Conjunctival Impression Cytology (CIC) in Febrile Children in Pediatrics Department of M. Djamil General Hospital, Padang

Marjis, and Hafid Ardy
Faculty of Medicine, Andalas University/DR. M. Djamil Hospital Padang,
West Sumatra, Indonesia

ABSTRACT

Objective: *Febrile children due to infectious disease will suffer relative deficiency of vitamin A, due to increased of vitamin A excretion via urine, non specific reaction to fever, and decreased food intake, thus absorption will also decrease. This study is to know the picture of conjunctival impression cytology (CIC) of febrile children.* ***Method:*** *Cases, Patients were hospitalized febrile children aged between 2-7 years old at the Pediatrics Department, M. Djamil General Hospital, Padang, in a period of two months :March and April 2003. Patiens nutritional states were evaluated using* Height, *Weight, and Age parameters used in a standard formula to be compared to normal standard WHO tabel. CIC test were done according to Tseng method. Examinations were done on the first day of hospitalization. As controle group, 16 eyes of normal non febrile children were used. The significance of differences of Nutritional state and CIC picture were statically analyzed.* ***Result:*** *In febrile group the result of CIC examination was statistical significant. But nutritional state, frequency of febrile in a year and duration of the febrile weren't. Further study will be needed with a larger number of patients and longer duration of fever.* ***Conclusion:*** *CIC examination of the conjunctiva is a simple test and have a good result to detect (relative) vitamin A deficiency in febrile patients.*

Keyword : Conjunctival Impression Cytology (CIC) in Febrile Children in Pediatrics

INTRODUCTION

The role of vitamin A in immunity to infectious diseases has long been suggested. Vitamin A has been known as "The anti-infective vitamin" since the 1920, but only in the last several years rigorous clinical trials and appropriately designed experiments in animals to demonstrate that vitamin A enhances immunity, thereby reducing childhood morbidity and mortality from infectious diseases were studied.[1,4]

Vitamin A deficiency also has long been recognized as a major public health problem in most developing countries.[1,2,4,7,20] According to Lietman et al in 1998 perhap vitamin A deficiency affect as many as 200 million children world wide, and it was suggested to be a factor in causing 500.000 cases of blindness and represents a cause of 1-2,5 million preventable death anually.[4,6] Vitamin A deficiency with xerophthalmia and subclinical manifestation likely to play a contributory role in a large proportion of childhood morbidity and mortality.[10,16,20]

It is well known that vitamin A is also essential for normal cellular diffrentiation, particularly of mucous-secreting epithelium. While mucous-secreting epithelium of many organ are involved, including the respiratory, genito-urinary and gastroentestinal tracts, changes are most readily observed in the conjunctiva. Beyond functioned in vision and maintenance of epithelium, vitamin A is also required for the healthy

maintenance of reproduction, immune function, growth and hematopoesis.[20]

Febrile children caused by infectious disease will show decrease plasma vitamin A level by increased vitamin A excretion via urine, decreased absorption was and non-spesific reaction to fever. In the other hand food intake is decreased and utilization is increased during febrile. As the consequence of these, children with normal vitamin A level will be altered to subclinical vitamin A deficiency, and children with subclinical vitamin A deficiency will be altered to full vitamin A deficiency. Many studies had shown that there were evidence that vitamin A deficiency most commonly happened in children with infectious disease or after it, if compared with normal children.[5,10,14]

Presently, the ocular changes of xerophthalmia are the most accessible pato-physiologic indicator of vitamin A deficiency. Clinical survey for xerophthalmia (full blown sign of vitamin A deficiency) may describes the pato-physiological state better, but requires large sample size. On the other hand, the sub clinical deficiency of children can not be detected by using this method. Measurement of serum vitamin A level requires invasive sampling procedure, sophisticated equipment, highly trained personal and method for preparing, storing and transporting the delicate samples that are impractical for most field surveys in developing countries. There was no significant correlation between serum vitamin A level and body store of the vitamin, except under severe depletion, and was shown that serum vitamin A level is not a direct indicator for individual physiologic state.[15,18,20]

Vitamin A is essential for the proper differentiation and maintenance of mucosal epithelium. Vitamin A deficiency causes loss of goblet cells and keratinizing metaplasia of the epithelium in respiratory, urinary and gastrointestinal as well as diffusely throughout the bulbar conjunctiva.[20]

Conjunctival impression cytologic was first found by Egbert in 1977,using cellulose acetate filter. Impression cytologic permits atraumatic sampling of superficial conjunctival epithelial cells for histologic examination.[9,10]

The Purpose of this study wasTo know the pictures of conjunctival impression cytology in febrile illness caused by infectious disease.

OPERATIONAL DEFINITION

Nutritional State Examination

Nutritional state is a value of comparison of the formulated parameters of weight and age (W/A) – height and weight (H/W) to normal WHO-NCHS nutritional state table. Good nutritional state children are those when compared to W/A and H/W standard are more than 80%. (cited from 12)

Conjunctival Impression Cytology

Conjuctival impression cytology was conducted to each children by using a previously described technique. In summary this technique is performed by applying 5x5 mm piece of cellulosa acetate filter paper (HAWP 304 FO, millipore corp. Bedford Mass.), to temporal bulbar conjunctiva of each eye. The filter paper was gently applied to the eye for 2 seconds and then removed with peeling motion. The filter paper with the adherent epithelial cells was fixated in 96% alcohol. After fixation, the specimens were stained with Periodic acid-schiff stain (PAS). All impresion cytologic specimens were examinated in masked fashion and staged according to the degree of squamous metaplasia according to Tseng's method.

Normal conjunctival impression cytology are stage 0 and 1.

Abnormal conjunctival impression cytology are stage 2-5.

Staging of Conjunctival Squamous Metaplasia

Stage	Criteria
0	Abundant goblet cells and mucin spots, small epithelial cells
1	Fewer goblet cells and mucin spots, small epithelial cells
2	Loss of goblet cells and mucin spots, enlarging epithelial cells
3	Enlarging and separating epithelial cells
4	Large, separate epithelial cells with scattered keratinization and pyknotic nuclei
5	Large keratinized epithelial cells with pyknotic nuclei or loss of nuclei

Febrile Frequency

Febrile frequency was how frequent these children suffer from febrile during the latest year.

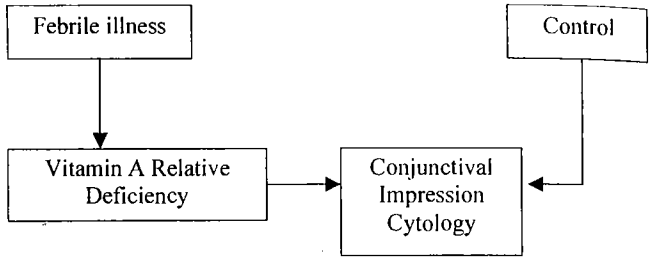
Duration of Febrile

Duration of febrile, is how long these children were febrile before the first day of hospitalization.

Subject and Method

Fourty eyes from twenty patients who are hospitalized caused by febrile illness at the Pediatrics Department of M. Djamil General Hospital, Padang in March to April 2003 were subjects for the study. Patient's age were between 2-7 years. Control group were 16 eyes from 8 ambulatory patients coming to the Dept. for booster immunization.

Examination of conjunctival impressio cytology and nutritional state were done at the first day of hospitalization . The results of the observations was then statistically analized by Chi-Square test which was corrected by Fisher's exact test for small sample.



RESULT AND DISCUSSION

Table 1. Result of Conjunctival Impression Citology and Nutritional state in Febrile Children

No.	M / F	Age (yr)	W (kg)	H (cm)	Nutri.State			Freq. of febrile	Duration (Day)	CIC OD	CIC OS
					W/A	W/H					
1	F	3	11,5	89	G	G	G	6	7	3	2
2	F	3	12	90	G	G	G	6	10	2	2
3	F	2	8,4	78	W	W	W	1	7	1	0
4	F	2	10	80	G	G	G	1	7	2	2
5	F	4,5	14	95	G	G	G	12	7	1	1
6	M	2	8	78	W	W	W	3	1	2	3
7	M	3	11	81	W	G	W	3	1	2	2
8	M	2	11	85	G	G	G	6	4	0	1
9	F	5	16	108	G	G	G	0	1	1	1
10	F	5	15	102	G	G	G	3	3	1	1
11	M	6	16	110	W	G	W	4	5	2	2
12	M	4	13	96	W	G	W	6	4	2	2
13	F	2,5	11	86	G	G	G	2	1	0	0
14	M	2,5	12,5	88	G	G	G	1	3	1	1
15	F	3	14	90	G	G	G	2	5	1	1
16	M	2,5	10	82	W	G	W	3	4	1	1
17	M	2,5	11	84	G	G	G	4	5	2	2
18	F	6	18	112	G	G	G	3	3	1	2
19	F	7	19	116	G	G	G	2	4	1	1
20	F	4	14	98	W	G	W	4	2	2	2

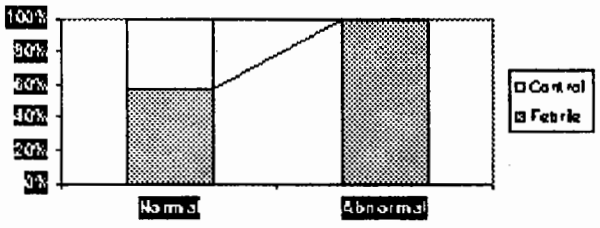
Tabel 2. Result of Conjunctival Impression Citology and Nutritional state in Control Group

No.	M / F	Age (yr)	W (kg)	H (cm)	Nutri. State			CIC OD	CIC OS
					W/A	W/H			
1	M	3,5	13	97	G	G	G	1	1
2	F	2,5	11	88	G	G	G	1	0
3	F	4,5	17	105	G	G	G	1	1
4	M	5	22	125	G	G	G	1	1
5	M	3	16	105	G	G	G	0	0
6	F	4,5	21	107	G	G	G	1	0
7	F	2	13	90	G	G	G	1	0
8	M	3	12	95	G	G	G	0	0

M = male ; F = female ; W = weight ; H = height ; G = good ; W = worse ; CIC = conjunctival Impression Cytology

Table 3. Correlation between Conjunctival Impression Cytology features of febrile and control group

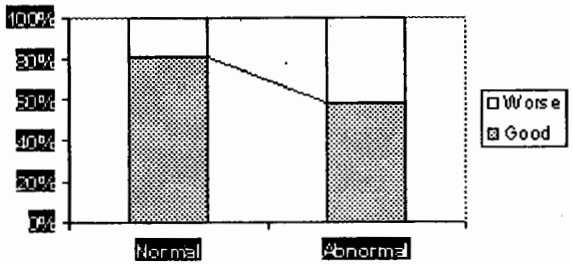
Group	Conjunctival Impression Cytology		Total
	Normal	Abnormal	
Febrile	21	19	40
Control	16	0	16
	37	19	56



X^2 : 9,481 DF : 1 P = 0,002

Table 4. Correlation between nutritional state and Conjunctival Impression Cytology features in febrile children

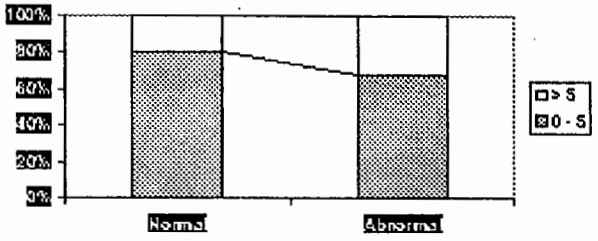
Nutritional State	Conjunctival Impression Cytology		Total
	Normal	Abnormal	
Good	17	11	28
Worse	4	8	12
	21	19	40



X^2 : 1, 574 DF : 1 P = 0,214

Table 5. Correlation between febrile frequency during the last one year and Conjunctival Impression Cytology features

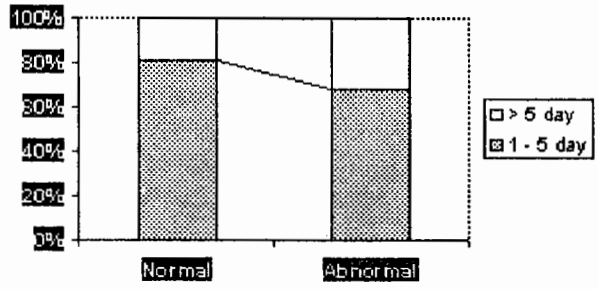
Febrile Frequency	Conjunctival Impression Cytology		Total
	Normal	Abnormal	
0-5	17	13	30
>5	4	6	10
	21	19	40



X^2 : 0,301 DF : 1 P = 0,583 corrected by Fisher exact test P = 0,473

Table 6. Correlation between duration of febrile and Conjunctival Impression Cytology features

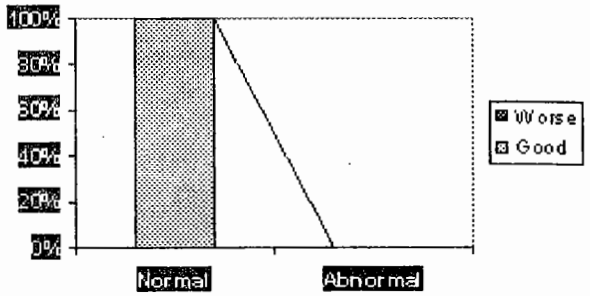
Duration of Febrile	Conjunctival Impression Cytology		Total
	Normal	Abnormal	
1-5 day	17	13	30
>5 day	4	6	10
	21	19	40



X^2 : 0,301 DF : 1 P = 0,583 corrected by Fisher exact test P = 0,473

Table 7. Correlation between Nutritional State and Conjunctival Impression Cytology in control group.

Nutritional State	Conjunctival Impression Cytology		Total
	Normal	Abnormal	
Good	16	0	16
Worse	0	0	0
	16	0	16



Vitamin A is one of micronutrients, which is essential for immunity, cellular differentiation, maintenance of epithelial surface, growth, reproduction, hematopoeisis and vision. This fat-soluble substance is found in foods from animal sources, including dairy products. Vitamin A can be ingested as its preformed state found in liver, cod-liver oil, or eggs. Provitamin A carotenoid can be ingested from sources like dark-green leavy vegetables, carrots, mangos and papayas. Retinol is esterified in the intestinal mucosa, packed into chylomicrons and carried to the liver via the lymphatic circulation. Provitamin A carotenoid such as b-carotene may be converted to retinal aldehyde through cleavage pathway by carotenoid-15, 15¢-dioxy genase or by an excentrie cleavage pathway. Approximately 90% of serum vitamin A in the body is stored in the liver as retinyl ester. Retinol is released from liver in a molar combination with retinol-binding protein and transthyretin and seems to enter certain target cells via specific receptor.[3,8,9,11,14,19] (figure 1).

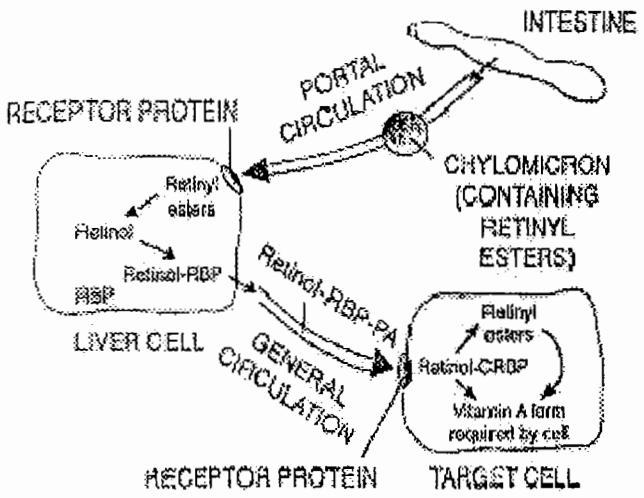


Figure 1. The processing and transport of vitamin A to its target cells.
RBP = retinol-binding protein; PA = prealbumin; CRBP = cellular retinol-binding protein. CRBP is used to bind retinol only inside cells.

In the cytosol, retinol undergoes conversion to retinoic acid within the nucleus, retinoic acid influences gene activation through specific receptors that belong to be superfamily of thyroid and steroid receptors (RAR) , act as transcriptional activators for specific target genes. RAR are expressed as several isoforms (referred to as RAR-a, RAR-b and RAR-g) for which all-trans retinoic acid acts as a ligand: for retinoid x receptor (RxR) (referred to as RxR-a, RxR-b and RxR-g) 9-cis retinoic acid acts as a ligand. Each RAR and each RxR has a specific DNA-binding domain by which it effects transcriptional activity. The DNA sequences required for the action of RAR and RxR are known as retinoic acid response elements (RARE). RAR and RxR as well as thyroid hormone and RxR, appear to form heterodimers and to effect trancriptional activity. (figure 2).[14,19]

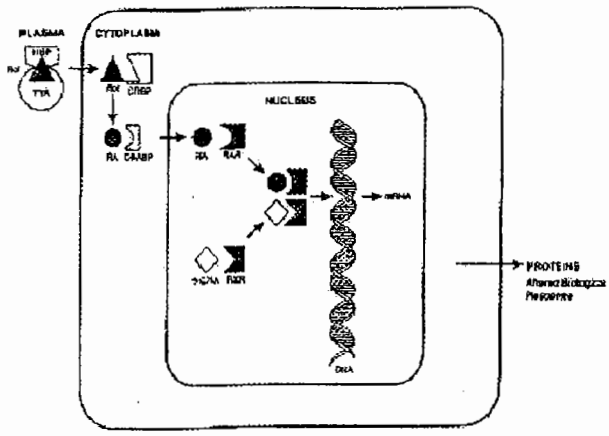


Figure 2. Metabolic pathway of vitamin A. Rol = retinol. RBP = retinol-binding protein. TTR = transthyretin. CRBP =cellular retinol-binding protein. RA = retinoic acid. CRABP = cellular retinoic acid binding protein. RAR = retinoic acid receptor. 9-CRA = 9-cis retinoid acid and RxR = retinoid x receptor. Thyroid hormone and vitamin D may also binnd with RxR.

Vitamin A deficiency is a co-factor for immunodeficiency disorder characterized by widespread alteration in immunity, including pathological alteration in mucosal surfaces, impaired antibody responses to challenge with protein antigens, changes in lymphocyte subpopulations, and altered T- and B- cell function. Vitamin A and its metabolites are immune enhancers that have been shown to potentiate antibody responses to T cell-dependent antigens, increase lymphocyte proliferation responses to antigens and mitogens, inhibit apoptosis and restore the integrity and function of mucosal surface.[14]

Ocular examination as a diagnostic method for vitamin A deficiency is well accepted. However, the clinical signs of xerophthalmia manifest is late, and so when they are noted, irreversible damage may already have been inflicated. Beside that, clinical examination is not specific. However, a biochemical assessment of vita-

min A state of a population is fraught with technical, analytical and cultural problem as blood needs to be extracted and analysed, careful storage and proper handling of blood samples in the field is challenging, and serum analysis is expensive. Thus, diagnosing vitamin A deficiency is complicated by issue of feasibility.[14,18,20]

Vitamin A deficiency both with clinical sign (xerophthalmia) and without one (sub clinical), can substantially increase the risk factor for childhood mortality from infectious and non infectious causes. In longitudinal study of 3000 Indonesian preschool children, the mortality rate among children with mild vitamin A deficiency was 4 to 12 times higher than among children with normal serum vitamin A level.[10,20]

In the other hand vitamin A serum level was influenced by infectious diseases sucs as diarhrea, respiratory tract infection, schistosomiasis, malaria, tuberculosis, leprosy, rheumatoid fever, measles and otitis media. Amount of depletion of serum vitamin A level in infectious disease influences the severity and duration of the disease. [3,15] In this study, conjunctival impression cytilogy is statistically different between febrile children and control group (table. 1).

Conjunctival impression cytology is a simple and reliable test for the detection of vitamin A deficiency. Amedee-Manesme et al in 1998 found that children with normal vitamin A serum have normal conjunctival impression cytology and children with vitamin A deficiency have abnormal conjunctival impression cytology. Natadisastra and co workers in 1987 proposed an abnormal conjunctival impression cytology in children with vitamin A deficiency.[9,10,13]

Nutritional state influences serum vitamin A level, but in this study we did not find any correlation between conjunctival impression cytology and nutritional state.[10,13] This might be due to that non of the sample members have suffered from severe nutritional state such as marasmic and kwashiorkor, and also the small sample size might leads to inaccurate conclusion (table 2).

Hadi et al in 1998 had found that frequency of infectious diseases influence serum vitamin A level. Duration of illness also influences the state of vitamin A serum.[5,14] In this study we didn't find the correlation between both factor and conjunctival impression cytology. This fact may be caused the same reason as stated above. (table 3-4)

CONCLUSION

The consequences of vitamin A deficiency include increased morbidity and mortality rate. The mechanism by which vitamin A deficiency increases morbidity and mortality is associated with increased rates of infectious disease made easier by disruption of mucosal barriers and by the decreasing humoral and cellular immune functions. In the other hand infectious diseases, such as respiratory disease, diarrhea, measles, tuberculosis, schistosomiasis etc. may leads to deficiency of vitamin A .

Conjunctival impression cytology is a simple and fairly sensitive method to detect deficiency of vitamin A in risk factor group such as febrile children, and children in community with bad sosio-economic condition, especially in endemic regions to identify risky group who requires vitamin A suplementation.

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