The Application Research of WBC Morphology Combined with NAP in the HFMD Co-Infection of Children

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Abstract

In this manuscript the authors have studied the application value of White blood cell morphology combined with neutrophils alkaline phosphatase staining integral value in treatment of pediatric HFMD combined with infection. They showed that comparing the NAP positive rate of HFMD co-infection with the NAP positive rate of other three groups respectively—reference group, HFMD group and anti-infection HFMD group—the t value is 25.7964, 28.2326, 28.3696 and the values of P are 0.0000; Comparing the NAP integral value of HFMD co-infection with the NAP integral value of other three groups respectively, which are reference group, HFMD group and anti-infection HFMD, the t value is 35.8687, 36.2664, 36.1374 and the values of P are 0.0000. They concluded that it is useful to apply WBC morphological examination combined with the positive rate and integral value of NAP staining to diagnose and treatment HFMD.

Keywords
HFMD Hand-Foot-and-Mouth Disease, WBC White Blood Cell Morphology, Leukocyte Morphology, Neutrophil Alkaline Phosphatase, Integral

1. Introduction

In recent years, hand-foot-and-mouth disease combined infection is a common pediatric disease in some hospitals especially in grassroots units [1] [2] [3]. WBC (White Blood Count) and leukocyte classification ratio is a clinical diagnostic criterion. However, due to WBC and leukocyte classification ratio varied from
individual to individual, from day to night and also be affected by mood swings
the test results of the method above cannot correctly reflect the status of patients
infection and identify the type of infection [4] [5] [6] [7] [8]. Bacterial culture
takes a lot time and it is also affected by drugs, sampling time, sampling sites and
sampling amount. Positive detection rate is low. It can hardly meet the require-
ments of clinical diagnosis [9] [10] [11] [12].

The purpose of this paper is to evaluate the clinical application value of the
method and to promote this method in other hospitals. The method is taking the
doctor’s diagnosis, WBC morphology and the integral value NAP positive rate as
an ideal and rapid detection index in the treatment of pediatric co-infection HFMD.
Below is the result.

2. Material and Method

2.1. The Demographic Characteristics of the Study Object Are
Shown in Schedule 1

Research Object: 150 cases of research objects are sampled from the sick children
in the Outpatients and inpatients of Pediatrics of our hospital from January
2017-October 2017.

According to the inspection results and illness they were divided into three
groups, the normal control group, HFMD group and HFMD co-infection. Each
group includes 50 cases. Demographic and clinical characteristics of the study
subjects are shows in Table 1. After treatment, we take the HFMD co-infection
group as Anti-infection HFMD group. The normal group is healthy children
who do medical examination in our hospital; the HFM group is children who
infected with hand, foot and mouth disease without other pathogen infection;
the HFMD concurrent infection group is children who not only infected with
hand, foot and mouth disease but also with other pathogen infection. Both Med-
ical ethics committee of our hospital and the parties concerned or their family
members agree that the 150 patients as the research object. The research is
without any legal disputes.

2.2. Diagnostic Criteria of Hand-Foot-and-Mouth Disease

1) Clinical diagnosis cases: child patient has some symptoms such as illness
urgent, fever, the palm of hands or feet with maculopapule and herpes; Oral
mucosa herpes; tetter with inflammatory flush around can be found on hip or
knees; There is less fluid in the blister; Patients feel pain obviously. Some child
patient may be accompanied by loss of appetite, cough, nausea, vomiting, runny
nose, headache and so on. a) some patient have the HFM symptoms as well as
muscle clonus or encephalitis, cardiopulmonary failure, delayed acute paralysis,
pulmonary edema, etc.; b) Infant patients in hand, foot and mouth disease epi-
demic areas has no HFMD typical performance but have a fever accompanied by
muscle clonus or encephalitis, cardiopulmonary failure, delayed acute paralysis,
pulmonary edema.
Table 1. Demographic and clinical characteristics of the study subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls</th>
<th>HFMD</th>
<th>HFMD concurrent infection</th>
<th>Anti-infection HFMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 50</td>
<td>n = 50</td>
<td>P value</td>
<td>n = 50</td>
</tr>
<tr>
<td>Age (years) mean ± SD</td>
<td>2.62 ± 0.65</td>
<td>2.64 ± 0.72</td>
<td>0.4422</td>
<td>2.65 ± 0.74</td>
</tr>
<tr>
<td>Gender, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32 (64.0)</td>
<td>33 (66.0)</td>
<td>0.8339</td>
<td>31 (62.0)</td>
</tr>
<tr>
<td>Female</td>
<td>18 (36.0)</td>
<td>17 (34.0)</td>
<td></td>
<td>19 (38.0)</td>
</tr>
</tbody>
</table>

2) Laboratory diagnosis cases: Laboratory diagnosis cases is the Clinical diagnosis cases which meet one of the below condition: a) Viral isolation: enterovirus can be found in feces, anal swab, pharynx swab, throat lotion, cerebrospinal fluid, herpes fluid or samples of lung, brain, spleen, lymph nodes and other organizations; b) Serologic testing: the detection of specific IgM antibody in patient’s serum is positive or IgG antibody is 4 time higher than normal level in acute phase and convalescence. c) Nucleic acid testing: Pathogen nucleic acid can be found in feces, anal swab, pharynx swab, throat lotion, cerebrospinal fluid, herpes fluid or samples of lung, brain, spleen, lymph nodes and other organizations.

2.3. Inspection Method

To Collect 1 - 2 ml venous blood for each of 150 children using disposable EDTA expansion condenser vacuum blood collection tube. Making 2 blood films, one is Wright staining and the other is staining according to the NAP kit instructions after 10% formaldehyde fixed 30 seconds after the blood dry.

The staff of lab identifies the morphological changes in the WBC and counting neutrophils positive rate and integral under the microscope. Statistical analysis of the changes of morphological changes in the WBC and NAP positive rate integral of the HFMD co-infection group.

2.4. Statistical Analysis

Do statistical analysis using SPSS19.0 statistical software, comparing the NAP positive rate and integral value of every group via t test result of two independent samples. Comparing the cases of Morphological change via result of $\chi^2$ or Fisher’s exact probability method, $P < 0.05$ is statistical significance.

3. Results

Among the 4 group patients, NAP positive rate of the normal control is $(23.58 \pm 11.89)\%$ and integral value is $28.18 \pm 13.82$ (Figure 1); NAP positive rate of HFMD group is $(22.8 \pm 10.49)\%$ and integral value is $26.92 \pm 11.9$; NAP positive rate of the HFMD co-infection group is $(77.96 \pm 8.99)\%$ and integral value is $332.7 \pm 58.42$; NAP positive rate of Anti-infection HFMD group is $(22.38 \pm 10.54)\%$ and integral value is $27.74 \pm 12.16$; Comparing the NAP positive rate of
HFMD concurrent infection group with the NAP positive rate of other three
groups respectively, those are reference group, HFMD group and Anti-infection
HFMD group, t value is 25.7964, 28.2326, 28.3696. The values of P are 0.0000;
comparing the NAP integral value of HFMD co-infection with the NAP integral
value of other three groups respectively, those are reference group, HFMD group
and Anti-infection HFMD, t value is 35.8687, 36.2664, 36.1374. The values of P
are 0.0000; there is no statistical significance to compare the NAP positive rate
and the NAP integral value among the normal control, HFMD group and An-
ti-infection HFMD. Abnormal lymphocytes and vacuoles degeneration occasion-
ally be observed from the result of White blood cell morphology of both the
normal control group and the HFMD group. Many abnormal lymphocytes, toxic
particles cavitating cells and Dohle body can be observed from the result of
White blood cell morphology of both HFMD concurrent infection group and
Anti-infection HFMD group. The comparison result of the incidence of abnor-
mal lymphocytes and vacuolar degeneration cells in White blood cell morphol-
ogy between the normal control group and the HFMD group shows that the t is
respectively 5.8005, 8.0225, the all P is respectively 0.0000. The comparison result
of the incidence of abnormal lymphocytes and vacuolar degeneration cells in
White blood cell morphology between the normal control group and shows that
t is respectively 15.3897, 18.7609, the all P is respectively 0.0000. Contrast the in-
cidence of abnormal lymphocytes and vacuolar degeneration cells in White
blood cell morphology between the normal control group and the Anti-infection
group we can see that t is respectively 2.5586, 1.8123, P is respectively 0.0060,
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0.0365; Contrast the incidence of abnormal lymphocytes and vacuolar degeneration cells in White blood cell morphology between the HFMD group and the HFMD concurrent infection group, we can see that t is respectively 7.0166, 16.7142, the all P are respectively 0.0000. The comparison result of the incidence of abnormal lymphocytes and vacuolar degeneration cells in White blood cell morphology between the HFMD group and Anti-infection HFMD group shows that it is 4.3740 and 7.7346 respectively, the all P are respectively 0.0000. The comparison result of the incidence of abnormal lymphocytes, toxic particles cavating cells, vacuolar degeneration cells and Dohle body in White blood cell morphology between the HFMD concurrent infection group and the Anti-infection HFMD group shows that t is respectively 16.0121, 28.0628, 18.5339, 6.0320, the all P are respectively 0.0000. Detail result is shown in below Table 2 and Table 3. White blood cell morphology is shown in below Figure 2.

Table 2. Comparison of NAP positive rate and integral value of 4 groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>The normal control group</th>
<th>The HFMD group</th>
<th>HFMD concurrent infection group</th>
<th>The anti-infection HFMD group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive rate %</td>
<td>23.58 ± 11.89</td>
<td>22.8 ± 10.49</td>
<td>77.96 ± 8.99</td>
<td>22.38 ± 10.54</td>
</tr>
<tr>
<td>Integral value</td>
<td>28.18 ± 13.82</td>
<td>26.92 ± 11.9</td>
<td>332.7 ± 58.42</td>
<td>27.74 ± 12.16</td>
</tr>
</tbody>
</table>

Table 3. The comparison of four groups’ WBC morphology.

<table>
<thead>
<tr>
<th>Group</th>
<th>The normal control group</th>
<th>The HFMD group</th>
<th>HFMD concurrent infection group</th>
<th>The anti-infection HFMD group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal lymphocytes (%)</td>
<td>1.52 ± 1.93</td>
<td>4.04 ± 2.39</td>
<td>6.84 ± 1.50</td>
<td>2.36 ± 1.29</td>
</tr>
<tr>
<td>Toxic particles cells (%)</td>
<td>0</td>
<td>0</td>
<td>40.14 ± 9.44</td>
<td>2.26 ± 1.41</td>
</tr>
<tr>
<td>Vacuolar degeneration cells (%)</td>
<td>0.96 ± 1.43</td>
<td>3.10 ± 1.23</td>
<td>19.76 ± 6.94</td>
<td>1.40 ± 0.95</td>
</tr>
<tr>
<td>Durer corpuscle (%)</td>
<td>0</td>
<td>0</td>
<td>2.90 ± 2.73</td>
<td>0.50 ± 0.68</td>
</tr>
</tbody>
</table>
4. Discussion

HFMD (hand-foot-and-mouth disease) a kind of infectious disease caused by intestinal virus serious damage to children’s healthy life. According to the report, more than 20 intestinal virus strain can cause HFMD, among which Coxsackie A16 (CA16) and enterovirus 71 (EV 71) is the most common [13] [14] [15].

Children under the age of five are susceptible to infection. Who infected usually has symptoms such as low thermal pain, anorexia, oral pain, hands, foot and mouth with small herpesis or ulcers. Most infected children whose immune system is strong can heal in a week, while the other few patients with complications such as pulmonary edema, aseptic meningoencephalitis, myocarditis, etc. [16] [17] [18]. Some severe individual patient’s disease develops rapidly, causing heart failure and death. Currently there is no particularly effective medicine for the treatment, most of the clinical treatment can only symptomatic treatment [19] [20].

This paper is mainly study the changes of White blood cell morphology of those HFMD concurrent infection and the integral value and positive rate of NAP and analyze and contrast the differences with the normal control group, HFMD group and the Anti-infection HFMD group. The results showed that Neutrophil alkaline phosphatase positive rate and integral value of HFMD infected with complications is higher contrast the normal control group, HFMD group and the Anti-infection HFMD group. The comparison results of positive rate: t are 25.7964, 28.2326, 28.3696, P are all 0.0000; The comparison results of integral value: t are 35.8687, 36.2664, 36.1374, P are all 0.0000; he comparison results of the other groups is no statistical significance, all P are less than 0.05, P < 0.05. That is to say, positive rate and integral value of NAP of those infected HFMD with complications changes a lot and become normal after anti-infection. This suggests that clinic those infected HFMD with complications should do anti-infection treatment as soon as possible so that avoiding aggravation infection and endangering life. So it has very important clinical significance for those with HFMD should do examination in time to exclude the concurrent infection.

This study also shows that WBC morphological changes of patient who with HFMD concurrent infection changes more obviously than the one of the regular group, the HFMD group and Anti-infection HFMD group. Abnormal lymphocytes and vacuoles degeneration occasionally be observed from the result of White blood cell morphology of both the reference group and the HFMD group. Many abnormal lymphocytes, toxic particles cavitating cells and Durer corpuscle can be observed from the result of White blood cell morphology of both HFMD concurrent infection group and the Anti-infection HFMD group. From comparison data of HFMD concurrent infection group and the others three groups, the regular group, the HFMD group and the Anti-infection HFMD, it can be found that the positive rate of abnormal lymphocytes, the t are respectively 15.3897, 7.0166, 16.0121, P are all 0.0000 and the positive rate of Vacuoles degeneration of cells, the t are respectively 18.7609, 16.7142, 18.5339, P are all 0.0000. Toxic
particles cavitating cells and Durer corpuscle was not found from test results of the WBC morphology of both the regular group and the HFMD group. From comparison data of HFMD concurrent infection group and the Anti-infection HFMD, it can be found that the positive rate of toxic particles cavitating cells and Durer corpuscle, the t are respectively 28.0628, 6.0320, P are all 0.0000. It is approved that changes of the leukocyte morphology of those HFMD concurrent infection is a very important measurement index for indicating and monitoring the status of WBC morphology. It enables doctor to timely intervention, so as to achieve therapeutic effect.

5. Conclusion

As mentioned above, it has very important clinical significance that taking the examination of leukocyte morphology and the positive and integral value of NAP as the diagnosis index and the prognosis monitoring for pediatric HFMD concurrent infection. It is worth to promote in other hospitals.

6. The Limitation of the Study

The sample cases only include the patient in our hospital, and the study result just reflect the local situations not other places. A lot of manpower material resources is necessary if study the situation of other places. What's more, due to selecting the sample cases take a long time and large span on the test time of all observation indexes, so there is some limitation in quality control.

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