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Therapeutic Assessment of Conjunctival Impression Cytology in Vitamin A Deficient Children

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Abstract: *This study was carried out to examine vitamin A deficiency using conjunctival impression cytology and to find out the effect of vitamin A supplementation in vitamin A deficient children attending out door patient of Ophthalmology Department, ANMMCH, Gaya. The study comprised of 200 children with complaint of night blindness and ocular changes. Conjunctival impression cytology was performed in all the children with the help of Millipore filter material. Children were given 200000 IU oil miscible vitamin A orally. They received an additional oral dose next day and cytology was repeated after 4 weeks. Maximum number of male (62.93 per cent) and female (65.47 per cent) children showed abnormal cytology at initial stage (0 week) of study. After 4 weeks of supplementation with vitamin A, significant number of children with abnormal cytology reverted to stage 0 and I i.e. normal category. This study showed that conjunctival impression cytology is simple, easy, accurate, low cost, non-invasive and precise with high acceptability method to detect vitamin A deficiency in children.*

Key Words: *Paediatric, Cytology, Vitamin A deficiency, Supplementation*

I. Introduction

Vitamin A is an essential nutrient needed in small amount for the normal functioning of the visual system, growth, development and maintenance of epithelial cellular integrity, immune function and reproduction. Vitamin A deficiency (VAD) is a major cause of childhood preventable blindness in India and other developing countries (1) and increases the risks of diseases from severe illness and even death from such a common childhood disease as diarrhea and measles. Approximately 228 million children are affected by VAD (2); it is the most common form of malnutrition leading to childhood co-morbidity and blindness worldwide, accounting to 1 to 3 million childhood. VAD appears to increase the risk of death even before xerophthalmia is apparent clinically (3). Children between the ages of 6 months and 6 years are highly vulnerable to VAD. The effects of vitamin A status on childhood mortality depend on the severity and prevalence of pre-existing VAD, concomitant nutritional disorders, and co-exiting infections (4). It is estimated that over one million deaths in children could be prevented each year with improvement to vitamin A nutrition (5). Conjunctival impression cytology (CIC) using cellulose acetate filter paper is an innovative technique to study the viability and surface characters of conjunctiva (6). The cells thus removed can be subjected to histological, immune histological, or molecular analysis. Egbert *et al*. first described this non- invasive method of studying conjunctival goblet cells in 1977 (7). Since then the technique has been used to evaluate several ocular surface disorders and modifications to the original technique have been introduced. It is non-invasive, easy to perform, and yields reliable information about the area sampled with minimal discomfort to the patient. Its use in research has experienced an enormous growth and has greatly contributed to the understanding of ocular surface pathology (8). The purpose of this study was to evaluate the conjunctival surface changes in children using CIC method and to find out the effect of vitamin A supplementation in VAD diagnosed children.

II. Materials And Methods

The study was conducted in the Ophthalmology Department, Anugrah Narayan Magadh Medical College, Gaya, Bihar. A total 200 cases of either sex with the history of night blindness and/ or showing signs of xeropthalmia, belonging to the age group of 1-12 years (where 1 year is 1 completed year and 12 years is 12 completed years), attending Ophthalmology out door patient were included. Children who had received Vitamin A within a period of last six months, and with congenital disorders, chemical eye burns, ocular injuries, microbial infections, ocular surgeries, Steven-Johnson syndrome, Sjogrens syndrome, retinitis pigmentosa and congenital stationary night blindness were excluded from the study. A detailed history regarding age, sex, socio economic status, immunization status, and history of diseases like diarrhoea, respiratory tract infection (RTI), measles, helimenthiasis were taken. Thorough clinical examination was done followed by CIC. The conjunctival cells samples were collected on Millipore filter material (CATNO GSWPO2500, LOT No. BM1HA3393) with

help of disk applicator. The applicator eliminates any hand contact with the filter paper specimen, applies a disk of paper of fixed area to the conjunctiva, reduces variations in applied pressure, improves cells adhesion and permits more precise targeting of sample sites. It was used for one inferotemporal and one inferonasal location. The cells were fixed by the previously prepared fixative solution containing 70 per cent ethyl alcohol, 37 per cent formaldehyde and glacial acetic acid in a 20:1:1 volume ratio. All the slides were dried and kept for staining. Procedure was explained to parent. They were reassured that it would be quick and painless procedure. Local anaesthesia was not needed. After taking the specimen, topical antibiotic was instilled in each eye. Staging of CIC was done according to method described by Natadisastra *et al*. (9).There are six stages. 0 and I were taken as normal while stage II to V were taken as abnormal cytology evidence of VAD.

Table 1:- Staging of conjuctival squamous metaplasia

Stage	Criteria
Stage - 0	Abundant goblet cells and mucin spots, small epithelial cells
Stage - I	Fewer goblet cells and mucin spots, small epithelial cells
Stage - II	Loss of goblet cells and mucin spots, enlarging epithelial cells
Stage - III	Enlarging and separating epithelial cells
Stage - IV	Large separate epithelial cells with scattered keratinization and pyknotic nuclei
Stage - V	Large keratinized epithelial cells with pyknotic nuclei or loss of nuclei

All the cases showing abnormal cytology were given 200000 IU oil miscible vitamin A followed by an additional oral dose next day as recommended by WHO. CIC was done before (0 week) and after 4 weeks of administration of vitamin A.

III. Results

Two hundred children aged 1-12 years were examined for evidence of vitamin A deficiency. There were 116 (58 per cent) male and 84 (42 per cent) female. Majority of the male (58.62 per cent) and female (57.14 per cent) respondents were 4-8 years old. It was also found that, 68 per cent of both male and female respondents were below poverty line. Majority (65.51 per cent) of male and female (61.90 per cent) children were not immunized. History of diarrhoea during the last two weeks was given by 80 per cent of male and 80.95 per cent of female children, while 20 per cent of male and 12 per cent of female were found to be suffering from RTI. Measles was reported by 8 per cent male and only 4 per cent female children whereas history of helimenthiasis was given by 8 per cent male children. None of the female children reported heliminthiasis (Table 2).

Table 2:- Demographic profile of the subjects (n=200)

Particulars	Male	Female
	No. (%)	No. (%)
Gender	116 (58)	84 (42)
Age (yrs.)		
1 - 4	44 (37.93)	24 (28.57)
4 - 8	68 (58.62)	48 (57.14)
8 - 12	4 (3.44)	12 (14.28)
Socio-economic status		
Below poverty line	80 (68.96)	68 (80.95)
Above poverty line	36 (31.03)	16 (19.04)
Immunization		
Yes	40 (34.48)	32 (38.09)
No	76 (65.51)	52 (61.90)
History of diseases (last 6 months)		
Diarrhoea	80 (68.96)	68 (80.95)
RTI	20 (17.24)	12 (14.28)
Measles	8 (6.89)	4 (4.76)
Helimenthiasis	8 (6.89)	0 (0)

Table 3:- CIC status of participants at baseline (0 week) and after 4 weeks of vitamin A supplementation

Stages	Male		Female	
	Before (0 week) No. (%)	After (4 weeks) No. (%)	Before (0 week) No. (%)	After (4 weeks) No. (%)
0	20 (17.24)	33 (58.92)	12 (14.28)	24 (55.81)
I	23 (19.82)	18 (32.14)	17 (20.23)	17 (39.53)
II	34 (29.31)	3 (5.35)	25 (29.76)	1 (2.32)
III	30 (25.86)	1 (1.78)	27 (32.14)	1 (2.32)
IV	7 (6.03)	1 (1.78)	3 (3.57)	0 (0)
V	2 (1.72)	0 (0)	0 (0)	0 (0)
Total	116	56	84	43

The results of CIC status of the children at 0 week and after supplementation of vitamin A are shown in Table 3. A total of 116 male children, 43 (37.06 per cent) showed normal cytology in which 17.24 per cent were in stage 0 and 19.82 per cent were in stage I whereas 73 (62.93 per cent) children showed abnormal cytology [stage II (29.31 per cent), stage III (25.86 per cent), stage IV (6.03 per cent) and stage V (1.72 per cent). So, 2 lac IU of oil miscible vitamin A with an additional oral dose next day was supplemented to 73 children presenting abnormal cytology. After 4 weeks, CIC was repeated. Results revealed that after supplementation, 56 cases were turned up during repeated CIC. It was observed that 51 (91.07 per cent) male children were treated as they showed normal cytology while only 5 (2.80 per cent) children showed abnormal cytology. Out of 84 female children, 29 (34.52 per cent) were in category of normal cytology whereas 55 (65.47 per cent) showed abnormal cytology [stage II (29.76 per cent), stage III (32.14 per cent) and stage IV (3.57 per cent)]. None of them were in stage V. So, 55 female children were given vitamin A orally. Repeated CIC results revealed that 43 female children were present in the follow up in which 2 (4.65 per cent) were found in the abnormal category, rest of them i.e. 41 (95.34 per cent) cases were treated as they showed normal cytology.

IV. Discussion

The ocular surface made up of stratified, non keratinizing cell layers, is covered by the tear film, which lubricates, hydrates, and protects the underlying epithelium. The innermost component of the tear film is mucus, composed mainly of secreted mucins, which are produced by conjunctival goblet cells, with potential additional contribution of membrane-spanning mucins expressed by corneal and conjunctival epithelia. Previous in vitro studies have indicated that vitamin A derivatives are required for maintaining mucosal cell differentiation, mucin production, and mucin gene expression (10). VAD is reported to be associated reduction in the goblet cell population and an increase in conjunctival epithelial cell mitosis in the eye. The tear quantity and quality are normal despite the formation of a real metaplasia in conjunctiva is a VAD (11).

Conjunctival impression cytology (CIC) is the technique of collection of the most superficial layers of the ocular surface by applying different collecting devices (usually filter papers) so that cells adherent to that surface are subsequently removed from the tissue and further processed for a diversity of techniques (12). It is fast, non invasive, easy to perform, and economical. In the present study, CIC was done on 200 children between aged 1-12 years. Results showed that 73 (62.93 per cent) of male children and 55 (65.47 per cent) of female children had an abnormal cytology. It was also found that VAD is more prevalent in male children. Clinical surveys among children have consistently documented VAD in children (13, 14). In various studies VAD is reported to be associated with diarrhea respiratory tract infection and measles. WHO recommended Vitamin A administration to prevent xerophthalmia in condition like severe protein energy malnutrition prolong diarrhea, ARI and measles.

In the present study, supplementation with 2 lac IU of oil miscible vitamin A was given orally with an additional dose on next day to the children showing abnormal cytology. Results showed a reversal of sign, symptoms and cytology towards normal in both male and female children, it become 91.07 per cent from 62.93 per cent in male children, while in female children it changes from 65.47 per cent to 95.34 per cent. These results are in agreement with Singh *et al*. (15) who also observed abnormal CIC in rural children which reverted to normal after administration of oral vitamin A in spite of clinical condition of persistent diarrhea. Natrajan *et al*. (16) also showed in their study that administration of oral vitamin A changes abnormal CIC to normal.

It is hereby suggested that CIC should be included as a screening test at the primary school level (or earlier) so that VAD is detected and corrected at an early stage. This will help in prevention of ocular lesions which might subsequently lead to blindness especially in the presence of precipitating factors like acute respiratory tract infections and diarrhoea. CIC can also serve as a useful technique for monitoring and evaluation of the vitamin A prophylaxis programme. Hence thought should be given to integration of this component in the primary health care system.

V. Conclusion

The results of the present study demonstrated that CIC technique is simple, safe, non-invasive and cost effective method to detect VAD in children.

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