Conjungtival Impression Cytology (CIC) In Diarrheal Children In Pediatrics Department of M. Djamil General Hospital, Padang – Indonesia Preliminary Report

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Objective: *Digestive system infection such as diarrhea might cause malabsorbtion and low intake of vitamin A or reverse. Vitamin A deficiency may result an increase in mortality rate. This study was performed to observe the picture of the Conjunctival Impression Cytology (CIC) in children suffers acutely from diarrhea.* ***Methods:*** *Patient's age were between 2-7 years old, and hospitalized because of diarrhea at the pediatrics Department of M. Djamil General Hospital, Padang, between March and April 2003. Patients nutritional state were evaluated using Height, Weight and Age parameters as compared to standard (WHO) table. CIC test were done according to Tseng method. Examinations were done on the first day of hospitalization. Nutritional state, CIC picture were statistically analyzed. CIC examination, were done on 22 eye of patients suffering from diarrhea, and as a control from 16 eye.*

Result: *In diarrhea group, the result of CIC examination was statistically significant different from that found in control group. But the nutritional state, frequency of diarrhea in a year, and duration of the diarrhea didn't showed a statistical significance. Further study might be needed with on a larger number of cases and longer duration of diarrhea.* ***Conclusion:*** *CIC examinations are a simple test that could be used to detect vitamin A deficiency in diarrhea patients.*

Keywords : Conjungtival Impression Cytology (CIC) In Diarrheal Children In Pediatrics

INTRODUCTION

Vitamin A is a micronutrient that is essential for immunity, cellular differentiation, and maintenance of epithelial surfaces, growth, reproduction and visual functions. Vitamin A, is a fat-soluble, heat-stable nutrient (retinol), derived from animal sources, certain fruits and vegetables. Vitamin A can be ingested in its preformed state in liver, cod-liver-oil, egg or as provitamin A carotenoids found in dark-green leafy vegetables, carrots, mangos, and papayas. Vitamin A forms the basic component of retinal pigments and plays a vital role in optimal health, growth, and development. [3, 4, 5, 11, 15]

Vitamin A deficiency (VAD) (if serum retinol <20 μg/dL or <0.7 μmol/L), also sub clinical VAD can substantially increase the risk for childhood mortality rate from infectious and noninfectious diseases (1–3). VAD impairs the mobilization and transportation of iron and is usually associated with anemia and retarded growth [3, 4].

Vitamin A deficiency might cause blindness, increased morbidity and mortality rate among preschool children in many developing countries [1, 3]. Vitamin A deficiency which is also known as xerophthalmia in ophthalmology is manifested by night blindness, conjunctival xerosis and/or Bitot's spots. According to a previous study, Vitamin A supplementation for children in endemic area , appeared to reduces the mortal-

ity rate among them, suggesting that sub clinical vitamin A deficiency (i.e., vitamin A deficiency without ocular manifestations of xerophthalmia), is associated with children mortality. Vitamin A deficiency was estimated to affect more than 124 million children world wide, and represents the cause of 1 – 2,5 million of preventable death from associated diseases such as diarrhea and respiratory disorder. Even in normal children, diarrhea might leads to sub clinical vitamin A deficiency due to gastro-intestinal epithelial function failure that leads to mal-absorption of the vitamin A [4,11,12,13,15,16].

Several methods had been tried, and each has their own limitations to characterize the vitamin A status of individuals or of the whole community. Previous study suggests that impression cytology may represent a simple and reliable test to detect vitamin A deficiency. The test was first performed by Egbert et al. in 1977. A variety of materials including cellophane tape, photographic film and various synthetic filters had been tried. The conjunctival impression cytology (CIC) is a non-invasive method of obtaining ocular surfaces epithelium for evaluation. The conjunctiva is touched with a material to which epithelial cells will adhere. The cells are then stained and examined. CIC test reflects histologic changes in certain cells of the conjunctiva related to vitamin A state. CIC reveals a gradual reduction of goblet cells and morphological changes of epithelial conjunctiva with the severity of vitamin A deficiency. The test is more practical than the invasive determination of serum vitamin A concentrations. However, the reproducibility and validity of the relatively new method CIC compared with other methods of assessing vitamin A status has been inconsistent, needs further investigation.[2,6,7,10,14]

The purpose of this study was to compare CIC examination between diarrhea and normal children.

MATERIALS AND METHOD

This Study was performed at the pediatrics Department, M. Djamil Hospital, Padang, Indonesia on March – April 2003.

Number of patient was 22. Ages between 2 -7 years old and hospitalized because of diarrhea. Control group were normal children who comes to the department for immunization. Examinations were done on the first day of hospitalization. Before the examination, an agreement was asked from the patient's parent, and they were asked to fill informed consent. Nutritional status and CIC picture were compared and statically analyzed with Chi Square test (X^2) which was switched to Fisher exact test due insufficient number of cases.

Nutritional Status

Nutritional status were evaluated using Height, Weight and Age parameters in a certain formula compared to standard WHO nutritional table (WHO-NCHS). Good nutritional state when is 80% or more, bad nutritional state when less than 80 %.[cited from 9]

Conjunctival Impression Cytology (CIC) Examination

For CIC, the mother held the child in her arms while the child was distracted by a brightly colored object. A small piece (5 x 5 mm) of filter paper (Whatman paper) was applied with blunt forceps to the temporal quadrant of the bulbar conjunctiva (approximately 3 mm from the limbus) and gently removed after two seconds. After fixation, the specimens were stained with Periodic Acid-Schiff (PAS). All impression cytologic specimens were examined in masked fashion, and their stages were determined according to the degree of squamous metaplasia by Tseng methods (table 1). Conjunctival impressions were then divided as normal and abnormal. The CIC results was normal if impressions result were stage 0 or 1, abnormal if the results were stage 2 to 5.[8]

Table 1. –Staging of Conjunctival Squamous Metaplasia

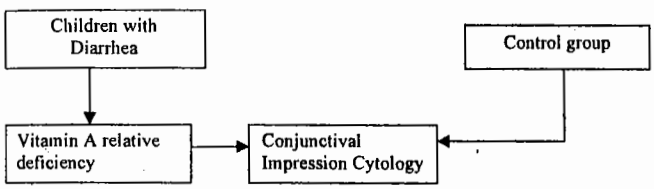
Stage	Criteria
0	Abundant goblet cells and mucin spots, small epithelial cells
1	Fewer goblet cells and mucin spot, small epithelial cells
2	Loss of goblet cells and mucin spot, enlarging epithelial cells
3	Enlarging and separating epithelial cells
4	Large, separate epithelial cell with scattered keratinization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

Frequency of Diarrhea

Diarrhea frequency was how many times the child suffered from diarrhea in the last 1 year.

Duration of Diarrhea

Duration of diarrhea was how long the child suffered from the diarrhea before hospitalized.



RESULTS

Twenty two children aged 2-7 years, male to female ratio 1,2 : 1 were take for sample (table.2), and 8 children were taken as control group (table 3). Nutritional states of all children were recorded. Conjunctival imprints were taken from both eyes.

Table.2 Results of CIC and nutritional state in children with diarrhea

No.	M / F	Age (y)	W (kg)	H (cm)	Nutr. status			Freq. (times)	duration (day)	CIC OD	CIC OS
					W/A	W/H					
1	M	2	9,4	84	B	G	B	6	2	2	2
2	F	5	15	105	G	G	G	0	1	2	2
3	F	3,5	13	98	G	G	G	2	1	3	2
4	F	2	12	85	G	G	G	2	1	1	1
5	F	5	14	110	B	B	B	8	1	2	2
6	M	5	14	103	B	G	B	0	1	2	2
7	M	3	11	90	B	G	B	4	1	1	2
8	F	5	14	105	B	G	B	2	1	1	1
9	M	2	9,4	81	B	G	B	9	3	1	1
10	M	5,5	19	112	G	G	G	0	1	0	1
11	M	4	13,5	105	G	G	G	10	3	3	3

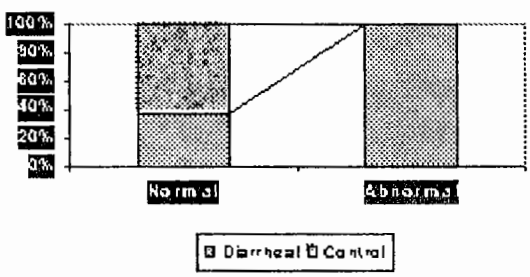
Table. 3 Results of CIC and nutritional status in control group

No.	M / F	Age (y)	W (kg)	H (cm)	Nutr. status			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 B: Bad

Table 4. Comparison of CIC result in diarrhea cases and Control group

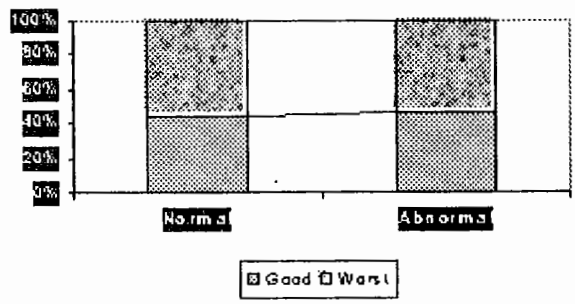
	conjunctival impression cytology		Total
	Normal	Abnormal	
Diarrhea	9	13	22
Control	16	0	16
	25	13	38



X^2 : 11,87 DF : 1 P = 0,05

Table 5. Comparison of nutritional status and CIC result in diarrhea children

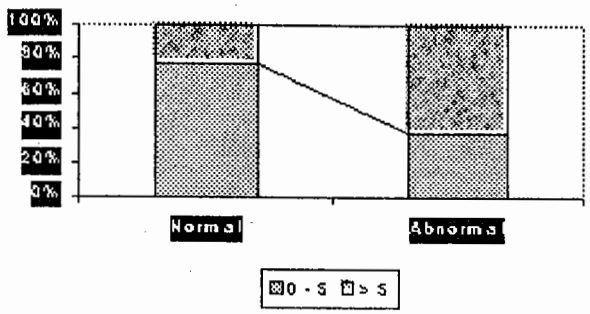
Nutritional status	conjunctival impression cytology		Total
	Normal	Abnormal	
Good	4	6	10
Bad	5	7	12
	9	13	22



X^2 : 0,127 DF : 1 P = 0,722 correction by Fisher exact test P= 1,000

Table 6. Comparison of frequency of diarrhea in the year and CIC result

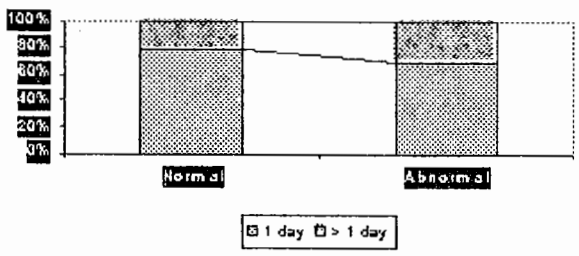
Frequency of diarrhea	conjunctival impression cytology		Total
	Normal	Abnormal	
0 – 5 times	7	5	12
> 5 times	2	8	10
	9	13	22



X^2 : 1,920 DF : 1 P = 0,166 corrected by Fisher exact test P= 0,099

Table 7. Comparison of the duration of diarrhea before hospitalized and CIC result.

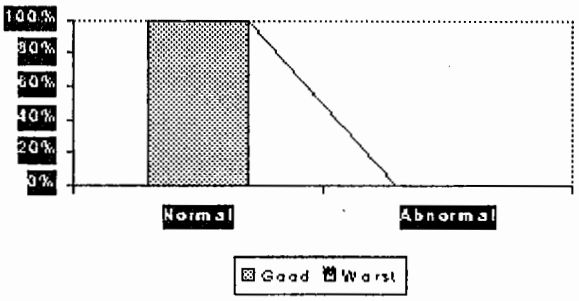
Duration of diarrhea	conjunctival impression cytology		Total
	Normal	Abnormal	
1 day	7	9	16
> 1 day	2	4	6
	9	13	22



X^2 : 0,002 DF : 1 P = 0,965 corrected by Fisher exact test P= 1,000

Table 8. Comparison of nutritional state and CIC result in control group

Nutritional status	conjunctival impression cytology		Total
	Normal	Abnormal	
Good	16	0	16
Bad	0	0	0
	16	0	16



DISCUSSION

The important functions of vitamin A are, its immunity supporting effect in developing T cell and B cell, thus influencing the severity of disease process and decreasing the morbidity of the infection, augmenting the differentiation of mucous epithelium cells.[11]

Many studies had shown that vitamin A level of the serum decreased in diseases such as respiratory tract infection, morbili and diarrhea, etc. The decreasing of vitamin A level in infectious diseases is in turn determined by the severity, duration and frequency of the disease.[11]

Table 4 shows that conjunctival impression cytology of children with diarrhea was statistically significant compared to children without diarrhea. It was revealed that children with diarrhea suffer relative vitamin A deficiency, similar to previous study.

According to Amedee-Manesme et.al (1998), children with normal vitamin A level will show normal conjunctival impression cytology result, and children with vitamin A deficiency show abnormality in their conjunctival impression cytology result.[14] Natadisastra

et. al. (1987) had found that abnormal conjunctival impression cytology results has a direct relationship with vitamin A deficiency.[8]

Nutritional state determines vitamin A deficiency incidence. Children with low nutritional status will show vitamin A deficiency, especially due to low intake and increased utilization. But no significant difference between nutritional status and conjunctival impression cytology was found in this study, this may be caused by too small amount of our sample.

Hadi A et.al. (1999) had found that the frequency of infection and diarrhea determines the degree of decrement of blood vitamin A level.[4] The more often the children suffer from diarrhea, the higher the possibility that the children will suffer from vitamin A deficiency due to low intake and absorption disorder of the vitamin A. Vitamin A deficiency on the other hand will also cause the children to get diarrhea easily. This study doesn't show significant relationship between frequencies of diarrhea within the time period of a year with conjunctival impression cytology result (Table 6.). This might be caused by the small amount of our sample and the inaccuracy of data presented by the parents. Most of the parents didn't precisely remember how many times their children suffer from the disease, especially those who have many children.

The more often children suffer from diarrhea, the lower intake they get, and the absorption of vitamin A will also become lower, so that the severity of the vitamin A deficiency will be increased. Table 7 shows that there's no significant relationship between duration of diarrhea and conjunctival impression cytology. This also caused by small amount of sample and inaccurate data from parents.

At least, when compared to the control group without diarrhea that shows normal conjunctival cytology result (table 8), this revealed that conjunctival impression cytology examination is sensitive enough to determine vitamin A deficiency in patients with diarrhea.

CONCLUSION AND SUGGESTION

Conjunctival impression cytology examination is a relative simple test and can be used to detect vitamin A deficiency in children with diarrhea. This study didn't have a firm conclusion yet about the relationship between nutritional status, diarrhea frequency and duration of diarrhea with conjunctival impression cytology. Because of the small amount of the sample and possible less accurate anamnesis, Further study might be needed with bigger sample size and longer period of time, and a more accurate recorded data card such as the so called "Kartu Menuju Sehat (KMS)" as guidance to determine nutritional status and diarrhea frequency, in endemic area of vitamin A deficiency.

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