Original Research Article

Conjunctival Impression Cytology in Vitamin A deficient children in a tertiary care hospital of Bihar: An observational, cross-sectional and comparative study

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Abstract

Background: Subclinical Vitamin A deficiency gets missed on routine clinical and biochemical examination. In this study conjunctival impression cytology (CIC) was used as a diagnostic tool to screen subclinical cases and to examine children had history of night blindness with signs and symptoms of Vitamin A deficiency. **Materials and methods:** This was an observational, comparative and cross-sectional study on 100 children of all gender and age group between 3 to 12 years. Demographic profile, immunisation status, history of present illness and drug history, history of any disease in the last 6 months, detailed family and maternal history during pregnancy, clinical examination and laboratory investigations including conjunctival impression cytology were evaluated and compared between fifty Vitamin A deficient children and fifty normal and healthy children. Data were interpreted using Chi square ($\chi 2$) test and Fisher's Exact test for significance by GraphPad Instat software. Relative risk was also calculated according to the comparative groups. **Results:** Study group versus control group, age group was 4-8 years (42%) vs 8-12 years (66%), 62% male preponderance vs same male & female ratio, had poor socio-economic conditions in 74% vs 52%, had immunization status 66% vs 90%, had history of infection in 54% vs 18% and had abnormal conjunctival impression cytology (CIC) in 90% vs 6% respectively. The relative risk between groups were statistically significant for different parameters. Abnormal conjunctival impression cytology found in 6% children in control group signifies that it is an important diagnostic tool for diagnosis of subclinical Vitamin A deficiency. **Conclusion:** Technique of conjunctival impression cytology can be used as a safe, non-invasive, cost effective method for screening of subclinical cases and also as a diagnostic tool to detect Vitamin A deficiency.

Keywords: Vitamin A deficiency, Night blindness, Xerophthalmia, Conjunctival impression cytology.

Introduction

Vitamin A is an essential nutrient needed in small amounts for the normal functioning of the visual system, growth and development, maintenance of epithelial cellular integrity, immune function and reproduction.

Vitamin A deficiency has long been recognised as a serious and widespread yet preventable nutrition deficiency in the world. Lack of Vitamin A in the child's diet leading to Xerophthalmia is a major public health problem in many developing countries, causing a third of a million children to go blind each year and contributing to 20% of all cases of blindness in India. 60% of these children die within a short time of losing their sight. [1]

Although Vitamin A deficiency can occur in any age group, the most serious effects are seen in preschool children. Vitamin A requirements are greatest due to rapid growth but their dietary intake is precarious and illnesses such as diarrhoea, acute respiratory infection, and measles depleting the Vitamin A resources are common. Vitamin A deficiency is not only responsible for blinding malnutrition but by now it is more than clear that it may be associated with high morbidity and mortality in children. According to WHO estimates, worldwide one child dies every minute from Vitamin A deficiency. [2]

Vitamin A supplementation of non – Xerophthalmic children has been shown to reduce mortality, thus suggesting the possibility that subclinical Vitamin A deficiency i.e. physiological deficiency without ocular manifestations of Xerophthalmia may be more common than it is believed to be and may be playing a role in higher mortality in this group. Clinical screening of preschool children is quite useful to diagnose Vitamin A deficiency well before Xerophthalmia becomes obvious. However subclinical Vitamin A deficiency is missed particularly in apparently normal children, who don't have any obvious signs of Vitamin A deficiency. Conclusive dietary, biochemical, or histological methods that reliably detect subclinical deficiency have been lacking until recently. The conjunctival changes of Xerophthalmia are the most accessible physiological index of Vitamin A status which is widely used to diagnose Vitamin A deficiency in high-risk patients as well as in population surveys. However, subclinical Vitamin A deficiency is missed on routine examination. Presently serum Vitamin A levels are the only objective method for the determination of Vitamin A deficiency. However, serum Vitamin A levels, suffer from poor correlation with body stores, except under conditions of severe depletion, and thus are not a direct indicator of individual physiologic status. Moreover, estimation of serum Vitamin A levels require invasive sampling procedure, sophisticated equipment and highly trained personnel which is impractical in developing countries.

Conjunctival impression cytology is a histological technique based on reduction of goblet cell numbers and abnormal epithelial cell characteristics of cellular impressions obtained from conjunctiva. Many workers like Wittpenn JR et al. (1986), [3] Natadisastra et al. (1987), [4] Luzaeu R et al. (1988), [5] Reddy M et al. (1991), [6] De Rojas MV et al. (1993), [7] Chowdhury S et al. (1996), [8] Singh M et al. (1997), [9] Divani SN et al. (1997), [10] Baudouin C et al. (1997), [11] suggested that conjunctival impression cytology is direct evidence that shows a substantial proportion of otherwise normal looking children are suffering from physiologic consequences of Vitamin A deficiency and suggests that the prevalence of Vitamin A dependent abnormal cellular differentiation is 5-10 times that of Xerophthalmia.

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In the present study, the endeavour is to employ conjunctival impression cytology as a method to screen and examine children giving history of night blindness and presenting with signs and symptoms of Vitamin A deficiency for the detection of the well described changes in the conjunctival epithelium.

Materials and methods

Study site: Department of Ophthalmology, Mata Gujri Memorial Medical College and Lion Seva Kendra Hospital, Kishanganj, Bihar.

Study duration: Two years from July 2011 to July 2013

Study design: This was an observational, analytical, comparative and cross-sectional study. This study was carried out on 100 children to compare the conjunctival impression cytology between 50 consecutive cases of night blindness (giving history by self or by parents) and/or showing signs and symptoms of Xerophthalmia with the matched 50 healthy children.

Inclusion criteria

1) Children of all gender and age group of 3-12 years.
2) Diagnosed cases of night blindness and/or signs and symptoms of Xerophthalmia were included in case group.
3) Healthy children of same age and gender without any history of night blindness, and/or signs or symptoms of Vitamin A deficiency and with no Xerophthalmia were included in control group.

Exclusion criteria

1) Children below 3 years and above 12 years of age either in case or control group
2) Children with congenital disorders of eye, chemical eye burns, ocular injuries, Microbial infections, ocular surgeries, Steven Johnson Syndrome, Retinitis Pigmentosa, Congenital stationary night blindness

Methodology

A) Information regarding demographic profile and immunisation status- Name, Age, Sex, Birth order, Father's name, Address, Religion, Residence – rural/ urban, Number of family members, Socioeconomic status- Below Poverty Line/ Above Poverty Line, Immunization History (Immunized against six vaccine preventable diseases)- Fully / Partially / Nil

B) A detailed history of present illness was taken

1) History of present illness

a. Night Blindness – Present / Absent
b. Duration
c. Associated complaints – Fever / Rash / Diarrhoea / Vomiting etc.
d. Complications of night blindness

2) Drug History

a. Vitamin A prophylaxis: Given / Not given / Not known
b. Route of Administration

C) A history of any disease in the recent past (in the last 6 months) was also taken- Diarrhoea / Measles / Respiratory Tract Infection / Helminthiasis

D) Detailed family and Maternal history during pregnancy was taken to rule out any familial disease/ genetic disease or congenital night blindness.

E) A thorough clinical examination and laboratory investigations were done

1) General physical examination- Body Weight & Other examinations

2) Systemic examination- Skeletal system, CVS, CNS, GIT

3) Ocular examination- The local examination of each eye to be done separately and positive findings to be noted down like Facial symmetry, Head posture, Forehead, Eyebrows, Orbit, Eyelids, Eyeball, Palpebral aperture, Lacrimal apparatus, Conjunctiva, Cornea, Limbus, Sclera, Anterior Chamber, Iris, Pupil, Lens, Vitreous, Visual acuity.

4) Ophthalmic laboratory examinations: - Intra ocular pressure, Refraction testing, Slit lamp examination, Staining (selected cases), Gonioscopy (selected cases), Fundoscopy (selected cases)

5) Haemoglobin estimation

6) Conjunctival Impression Cytological examination (of the affected eye) After initial examination the repeated conjunctival impression cytology was done at intervals of 2 weeks and 4 weeks. Conjunctival Impression Cytology was graded as normal or abnormal based solely on the presence or absence of goblet cells. Each child was staged according to the lowest stage of the two of their specimens. If the specimen from either eye was normal then the child was graded as normal.

a) Eye examined – Right / Left
b) Epithelial cells
   i. Size : large / small
   ii. Shape : normal / abnormal
   iii. Number : numerous / reduced
   iv. Relative separation : in masses / separated
c) Goblet cells : present /absent

Statistical analysis: The results obtained were statistically analysed using Graph Pad Instat software. Data were interpreted using Chi square ($\chi 2$) test and Fisher's Exact test for significance. Relative risk was also calculated according to the comparative groups.

Results

Table 1: Age and sex distribution of study (case) group and control group

Age group	Study (case) group (n=50)				Control group (n=50)			
	Male	Female	M:F	Total (%)	Male	Female	M:F	Total (%)
3 to <4 years	4	6	0.66:1	10 (20%)	4	2	2:1	6 (12%)
4 to <8 years	15	6	2.5:1	21 (42%)	6	5	1.2:1	11 (22%)
8 to 12 years	12	7	1.7:1	19 (38%)	15	18	0.83:1	33 (66%)
TOTAL	31	19	1.63:1	50 (100%)	25	25	1:1	50 (100%)
Chi square ($\chi 2$) test: p value- 0.3138- Not significant The Row/Column association is not statistically significant Relative risk-1.282								

Table 2: Case distribution of the study group (history and ocular examination)

	3-<4 YEARS	4-<8 YEARS	8-12 YEARS	TOTAL
Complaints of night blindness without any ocular signs and symptoms	2	9	5	16
Complaints of night blindness with specific signs of Xerophthalmia	8	12	14	34
Total	10	21	19	50
Chi square ($\chi 2$) test: 2.081, p value 0.3532- Not significant The Row/Column association is not statistically significant				

Table 3: Age and sex wise distribution of abnormal vitamin A status (abnormal conjunctival impression cytology)

		Study group (n=50)		Control group (n=50)	
		Normal	**Abnormal**	**Normal**	**Abnormal**
Age	3-<4 years	1 (10%)	9 (90%)	5 (83.3%)	1 (16.7%)
	4-<8 years	2 (9.5%)	19 (90.5%)	10 (90.9%)	1 (9.1%)
	8-12 years	2 (10.5%)	17 (89.5%)	32 (97%)	1 (3%)
	TOTAL	5 (10%)	45 (90%)	47 (94%)	3 (6%)
	Fisher's exact test: two-sided p value <0.0001-extremely significant The Row/Column association is statistically significant Relative risk- 0.1064				
Sex	MALE	3 (9.7%)	28 (90.3%)	24 (96%)	1 (4%)
	FEMALE	2 (10.5%)	17 (89.5%)	23 (92%)	2 (8%)
	TOTAL	5 (10%)	45 (90%)	47 (94%)	3 (6%)
	Fisher's exact test: two-sided p value 0.554-Not significant The Row/Column association is not statistically significant Relative risk- 1.867				

Table 4: Socio-economic status of parent and immunization status of children

		Study group (n=50)			Control group (n=50)		
		Male (n=31)	**Female (n=19)**	**Total (n=50)**	**Male (n=25)**	**Female (n=25)**	**Total (n=50)**
Socio economic status	Below poverty line	22 (71%)	15 (79%)	37 (74%)	12 (48%)	14 (56%)	26 (52%)
	Above poverty line	9 (29%)	4 (21%)	13 (26%)	13 (52%)	11 (44%)	24 (48%)
	Fisher's Exact test: two-sided p value- 0.0377- Significant The Row/Column association is statistically significant Relative risk-1.672						
Immunization status	Immunized	21 (68%)	12 (63%)	33 (66%)	23 (92%)	22 (88%)	45 (90%)
	Unimmunized	10 (32%)	7 (37%)	17 (34%)	2 (8%)	3 (12%)	5 (10%)
	Fisher's Exact test: two-sided p value- 0.0070- Very Significant The Row/Column association is statistically significant Relative risk-0.5475						

Table 5: History of some common infections in the past 6 months

Disease	**Study group (n=50)**		**Control group (n=50)**	
	With history	**%**	**Without history**	**%**
Diarrhoea	18	36%	4	8%
Respiratory tract infection	5	10%	3	6%
Measles	1	2%	0	0%
Helminthiasis	3	6%	2	4%
Total	27	54%	9	18%
Fisher's Exact test between with history and without history of infections: two-sided p value- 0.0003- Extremely Significant The Row/Column association is statistically significant Relative risk- 2.087				

Discussion

In the present study 20% of the children in the study group were between 3-4 years of age, 42% were between 4-8 years of age and 38% of the children were between 8-12 years of age. With regards to the sex distribution there was a male preponderance in the study group, the male to female ratio being 1.6:1. The percentage of male children was 62% and the female children were 38%. In a similar study [3,4] reported 59.6% males and 40.4% females.

In the present study cases of night blindness [32%] was outnumbered by the cases presenting with obvious ocular changes of Xerophthalmia [68%]. The incidence of night blindness was more for children between 8-12 years of age, while in the age group of 8-12 years the incidence of ocular changes was more.

Incidence of night blindness and ocular changes in the study group with low immunization coverage 66% was found to be higher than those children in the control group with higher [90%] coverage of immunization, which is statistically significant (P<0.001). In the study group the incidence of night blindness in the age group of 3-4 years was lower than that of the children in the age group of 4 years and above. It is probably due to a lower sample size and due to difficulty in determining night blindness in very young individuals due to lack of vocal expression characterizing night blindness.

In the present study, most of the children in the study group belonged to low socio-economic status (below poverty line) [74%], while the percentage of children below poverty line was 52% in the control group. This shows a strong correlation of a higher degree of poverty with Vitamin A deficiency and is consistent with the WHO report, Nutrition for Health and Development, 2000 [12] where nutritional intake was directly related to the prevalence of Vitamin A deficiency. They found that the incidence of Vitamin A deficiency in Protein Energy Malnutrition was highest in South East Asia where upto 70% malnourished children had eye signs of Vitamin A deficiency.

In the present study, the collection of conjunctival specimens was done according to the technique as suggested by Luzaeu R et al. (1988), [5] Chowdhury S et al. (1996), [8] which obviates the need for lid speculum, thus making the procedure less frightening for the child. No topical anaesthesia was used.

In the present study, 45 children in the study group had abnormal cytology [90%] and 3 children in the control group had abnormal cytology [6%]. These results were comparable with studies of

previous workers like Divani SN et al. (1997) [10] who got 78% abnormal cytology in children with history of night blindness. Singh M et al (1977) [9] found 68.2% abnormal CIC in healthy primary school children without any apparent signs of Xerophthalmia. Dewan et al (1995) [13] found 40.4% of abnormal cytology in normal children between 6-24 months of age. Natadisastra et al (1987) [4] found 23% of abnormal cytology in clinically non Xerophthalmic children. Reddy M et al (1989) [6] found abnormal cytology in 25% of children with normal eyes. Singh et al (1997) [9] found abnormal cytology in 25% of school aged children. Vadrevu VLD et al. (1994) [14] observed 44% of abnormal cytology in clinically normal group.

In the present study, the study group comprising of 50 children with history of night blindness only 10% of children showed normal conjunctival cytology as compared to the control group where 94% children showed normal conjunctival impression cytology which can be attributed to the fact that night blindness is the earliest symptom of Vitamin A deficiency. The results thus show a strong relationship between Vitamin A deficiency and abnormal conjunctival impression cytology where (P<0.001) which is statistically significant, as found by previous workers.

Sommer A et al. (1984) [15] reported that Diarrhoea occurs in Vitamin A deficient states and responds quickly to Vitamin A. Also reported that children with mild Xerophthalmia developed diarrhoea, respiratory tract infection at three times the rate of children with normal eyes.

Following several studies it has been found that diarrhoea especially repeated and prolonged is a risk factor for Vitamin A deficiency in children whose liver stores are low.

In the present study most of the children with night blindness [69%] and all the children with ocular signs and symptoms were found to have abnormal CIC, thus corroborating the study objective that abnormal Vitamin A status leads to abnormal CIC, and vice versa. Resnikoff S et al. (1987) [16] had found abnormal Vitamin A status in cases with night blindness.

In the present study positive history of disease/infection was given by 27 children in the study group within the last 6 months. Out of this 18 children gave history of diarrhoea [67%]; which emerged as the single most important factor for triggering night blindness and clinical manifestation of Xerophthalmia which is a statistically important cause of Vitamin A deficiency in our present study (P<0.01). Also, history of respiratory tract infection [18%], measles [4%], helminthiasis [11%] was given and which have been found to be statistically significant cause of Vitamin A deficiency and its manifestation when compared to the control group (P<0.001). These infections/diseases are some of the common etiological factors for future Vitamin A deficiency in the children; and thus, producing abnormal CIC in the present study. These results were comparable with those of the previous workers.

Sommer et al. (1984) [15] reported a high prevalence of Xerophthalmia with respiratory tract infection, whooping cough, tuberculosis and diarrhoea. Also observed that children with recent history of measles were 11 times more likely to develop Xerophthalmia. Reported a history of helminthiasis in recent past in 85% cases of Xerophthalmia. Found that dewormed children showed a high rising serum of Vitamin A level in Xerophthalmic children in absence of Vitamin A supplementation.

In the present study it was observed that malnutrition was more common in children with abnormal Vitamin A status. This was shown by previous workers that higher incidence of Xerophthalmia among malnourished children. The higher incidence of malnutrition in children with both normal and abnormal Vitamin A status in the present study is indicative of a poor health and socio-economic status in our community.

Conclusions

The technique of conjunctival impression cytology can be used as a safe, non-invasive, cost effective method for screening of subclinical cases and also as a diagnostic tool to detect Vitamin A deficiency, by observing the conjunctival changes in Vitamin A deficiency. Improvement of general nutrition, intake of foods containing adequate amounts of Vitamin A and incorporation of Vitamin A prophylaxis along with routine immunization will go a long way to prevent Vitamin A deficiency in our vulnerable patient population.

Conflict of Interest: Nil

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