Conjunctival 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 diar-
rea might cause malabsorption and low intake of vi-
tamin 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 Cy-
tology (CIC) in children suffers acutely from diarrhoea.

Methods: Patient’s age were between 2-7 years old,
and hospitalized because of diarrhoea 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 pa-
rameters as compared to standard (WHO) table. CIC
test were done according to Tseng method. Examina-
tions were done on the first day of hospitalization. Nu-
tritional state, CIC picture were statistically analysed.
CIC examination, were done on 22 eye of patients suf-
ferring from diarrhoea, and as a control from 16 eye.

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

Keywords: Conjunctival 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 nu-
trient (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 provi-
tamin A caroteinoids found in dark-green leafy veg-
etables, carrots, mangoes, and papayas. Vitamin A forms
the basic component of retinal pigments and plays a
vital role in optimal health, growth, and development.

Vitamin A deficiency (VAD) (if serum retinol <20
µg/dL or <0.7 µmol/L), also sub clinical VAD can sub-
stantially 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
(4).’

Vitamin A deficiency might cause blindness, in-
creased morbidity and mortality rate among preschool
children in many developing countries (5-7). Vitamin A
deficiency which is also known as xerophthalmia in
ophthalmology is manifested by night blindness, con-
njunctival xerosis and/or Bitot’s spots. According to a
previous study, Vitamin A supplementation for chil-
dren in endemic area, appeared to reduce the mortal-
ity rate among them, suggesting that sub clinical vita-
min A deficiency (i.e., vitamin A deficiency without
ocular manifestations of xerophthalmia), is associated
with childhood mortality. Vitamin A deficiency was es-
timated to affect more than 124 million children world-
wide, and represents the cause of 1 − 2.5 million of
preventable deaths from associated diseases such as di-
arrhea and respiratory disorder. Even in normal chil-
dren, diarrhea might lead to sub clinical vitamin A
deficiency due to gastro-intestinal epithelial function
failure that leads to mal-absorption of the vitamin A
(14,23,22,13,11).

Several methods have been tried, and each has their
own limitations to characterize the vitamin A status of
individuals or 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 Eggert et al. in 1977. A
variety of materials including cellophane tape, photo-
graphic film and various synthetic filters had been tried.
The conjunctival impression cytology (CIC) is a non-
invasive method of obtaining ocular surfaces epithe-
lium 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 histo-
logic changes in certain cells of the conjunctiva related
to vitamin A status. 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 determina-
tion of serum vitamin A concentrations. However, the
reproducibility and validity of the relatively new method
CIC compared with other methods of assessing vita-
min A status has been inconsistent, needs further in-
vestigation(1,2,10,14).

The purpose of this study was to compare CIC ex-
amination between diarrhea and normal children.

MATERIALS AND METHOD

This study was performed at the pediatrics depart-
ment, M. Djamal 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 agree-
ment 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 (X2) which was switched to Fisher ex-
tact test due insufficient number of cases.

Nutritional Status

Nutritional status were evaluated using Height,
Weight and Age parameters in a certain formula com-
pared to standard WHO nutritional table (WHO-NCHS).
Good nutritional state when is 100% or more, bad nutri-
tional state when less than 60%.

 Conjunctival Impression Cytology (CIC) Examination

For CIC, the mother held the child in, her arm while
the child was distracted by a brightly colored object. A
small piece (5 x 5 mm) of filter paper (Whatman pa-
per) was applied with lint freeg to the temporal
quadrant of the bulbar conjunctiva (approximately 7 mm
from the limbus) and gently removed after two seconds.
After fixation, the specimens were stained with Peri-
odic Acid-Schiff (PAS). All impression cytologic speci-
mens were examined in masked fashion, and their ages
were determined according to the degree of squamous
metaplasia by Tseng methods (table 1). Conjunctival impres-
sions were then divided as normal and abnor-
mal. The CIC results was normal if impressions result
were stage 0 or 1, abnormal if the results were stage 2
to 5.
Table 1. Staging of Conjunctival Squamous Metaplasia

<table>
<thead>
<tr>
<th>Stage</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Abundant goblet cells and mucin spots, small epithelial cells</td>
</tr>
<tr>
<td>1</td>
<td>Fewer goblet cells and mucin spot, small epithelial cells</td>
</tr>
<tr>
<td>2</td>
<td>Loss of goblet cells and mucin spot, enlarging epithelial cells</td>
</tr>
<tr>
<td>3</td>
<td>Enlarging and separating epithelial cells</td>
</tr>
<tr>
<td>4</td>
<td>Large, separate epithelial cell with scattered keratinization and pyknotic nuclei</td>
</tr>
<tr>
<td>5</td>
<td>Large keratinized epithelial cells with pyknotic nuclei or loss of nuclei</td>
</tr>
</tbody>
</table>

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 taken 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

<table>
<thead>
<tr>
<th>No.</th>
<th>M/F</th>
<th>Age (y)</th>
<th>W (kg)</th>
<th>H (cm)</th>
<th>Nutr. status</th>
<th>Freq. (times)</th>
<th>duration (day)</th>
<th>CIC</th>
<th>CIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>2</td>
<td>9.4</td>
<td>84</td>
<td>B G B</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>5</td>
<td>15</td>
<td>105</td>
<td>G G G</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>3.5</td>
<td>13</td>
<td>98</td>
<td>G G G</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>2</td>
<td>12</td>
<td>85</td>
<td>G G G</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>5</td>
<td>14</td>
<td>110</td>
<td>G B B</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>5</td>
<td>14</td>
<td>103</td>
<td>G B B</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>3</td>
<td>11</td>
<td>90</td>
<td>B G G</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>5</td>
<td>14</td>
<td>105</td>
<td>G G B</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>2</td>
<td>9.4</td>
<td>81</td>
<td>G G B</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>5.5</td>
<td>19</td>
<td>112</td>
<td>G G G</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>4</td>
<td>13.5</td>
<td>105</td>
<td>G G G</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Indonesian Food and Nutrition Progress, 2003 Vol. 10 no 1
Table 3 Results of CIC and nutritional status in control group

<table>
<thead>
<tr>
<th>No.</th>
<th>M / F</th>
<th>Age (y)</th>
<th>W (kg)</th>
<th>H (cm)</th>
<th>Nutritional status</th>
<th>CIC OD</th>
<th>CIC OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>3.5</td>
<td>13</td>
<td>97</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>2.5</td>
<td>11</td>
<td>88</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>4.5</td>
<td>17</td>
<td>105</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>5</td>
<td>22</td>
<td>125</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>3</td>
<td>16</td>
<td>105</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>4.5</td>
<td>21</td>
<td>107</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>2</td>
<td>13</td>
<td>90</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>3</td>
<td>12</td>
<td>95</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>

M: Male  F: Female  W: Weight  H: Height  G: Good  B: Bad

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

<table>
<thead>
<tr>
<th></th>
<th>Conjointival impression cytology</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>13</td>
</tr>
</tbody>
</table>

X²: 11.87  DF: 1  P = 0.05

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

<table>
<thead>
<tr>
<th>Nutritional status</th>
<th>Conjunctival impression cytology</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Good</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Bad</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Good</td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>

X²: 0.127  DF: 1  P = 0.722 correction by Fisher exact test P= 1.000

Indonesian Food and Nutrition Progress, 2003 Vol. 19 no.1
Table 6. Comparison of frequency of diarrhea in the year and CIC result

<table>
<thead>
<tr>
<th>Frequency of diarrhea</th>
<th>Conjunctival impression cytology</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>0 – 5 times</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>&gt; 5 times</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>

X²: 1.920            \( \text{DF} = 1 \) \( P = 0.166 \) corrected by Fisher exact test \( P = 0.099 \)

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

<table>
<thead>
<tr>
<th>Duration of diarrhea</th>
<th>Conjunctival impression cytology</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>1 day</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>&gt; 1 day</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>

X²: 0.002            \( \text{DF} = 1 \) \( P = 0.965 \) corrected by Fisher exact test \( P = 1.000 \)

Discusssion

The important functions of vitamin A are, its immuni

ty 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.\(^{15}\)

Many studies have shown that vitamin A level of the serum decreased in diseases such as respiratory tract infection, malaria 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.\(^{16}\)

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 Anodee-Manesme et al (1999), 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.\(^{16}\) Natadisastra
et. al. (1987) had found that abnormal conjunctival impression cytology results has a direct relationship with vitamin A deficiency.  

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. 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 Menjua Sehat (KMS)" as guidance to determine nutritional status and diarrhea frequency, in endemic area of vitamin A deficiency.

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