			0.21
Male	1004	34.9	0*			683	21.0	0*			21	1.2	0*		
Female	654	22.7	0.87	0.74, 1.01		520	16.0	1.31	1.10, 1.56		9	0.5	0.61	0.27, 1.35	
Age group					0.00					0.00					0.28
0-<1 y	180	6.3	2.51	1.84, 3.42		37	1.1	0.09	0.06, 0.13		3	0.2	1.82	0.48, 6.87	
1-<3 y	296	10.3	1.24	1.00, 1.52		102	3.1	0.11	0.09, 0.14		4	0.2	1.08	0.33, 3.51	
3-<6 y	474	16.5	1.34	1.12, 1.61		308	9.5	0.36	0.30, 0.44		13	0.7	2.16	0.93, 5.01	
6-14 y	708	24.6	0*			756	23.2	0*			10	0.6	0*		
Season					0.00					0.00					0.01
Autumn	382	13.3	1.68	1.35, 2.08		235	7.2	6.21	4.81, 8.01		16	0.9	3.34	1.20, 9.28	
Winter	366	12.7	1.09	0.90, 1.33		453	13.9	16.66	12.96, 21.41		6	0.3	1.44	0.43, 4.78	
Spring	358	12.4	1.15	0.94, 1.42		359	11.0	9.91	7.76, 12.66		3	0.2	0.7	0.16, 2.97	
Summer	552	19.2	0*			156	4.8	0*			5	0.3	0*		

Comparison was made by Pearson's  $\chi^2$  test; OR (Odds Ratio) with 95% CI (Confidence Interval) was calculated by logistic regression; \* is set to 0 because it is redundant.



### Discussion:-

Micronutrients play a crucial role in the health and well-being of any individual. These have even more special importance in children as they are growing and developing. The need for vitamin is different for different age groups, different for different gender. Our study showed the prevalence of vitamin A deficiency of 57.6% showing more than half children deficient in vitamin A. This was coinciding with the study done by Chun yang et al [6] where it was found that most of part of China was having more prevalence of vitamin A deficiency. This could be due to the changing pattern of feeding habit. There is an increasing trend of consumption of junk foods among children. There have been vast changes in the food and beverage. This diversity of food habit was also explained by the study done by Wang X et al [7] where he explained about the changing trend of feeding habit among Chinese children. This could explain the same result we had in our study. Western world children are also having similar changes in vitamin level which was explained in a study done by Mensrik et al [8] in children of UK, Poland and some western countries. They also have such a change of feeding habit. Generally, boys are more active in their growing phase. They are more sports lovers and enjoy outdoor activities. This makes the boys need more micronutrients. One of the studies showed that the boys would need more vitamin A than girls as growth rates are higher before 10 years of age [9], which was coinciding with the result shown in male children of our study. Our study revealed that vitamin A deficiency is more common in younger children. The children from transition of breast feeding to other food had shown this pattern of vitamin A deficiency and the level is not fulfilled as children grow rapidly [10] which was coinciding with the meta-analysis done by Peige Song et al [11] where prevalence of VAD in younger children was seen. This could be due to the fatty food pattern and lowering activities among children in present days.

Vitamin D intake in American children was below 21% as done by Mansbach et al [12], whereas the study done by Wang et al [13] in Wenzhou in China showed the prevalence of 38%. The study said that there was supply of vitamin D due to sea fish which have a good amount of vitamin D. Our study showed 36.9% of vitamin D deficiency. This may be due to lack of fresh food in northern parts and lack of sea food throughout the year. Jiamsui has less fresh food. Vegetables and poultry are imported from the south part. Wang LL study also showed the similar scenario of vitamin D deficiency. This study reflects the prevalence of vitamin D all over China. We had a significant difference between male and female children which coincides with the study done by Saintonge et al [14]. This could be due to less outdoor activities of female than boys. Chances of osteoporosis in females are more which was shown in one of the studies done in Beijing coinciding with higher prevalence of VDD in females [15]. Our study found the difference in season was significant with higher prevalence in winter and lesser prevalence of VDD in summer. Such a pattern of variation was shown in the study done in Shanxi province by Strand MA et al [16]. Probably due to low sunlight, wearing of more clothes due to longer winter and less outdoor exposure could explain the low levels of vitamin D in

northern part in winter season. More explanation matches with the result where vitamin D deficiency seen in summer is low and more vitamin D deficiency was prevalence in winter as extreme cold and longer snow covering remained for longer winter. Study done by Zhu et al [17] had shown that there was increasing vitamin D deficiency with increase of age. Study done by Schleicher et al [18] had shown that vitamin D was more common in 6-17 year children group than 1-11 years age group in US. Similarly study done by Sarafin k et al [19] in Canada had also shown more prevalence of vitamin D in 14-18 years of age group. Our study coincided with these two studies done out of China as we had similar older age group of children having more vitamin D deficiency. It may be due to more school work for children so that they won't get more time for physical activities. Older children generally take more junk foods which were relatively nutrients deficient. Infants were having high level of vitamin D. This may be due to supplementation of formula feed.

Vitamin E is an antioxidant which is very essential to human body. There was no clear cut trend of logics regarding the severity of vitamin E deficiency on basis of region, longitude, latitude and climate, prevalence was mostly affected by culture, life style of individual, nutritional pattern of children and presence of any infection in children during all time of sampling. When there was increased state of oxidative stress and lesser supply of antioxidants then patients showed bad prognosis [20, 21]. Our study did not show the significance differences with age group and gender. Study done by Khatib IM et al [22] showed the prevalence of 17.1% of VED when cut off was 5mg/L and 89.2% of VED when cut off was 7.5mg/L in Jordan children. Farrell PM et al [23] showed Low vitamin E level in children ranging from 3-9 mg/L. Males supposed to have more vitamin E deficiency than females mostly due to PUFAs intake, alcoholic pattern and smoking pattern more in male. Females had higher level of tocopherol than males which was shown by the study done by Kang MJ et al [24]. The more the breast feeding, the lesser are the chances of having vitamin E deficiency. This was explained by study of Sinvichayakul et al [25] which showed that duration of breast feeding was having positive correlation with level of vitamin E. Study done by Winklhofer-Roob BM et al [26] showed that vitamin E had influence by age, sex and season of sampling. Season of sampling affected alpha tocopherol high in winter and gamma tocopherol higher in winter and spring than other season. Study done by Fares S et al [27] had shown that one fourth of children were having low vitamin E levels. Our study showed significance with season. It couldn't be explained why real significance exists in autumn season. Some researches done in animal has shown that there is decline of vitamin A and E in cow milk from autumn to winter [28]. One of the researches done in sheep has shown the prevalence of vitamin E was more in autumn due to deficiency of grass. These two can explain that the intake of this deficient food lead to vitamin E deficiency.

## Conclusions:-

The prevalence of vitamin A deficiency and vitamin D deficiency is high in children of Jiamusi city area. The prevalence increases as child grow up. Vitamin D deficient more in winter whereas vitamin A deficient more in summer. Vitamin E was more deficient in autumn season. Supplement of vitamin A, D to elder children would reduce morbidity and mortality.

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