Assessment of Nutritional Status of Clinical Patients by Determining Normal Range of Oral Mucosal Apoptosis and Proliferation Rate

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Summary: The normal range of oral mucosal cell apoptosis and proliferation rate through a larger sample of non-malnourished crowd was investigated, and the nutritional status of clinical patients was assessed. Of 194 clinical patients selected according to “NRS2002” guidance, there were 167 non-malnourished patients and 27 malnourished cases, respectively. Twelve patients with toxic reactions of grade III after postoperative chemotherapy (POC) were chosen. The oral mucosal epithelial apoptosis and proliferation rate were measured by using flow cytometry. The statistical significance was processed by using unpaired t-test. The results showed that there was no significant difference in gender, age, and body weight between malnourished and non-malnourished groups. The normal range of oral mucosal epithelial apoptosis and the proliferation rate was (27.50±1.50)% and (15.12±1.68)% in non-malnourished patients, and that was (19.90±4.14)% and (6.66±5.68)% in the malnourished patients, respectively. It is concluded that the normal range of oral mucosa cell apoptosis and proliferation rate is achieved, which can not be influenced by gender, age, weight and other factors, and could be used as a sensitive and accurate index to assess the nutritional status of clinical patients.

Key words: oral mucosal epithelia; nutrition; apoptosis; proliferation

Malnutrition is one of the most common issues during the surgical treatment procedure[1]. Since last century, novel idea and modern technology had made revolutionary improvement in the field of surgical nutrition support. However, little progress has been made to improve the way to assess the nutrition status and provide necessary guidance. Traditional approaches for nutrition guidance, such as collecting disease history, measuring body index, and laboratory test, usually need systematic evaluation, and lack of accuracy and immediacy made it hard to monitor the nutrition status of patients in a dynamic way[2]. Without a sensitive and accurate approach to monitor nutrition status in a real-time fashion, it is very difficult to fully unfold the potential of modern nutrition support technique. At the mean time, a certain number of surgical patients have different levels of malnutrition caused by the disease itself, infection or surgical procedure[3-5]. Usually, immune system deficiency, slow recovery after surgery, and certain complications occur as a result of ignorance and/or delay in the monitoring of nutrition support of surgical patients. So how to monitor nutrition status of patients in terms of precise timing and accuracy and how to determine the duration of nutrition support coverage had become urgent issue for surgeon to deal with.

Cell is the fundamental unit for all metabolism process. In tissue, cell has very active metabolism activity, such as epithelial tissue, cells constantly produce raw material for protein and nucleic acid synthesis to maintain the process of synthesis of DNA, RNA and protein, which in turn keeps up the need for cell proliferation and metabolism. Lack of this raw substrate will directly affect the status of cells. In our previous study, we have demonstrated that monitoring cell status can provide better assessment for the status of body nutrition level[6]. Oral mucosal epithelial cell belongs to non-keratinized stratified squamous epithelium, can rapidly refresh between generations, and is very sensitive to the deficiency of nutrition substrate and can be easily collected. All these features provide us a novel approach to determine the nutrition status of patients in a quick and accurate fashion[7]. Previous studies have demonstrated the significant correlation between oral mucosal epithelial cell apoptosis rate and three proteins (retinol binding protein, transferrin and albumin), suggesting that the oral mucosal epithelial cell apoptosis rate is not only consistent with classic serologic index, but also serves as a much sensitive nutrition index[8]. In current study, the normal range of oral mucosal epithelial cell apoptosis and proliferation rate is established through testing a larger sample of non-malnourished crowd, and the index is used for further evaluation of nutrition status of patients, and to provide a novel approach for monitoring clinical nutri-
tion status in a more rapid and accurate way.

1 MATERIALS AND METHODS

1.1 Clinical Data
Of 194 clinical patients selected according “NRS2002” guidance, there were 167 non-malnourished patients including 103 males and 64 females with an average age of 47.79±3.85 years old, and 27 malnourished patients including 17 males and 10 females with an average age of 53.47±11.75 years old. Twelve patients with toxic reactions of grade III after postoperative chemotherapy (POC) were chosen, including 7 males and 5 females.

1.2 Collection of Mucosal Epithelial Cells
Every morning, after rinsing mouth with saline first, the mucosal epithelial cells of patients were collected through scrubbing mouth with sterile cotton swab several times gently. Then the swab was washed with saline 3 to 5 times. The saline solution was then passed through a 500-screen mesh. Cell suspension was further prepared through differential centrifugation, fixed with 80% cold ethanol, and keep at -20°C for overnight storage.

1.3 Detection of Apoptosis Rate by Using Sub-G1 Peak
After fixation, cells were washed with PBS twice first, then re-suspended with 80 μL PC buffer (192 mL 0.2 mol/L Na2HPO4 mixed with 8 mL 0.1 mol/L citric acid, then adjusting pH to 7.8) and incubated for 30 min in ice. Cells were washed with PBS once, then stained with 10 μg/mL PI and 0.1% RNaseA (Sigma, USA) in dark for 20 min. Cells were measured by flow cytometry using sub-G1 peak method established by Gong et al. [9-10]. Data were analyzed by Cellquest software (Becton Dickson, USA).

1.4 Measurement of Proliferation Rate by Using Ki-67/DNA Double Index
Fixed mucosal epithelial cells were separated first with centrifugation at 800 r/min for 5 min. After washing with PBS twice, cells were treated with 2.5 g/L TritonX-100 for 5 min. After washing twice with PBS, cells were incubated with 100 μL mouse anti-human Ki-67 monoclonal antibody (diluted with 10 g/L BSA) (0.25 μg antibody per 5×10^5 cells) at 4°C. The next day, cells were first washed with PBS once, then incubated with FITC-labeled goat anti-mouse IgG antibody (DAKO, USA) (1:40 dilution with 10 g/L BSA) for 30 min at room temperature. Cells were then washed with PBS once and stained with 10 mg/L PI and 0.1 g/L RNaseA for 20 min at room temperature in the dark. Cells were evaluated with FACSsort (BD, USA) with excitation at 488 nm. Data were analyzed with Cellquest software. Isotype control antibody was also purchased from DAKO (USA).

1.5 Nutrition Indicators for Mал nourished Patients
Retinol-binding protein, transferrin and albumin were measured by using ELISA following the manufacturer instructions[11].

1.6 Statistical Analysis
All data were presented as mean±standard error. The statistical significance was evaluated by using SPSS10.0 statistical software. P<0.05 was considered to be statistically significant.

2 RESULTS

2.1 Normal Range of Oral Mucosal Epithelial Cell Apoptosis Rate of Non-malnourished Patients and Impact of Gender, Age and Body Weight
The normal range of oral mucosal epithelial cells apoptosis rate and the impact of gender were shown in table 1. The apoptosis rate of normal oral mucosal epithelial cells (93% confidence interval, CI) was (27.50±1.50%). The unpaired t-test revealed that gender had no significant impact on the apoptosis rate.

<table>
<thead>
<tr>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total (%)</th>
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<tbody>
<tr>
<td>28.64</td>
<td>25.67</td>
<td>27.50</td>
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The impact of age on the oral mucosal epithelial cell apoptosis rate was shown in table 2. 167 non-nourished patients were divided into two age groups. The unpaired t-test indicated that there was no significant difference in the oral mucosal epithelial cell apoptosis rate between two age groups (24-49 year age group vs. 50-74 year age group) (P>0.05), suggesting no impact of age on the oral mucosal apoptosis rate.

<table>
<thead>
<tr>
<th>Mean (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.79</td>
<td>27.50</td>
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The impact of body weight on the oral mucosal epithelial cell apoptosis rate was shown in table 3. 167 non-nourished patients were divided into two body weight groups. The unpaired t-test indicated that there was no significant difference in the oral mucosal epithelial cell apoptosis rate between two body weight groups (50.0-67.5 kg group vs. 67.6-85.0 kg group) (P>0.05), suggesting no impact of body weight on the oral mucosal epithelial cell apoptosis rate.

<table>
<thead>
<tr>
<th>Mean (%)</th>
<th>Total (%)</th>
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<tbody>
<tr>
<td>25.00</td>
<td>27.50</td>
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The apoptosis rate of oral mucosal epithelial cells in malnourished patients (95% CI) was (19.90±4.14%).

2.2 Normal Range of Oral Mucosal Cell Proliferation Rate in Non-malnourished Patients and Impact of Gender, Age and Body Weight
The normal range of oral mucosal epithelial cell proliferation rate and the impact of gender were shown in