European Journal of Molecular & Clinical Medicine

ISSN2515-8260 Volume 09, Issue 01, 2022

Study of subclinical Vitamin A deficiency in suspected group of children by conjunctival impression cytology at a tertiary care centre in South India

[1]Dr. Vasanth Kumar D. L, [2]Dr.Navya, [3]Dr. Bheemaraya Shivasharana, [4]Dr. Ravindra B Patil

[1,3]Assistant Professor, Department of Paediatrics, SIMS, Shivamogga, Karnataka, India
[2]Senior Resident, Department of Paediatrics, SIMS, Shivamogga, Karnataka, India
[4]Professor& Head, Department of Paediatrics, SIMS, Shivamogga, Karnataka, India

Corresponding Author: Dr. BheemarayaShivasharana (bs43ghawanalli@gmail.com)

Abstract

Introduction: Vitamin A is required throughout the lifecycle.Vit A deficiency and Xerophthalmia occur throughout much of the developing world and are linked to under-nourishment and complicated by illness. In developing countries, it is estimated that 5 lakh preschool children become blind every year owing to Vitamin A Deficiency and many of them will die because of increased vulnerability to infections, especially measles, diarrhea & pneumonia. In southern districts of Karnataka, including Shivamogga, the burden of malnutrition, respiratory infection, diarrhea, measles and post-measles infections are high leading to significant morbidity and mortality. Majority of the studies related with Vitamin A deficiency are mostly concerned with clinical signs and symptoms of vitamin A deficiency. However subclinical Vitamin A deficiency is often missed. The present study is aimed to detect subclinical Vitamin A deficiency in children at risk, so that supplementation of Vitamin A in early stage may bring down the burden of preventable morbidity and mortality.

Objectives: The present study is undertaken to study subclinical Vitamin A deficiency at SIMS, Shivamogga, to know the–

1. Relative prevalence of subclinical Vitamin A deficiency in predisposed conditions.
2. Prevalence of abnormal Conjunctival impression cytology in predisposed conditions.

Methods: This cross-sectional study was conducted in tertiary care hospital from January 2020 to December 2020. Children of 1 to 12 years of age with under nutrition, recurrent respiratory infections, recurrent diarrhea, recurrent UTI, measles and post measles infections, admitted in SIMS Shimoga were included in the study. A detailed clinical evaluation (history & examination) and relevant laboratory investigations with CIC was done for all cases as per proforma. The obtained data was entered in Microsoft-excel and analysed with SPSS software for Prevalence, Proportion, Chi square test, Trend analysis.

Results: Out of 250 children included in the study, 108(43.2%) were in the age group of 4 to 6 years. Total no of male children were 140(56%) and that of female were 110 (44%). Majority of the children were from rural area (59.2%). urban slum (27.2%), urban (13.6%). Majority of the cases were from lower SES. 61.6% from class 3, 22% from class 2 and 16.4% from class 4 of modified B.G. Prasad classification. Among the cases examined, majority belonged to severe grades of PEM (43.2%). Recurrent LRTI (19.2%), recurrent diarrhea (20%), recurrent UTI (12.8%), measles and post measles bronchopneumonia (4.8%). Majority of the cases had CIC grading of 3 & 2, with 42% grade 3, 39.2% grade 2, 10.8% grade 1 and 8% grade 0.

All age groups were equally affected and all of them had subclinical VAD with average of 81.2%. Both male and female children, almost equally had subclinical VAD. Male 82.1% & female 80%.There was no difference noticed in subclinical VAD with respect to locality of the children. Rural children were affected 83.1%, urban slum 80.9% and Urban 78.6%. With respect to SES, 90.2% of children with class 4, 78.6% of class 3 & 81.8% of class 2 had subclinical VAD. There was no difference in children having subclinical VAD with respect to SES.

PEM was the most common presentation in suspected group with total of 108 cases. Among them, 83.3% had subclinical VAD. Among the other predisposed groups, children with recurrent diarrhea 88%, recurrent LRTI 82.3%, recurrent UTI 65.5%, measles 100% and post measles bronchopneumonia 62.6%, had subclinical VAD. The occurrence of subclinical VAD was significantly associated with severe grades of PEM, recurrent LRTI, diarrhea, UTI, Measles & post Measles bronchopneumonia.

Conclusion: Subclinical VAD was largely prevalent in suspected group of children, with severe grades of PEM, recurrent diarrhea, LRTI, UTI, Measles & post measles bronchopneumonia. Both male & female children were equally affected. Predisposing conditions were more prevalent in lower socio-economic status. All classes were affected equally in a given disease condition.

Keywords: Subclinical VAD, recurrent LRTI, diarrhea, UTI, Measles, post Measles bronchopneumonia

Introduction

Vitamin A is required throughout the lifecycle. Its role begins with fundamental biologic activities of the cells, such as cell division, cell death and cell differentiation. It affects many physiologic processes, including reproduction, growth, embryonic and fetal development, in addition to respiratory, gastrointestinal, hematopoietic and immune functions [1, 7].The role in immune function and host defense is particularly important in developing countries, where Vitamin A supplementation and therapy reduces the morbidity and mortality. A deficiency and Xerophthalmia occur throughout much of the developing world and are linked to under-nourishment and complicated by illness [2,6]. In developing countries, it is estimated that 5 lakh preschool children become blind every year owing to Vitamin A Deficiency and many of them will die because of increased vulnerability to infections, especially measles, diarrhea & pneumonia [3]. Over all prevalence of vitamin A deficiency in India is up to 6%, in some backward pockets, up to 12%. Vitamin A deficiency is a major public health problem in many parts of the world with more than 124 million children estimated to be affected and represents a source of 1 to 2.5 million deaths preventable by improvement of vitamin A nutriture[4]. Vitamin A deficiency is one of the major causes for preventable blindness in India. Subclinical Vitamin A deficiency has deleterious effect on child's health predisposing to recurrent infections, diarrhea and poor overall growth[5]. Diarrhea, malnutrition, respiratory infections are killer diseases in pediatric population of India.

In southern districts of Karnataka, including Shimoga, the burden of malnutrition, respiratory infection, diarrhea, measles and post measles infections are high, leading to significant morbidity and mortality. Majority of the studies related with Vitamin A deficiency are mostly concerned with clinical signs and symptoms of vitamin A deficiency. However subclinical Vitamin A deficiency (i.e.Physiologic deficiency without ocular manifestation of Xerophthalmia) is often missed. The present study is aimed to detect subclinical Vitamin A deficiency in children at risk, so that supplementation of Vitamin A in early stage may bring down the burden of preventable morbidity and mortality.

Methods

This cross-sectional study was conducted in tertiary care hospital from January 2020 to December 2020. Children of 1 to 12 years of age with under nutrition, recurrent respiratory

infections, recurrent diarrhea, recurrent UTI, measles and post measles infections, admitted in SIMS Shimoga were included in the study. Exclusion criteria were as follows: Child with Xerophthalmia/Trachoma/active conjunctivitis/congenital heart disease/ immunodeficiency disorder. Child who has received non dietary supplementation of Vitamin A within past 6 months. Child with anatomical defect of urinary tract and respiratory tract. A child who is very sick. Child not co-operative for the procedure. Parents not giving consent for the procedure.A detailed clinical evaluation (history & examination) and relevant laboratory investigations (Complete haemogram, Chest roentgenogram, Urine routine microscopy and culture sensitivity Stool routine microscopy, HIV and Optional investigations like 2 D echo, DMSA scan, MCU, LFT, USG abdomen, CT Scan) with CIC was done for all cases as per proforma.

Among the cases included in study group, a detailed ophthalmic examination was done at firstand re-examined by an ophthalmologist. Any child with clinical evidence of Xerophthalmia was excluded from the study and was given vitamin A supplementation of 2 Lakh I.U. And the same dose was repeated the next day.

Based on detailed clinical history, examination and relevant laboratory investigations children were diagnosed to be having severe grades of PEM, recurrent LRTI, recurrent/persistent diarrhea, recurrent UTI, Measles and post measles bronchopneumonia. Later all the cases were subjected to conjunctival impression cytology.

Procedure of conjunctival impression cytology

All the children and/or their parents were informed about the procedure in their own understandable language and written informed consent was taken before proceeding with the procedure.

All the children were instilled a drop of proparacaine (0.5%) topical anesthetic drop, two minutes prior to the procedure. Cellulose acetate filter paper disc (pore size 0.45µm; Sartorius GmbH.3400Gottingen.w.germany) was cut into 4 parts diametrically. Blunt forced was used to hold the cut filter paper. Subjects were instructed/distracted to open eyes and direct their gaze to the opposite side. The assistant was asked to hold the lids wide open. Rough side of the filter paper was pressed to the temporal aspect of bulbar conjunctiva. A gentle pressure was applied with the cotton end of an ear bud for few seconds.

Then the filter paper was removed with smooth peeling motion & was pressed over the clean dry glass slide with help of cotton end of an ear bud to transfer the material over the glass slide. Similar procedure repeated in the other eye. The glass slide was marked with diamond marker, and then the slide was air dried and kept in coplin jar with 70% isopropyl alcohol for fixing the material for 15 minutes. For staining the smears modified PAS staining technique was used and after staining the slides were air dried and studied under light microscope. The cytology was studied and graded for epithelial metaplasia as per Nelson grading system. The obtained data was entered in Microsoft-excel and analysed with SPSS software version v27 for Prevalence, Proportion, Chi square test, Trend analysis.

The study was approved by the institutional ethical committee.

Results: The total number of children included in the study was 250. Table no 1 to 5 shows various results of our study. Maximum number of children were in the age group of 4 to 6 years (43.3%). Among the total cases, 140(56%) were males and 110 (44%) were females. Male to female ratio was 1:0.78.

Cases from rural area were 148 accounting to 59.2% of total admissions. 27.2% were from urban slums and 13.6% were from urban area. Among the cases studied, majority were from class 3 socio economic status, accounting for 61.6% of the total. 22% were from class 2 and 16.4% were from class 4. Among the cases admitted majority were with severe PEM with total of 108 cases accounting to 43.2% of the total admissions. Cases with recurrent LRTI were 19.2%, recurrent diarrhea were 20%, recurrent UTI were 12.8%, measles 0.4% and post

measles bronchopneumonia were 4.4%.The CIC grading showed majority were of grade 3 and grade 2, accounting for 42% and 39.2% respectively. & 20% were of grade 1 and grade 0.Among the cases evaluated for subclinical vitamin A deficiency, 203 out of 250 were positive, accounting 81.2% with subclinical VAD. Only 18.8% had normal CIC. Among the age groups affected with subclinical VAD, children from age group of 4 to 6 were affected more (91 cases) but it was not statistically significant. Further it was seen that no age group was protected from VAD and all age groups were affected around 80%. Comparing the gender with subclinical VAD, it is seen that, both male and female are equally affected. No sex is protected from VAD and the prevalence rate in this study is ≥80%. Here in this study, 83.1% of the children from rural area were affected. 73.5% and 80.9% of the Children from urban area urban slum were affected respectively.

In the present study, children from all socio-economic status were affected almost equally when they were predisposed. In Children with class 2 SES, 81.8% had subclinical VAD. In Children with class 3 SES, 78.6% and children from class 4 SES, 90.2% had subclinical VAD. The present study clearly shows the study group had significant sub clinical VAD, 88% of affected cases in children with recurrent diarrhea, 82.2% in children with recurrent LRTI, 65.5% in children with recurrent UTI, 100% in children with measles, 62.6% in children with post measles bronchopneumonia, 83.3%in children with severe grades of PEM had subclinical VAD. It is seen that subclinical VAD in study group is statistically significant with p value < 0.05.

Table 1: Age and Gender wise distribution of cases

Age group	Gender		Percentage
	Male	Female	
1-3 years	33 (23.6%)	24 (21.7%)	57 (22.8%)
4-6 years	69 (43.3%)	39 (35.4%)	108 (43.2%)
7-9 years	24 (17.2%)	22 (20.0%)	46 (18.4%)
10-12 years	15(10.9%)	24 (21.9%)	39 (15.6%)
Total	140(100%)	110(100%)	250 (100.0%)

Chi square value-7.95 df-3 p value-0.04

Table 2: Distribution based on diagnosis

Diagnosis	Frequency	Percentage
Recurrent LRTI	48	19.2%
Recurrent diarrhoea	50	20.0%
Recurrent UTI	32	12.8%
PEM	108	43.2%
Measles	01	00.4%
Post measles bronchopneumonia	11	04.4%
Total	250	100%

Table 3: Distribution based on CIC grading

CIC grading	Frequency	Percentage
Grade 0	020	08.0%
Grade 1	027	10.8%
Grade 2	098	39.2%
Grade 3	105	42.0%
Total	250	100%

Table 4: Distribution based on Sub clinical VAD

Sub clinical VAD	Frequency	Percentage
Present	203	81.2%
Absent	047	18.8%
Total	250	100%

Table5: Relation between Diagnosis and subclinical VAD

Diagnosis	Sub clinical VAD		Percentage
	Present	Absent	
Recurrent diarrhoea	44 (88.0%)	06 (12.0%)	50 (100%)
Recurrent LRTI	40 (82.3%)	08 (17.7%)	48 (100%)
Recurrent UTI	21 (65.6%)	11 (34.4%)	32 (100%)
measles	01 (100%)	00	01 (100%)
Post measlesbronchopneumonia	07 (62.6%)	04 (37.4%)	11 (100%)
PEM	90 (83.3%)	18 (16.7%)	108 (100%)
Total	203 (81.2%)	47 (18.8%)	250 (100.0%)

P value - <0.05 for trend analysis.

Table 6: Age distribution of present study compared with others

Age group	Present study	Reddy*et al.*	Somsanguan*et al.*
≤ 6yrs	66%	37.8%	51%
≥ 6yrs	34%	62.2%	49%

Table 7: Abnormal CIC comparison to other studies

Age group	Present study	Reddy *et al.*	Somsanguan*et al.*
≤ 6yrs	84.8%	35.5%	40.2%
≥ 6yrs	77.6%	25.5%	17.3%
Total	81.2%	30.5%	28.75%

Discussion

The present study was conducted in the Department of Paediatrics, SIMS, Shimoga, fromJanuary 2020 to December 2020, to study the subclinical VAD, in suspected group of children, by conjunctival impression cytology.

Recent reports have documented significant improvement in mortality & morbidity of antixerophthalmic children receiving vitamin A supplements, suggesting the existence of subclinical but not physiologically significant VAD. Vitamin A is essential for proper differentiation & maintenance of mucosal epithelium. Absence of vitamin A causes loss of goblet cells and keratinizing metaplasia of the epithelium. The process occurs on mucosal surfaces of the respiratory, urinary and gastrointestinal tracts as well as diffusely throughout the bulbar conjunctiva.

Table 6 and 7 shows comparison of our study with other studies [8-9].

Age incidence

Out of 250 cases in the study, 66% were below the age of 6 years. Two studies were community based studies whereas the present study was hospital based[10]. The male to female ratio in the present study was 1:0.78 and majority of the children were from lower socio-economic status and belonging to rural areas. Other studies have not commented in these aspects.

Prevalence of subclinical VAD

It is seen that the abnormal CIC in present study is 81.2%, which is much greater than other two studies; the present study was conducted in suspected group of children whereas in other

studies, the subjects were from general population where both normal and Xerophthalmic children were studied. Cases considered in present study were not having established eye features of VAD, but were subclinical cases where the underlying VAD has predisposed them for recurrent infections and poor growth.

Socio-economic status & subclinical VAD

The present study considered children from all SES, the prevalence of subclinical VAD in suspected group of children didn't vary with the child's SES. It was seen that the underlying VAD which had not manifested clinically had predisposed the child to his present clinical condition. So early detection and treatment of underlying deficiency would have prevented the child from suffering the present morbidity. In present study abnormal CIC in class 2 SES was 81.8%, class 3 was 78.6% and class 4 was 81.2%.

Gender and subclinical VAD

In the present study the male to female ratio was 1:0.78 which was statistically comparable. The presence of subclinical VAD in suspected group of children with respect to gender was, males were affected 82.1% & that of female was 80%, where the difference is statistically not significant with p value of 0.66. Thus both males & females were affected equally in a given condition. In largest hospital series ever reported from the eye hospital of Dr. Yap Kie Tiong in Jogjakarta, Indonesia[10]. Male preponderance was found with VAD, in children admitted to hospital with various illnesses. Among the 6300 cases, the male: female, ratio varied with age. It was 1.4:1.0 in the preschool age period and 6.0:1.0 at around 10 years of age. But the report has not commented on subclinical VAD.

Locality and subclinical VAD

The presence of subclinical VAD was, 83.1% in rural children, 73.5% in urban children and 80.9% in urban slum children. The difference in subclinical VAD of children from differentlocality was statistically insignificant with p value of 0.43; denoting children from all areas are affected equally in a predisposed condition.

Similar study conducted by Somsanguan*et al*. at Chiang Mai[11] in 1996, to detect & compare subclinical VAD in Hill Tribe and Urban children, that the hill tribe children had moreprevalence of abnormal CIC (55%) than the urban group (29%). There was no significant difference in children suffering from subclinical VAD with respect to their age group, gender and locality, under given condition. Because of the underlying subclinical VAD, all children are predisposed to recurrent infections and poor growth.

Disease conditions & subclinical VAD

In the present study the occurrence of subclinical VAD in suspected group of children was as follows. In children with recurrent diarrhea subclinical VAD was 88%, in children with recurrent LRTI, 82.3%, recurrent UTI, 65.6%, with measles, 100%, with post measles bronchopneumonia, 62.6% and with severe grades of PEM, 83.3%. The above results are comparable with the WHO statistics 2009.

Similar study conducted by D.K.Singh *et al*. [12]in 2011 at central U.P to detect subclinical VAD by CIC in 6 months to 8 years old children attending hospital, with chronic diarrhea. The study showed abnormal CIC in 51.7% of children. In 1990, a study by Natarajan *et al*. [13] showed that abnormal CIC in children with persistent/chronic diarrhea to be 73%.

In 2006, a study by Vivek D *et al*. [14] at Kalavati Saran children hospital, New Delhi; 40.4% of children had subclinical VAD detected by abnormal CIC. In a study by Natadisastra*et al*. [15] in 1987, it was seen that 93% of children with severe grades of PEM and recurrent diarrhea had abnormal CIC. Thus subclinical VAD is highly prevalent in children with recurrent diarrhea, LRTI, UTI & severe grades of PEM.

Conclusion

Subclinical VAD was largely prevalent in suspected group of children, with severe grades of PEM, recurrent diarrhea, LRTI, UTI, Measles and post measles bronchopneumonia. Most common age group affected was 4 to 6 years. Both male and female children were equally affected. In a given disease condition, children from Urban, Rural and Urban Slums were equally affected with subclinical VAD. Predisposing conditions were more prevalent in lower socio-economic status. All classes were affected equally in a given disease condition.

References

1. Devendra WK, Vasudeo ND, Sanjay P Zodpey, Kumbhalkar DT, Manisha RK. Subclinical vitamin A deficiency in Under Six children in Nagpur, India. Southeast Asian J Trop Med Public Health.1998;29(2):289-292.
2. Kjolhede, Anne M Gadomski, John Wittpenn, Jesus Bulux, Anamaria R Rosas,Noel W Solomons*et al*. Conjunctival impression cytology: feasibility of field trial to detect subclinical Vitamin A deficiency. Am J Clin. Nutr.1999;49:490-4.
3. Louis Rosenfeld. Vitamin-vitamin. The early years of discovery. Clinical Chemistry. 1997;43(4):680-685.
4. Underwood BA. Methods for assessment of vitamin A status. J Nutr. 1990;120:1459-63.
5. Kothari G, Bhattacharjee LI, Naik EG, Bhullar PK, McLaren DS.Assessment of vitamin A nutriture in preschool children: a multi-approach. J. Trop. Pediatr. 1995;41:290-294.
6. Vitamin A history. URL: http://www.news-medical.net/health/Vitamin-A- History.aspx.
7. Gopalan C, Venkatachalam PS, Bhavani B.Studies of vitamin A deficiency in children. Am. J Clin. Nutr. 1960;8:833-840.
8. Reddy V, Rao V,Arunjyothi, Reddy M. Conjunctival impression cytology for assessment of vitamin A status. Am. J Clin. Nutr.1989;50:814-817.
9. SomsanguanAusayakhun, Nobutaka Yamada, Yoshinori Shimizu *et al*. Assessment of Vitamin A status in Urban children of Chiang Mai using Conjunctival impression cytology. Thai J Ophthalmol.1996 July-Dec;33(3):139-144.
10. Yap Kie Tiong. Brit. J Ophthal. 1956;40:502.
11. SomsanguanAusayakhun, Nobutaka Yamada, Yoshinori Shimizu, PrapatsornPatikulsila, ApinyaLuxruji, PipatAnuchatrakul. Conjunctival impression cytology to detect subclinical Vitamin A deficiency-comparison between hill tribes and urban children. Thai J Ophthalmol.1996 July-Dec;2:95-100.
12. SinghDK,GaganeN. GVS. Murthy. Evaluation of vitamin A status by conjunctival impression cytology. Indian Pediatrics. 2011;30:57-578.
13. Natarajan U, Sankaranarayanan A,Walia BN,Ganguly NK. Conjunctival impression cytology in children with persistent diarrhea as indicator of pre-clinical vitamin A deficiency Indian Pediatr. 1990 May;27(5):437-42.
14. Sehgal.P, Kumar.B, Sharma.M, Salameh A.A, Kumar.S, Asha.P (2022), Role of IoT In Transformation Of Marketing: A Quantitative Study Of Opportunities and Challenges, Webology, Vol. 18, no.3, pp 1-11
15. Kumar, S. (2020). *Relevance of Buddhist Philosophy in Modern Management Theory*. *Psychology and Education*, Vol. 58, no.2, pp. 2104–2111.
16. Roy, V., Shukla, P. K., Gupta, A. K., Goel, V., Shukla, P. K., & Shukla, S. (2021). Taxonomy on EEG Artifacts Removal Methods, Issues, and Healthcare Applications. Journal of Organizational and End User Computing (JOEUC), 33(1), 19-46. http://doi.org/10.4018/JOEUC.2021010102
17. Shukla Prashant Kumar, Sandhu Jasminder Kaur, Ahirwar Anamika, Ghai Deepika, MaheshwaryPriti, Shukla Piyush Kumar (2021). Multiobjective Genetic Algorithm and Convolutional Neural Network Based COVID-19 Identification in Chest X-Ray Images,

Mathematical Problems in Engineering, vol. 2021, Article ID 7804540, 9 pages. https://doi.org/10.1155/2021/7804540

18. Vivek Devan. Miscellaneous Session-II. AIOC Proceedings, 2006, 416-418.

19. NatadisastraG,WittpennJR, West KPJr., Muhilal, Sommer A. Impression cytology for detection of vitamin A deficiency. Arch. Ophthalmol.1987;105:1224-1228.