E-ROSETTE PHENOMENON FOR THE EARLY DIAGNOSIS OF NEOPLASTIC CONDITIONS IN BUFFALOES

Pradeep Kumar1*; Prahlad Ubhale2; Y. Hari Babu3

ABSTRACT

A study was undertaken to diagnose neoplastic conditions during early stages of development in buffaloes based on change in T-lymphocyte count. In this study, total leukocyte count (TLC) and differential leukocyte count (DLC) were used to find the elicitation of immune response to neoplastic conditions and E-rosette formation by T-lymphocytes with sheep RBCs was used to differentiate T-lymphocyte and B-lymphocytes and enumerate T-lymphocytes. The increase in total leukocyte count (TLC) indicated that there was activation of specific immunity against tumor. The increase in lymphocyte count in differential leukocyte count (DLC) indicated that lymphocytes were activated during neoplastic conditions. A simple but reliable methodology of E-rosette formation for differentiation and enumeration of T-lymphocytes showed increase in T-lymphocyte population. This emphasizes that during early stages of neoplastic conditions there is increase in T-lymphocyte population and the condition can be diagnosed by counting the E-rosettes. The technique could well be used to diagnose and monitor the neoplastic conditions.

Keywords: E-rosette, T-lymphocytes, Buffalo, Neoplasm

Currently, the use of surface markers is receiving widespread application in clinics for both diagnosis and management of many diseases. Further, one of the most important discoveries is that T-lymphocytes form spontaneous E-rosettes with sheep erythrocytes (S RBCs), proving one of the simplest biological markers for identifying T-lymphocytes (Dow, et. al., 1977, Vijay, et.al., 1983 and Francis and Thomas, 1974). The surface markers on T-lymphocytes responsible for rosette formation are CD3 and CD11. The ligand for the surface marker CD3 is CD58 which is extensively present on sheep erythrocytes. Hence sheep RBCs readily form rosettes with T-lymphocytes (Tizard, 1996).

Most commonly occurring neoplastic conditions in bovines (cattle & buffaloes) are eye orbit (33.6%), tumor of eyelid, conjunctiva, lacrimal galand (14.3%) (Theilen and Madewell, 1987). Survival of a patient

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with cancer/neoplasm is dependent mainly on localization, histogenesis and stage of the disease at the time of diagnosis. Diagnosis of the neoplasm cases at the early stages forms a major phenomenon for the treatment and recovery of the patient. Considering the importance of T-lymphocytes in anti-tumor immunity, the present research work has been undertaken to differentiate T-lymphocytes from B-lymphocytes using E-rosette formation of with sheep RBCs and then assessing T-lymphocyte population in the blood vis-à-vis with the occurrence of neoplasms.

**Source of Sheep Erythrocytes:**

The blood from sheep (UAS breed) from veterinary college, Bidar was collected by using Alsever’s anti-coagulant.

**Preparation of sheep RBC suspension (0.5%):**

Sheep RBCs collected were washed thrice with PBS and then 0.5% RBC suspension was prepared by adding 0.5 ml of sheep RBCs in 100 ml PBS.

**Lymphocytes from buffaloes with neoplastic condition**

The blood of 42 buffaloes with neoplastic condition (35 eye tumor and 7 leg tumor) was collected and lymphocytes were isolated. The lymphocytes from blood of healthy buffalo were collected as control.

**Isolation of peripheral blood leucocytes:**

Separation of peripheral blood leucocytes from the blood was carried out according to the method of Boyum, 1968 by density gradient centrifugation with some modifications (Veerupaksh, 1999). The white opaque ring obtained was taken out carefully with Pasteur pipette in a centrifuge tube. If any RBCs present were lysed by adding 4-5 ml of 0.87% ammonium chloride to the tubes and centrifuged at 200 rpm for 10 min. The mononuclear pellet was collected and resuspended in 5 ml of RPMI and centrifuged at 2000 rpm for 10 min. The pellet was resuspended in RPMI.

**E-rosette formation:**

Equal volumes of the lymphocyte suspension and 0.5 % SRBCs (0.5 ml each) were mixed, centrifuged at 2500 rpm for 10 min and maintained at 4°C for 40 min. The supernatant was removed and lymphocyte-SRBC pellet was resuspended in RPMI. 2% glutaraldehyde in normal saline was added to the suspension (lymphocyte-SRBCs) to get final concentration of 0.66% and mixed thoroughly. The mixture was taken in serological test tubes and centrifuged at 600 rpm at 4°C for 15 min. The supernatant was removed and pellet was resuspended in PBS. A drop of above suspension was put on a micro slide, slide was prepared and E-rosettes were counted under the microscope.

Fig 1

E-Rosette Formation
Total leukocyte count and differential leukocyte count

From the blood samples collected TLC and DLC were done by using Coles, 1986 method.

The average TLC (Table 1) in the buffaloes with neoplastic condition showed $2.23 \times 10^4$ cells/mm$^3$ in comparison to normal cell count $1.08 \times 10^4$ cells/mm$^3$ indicating remarkable increase in the leukocyte count. The results of DLC (Table-2) showed increase in the count of lymphocytes (74%) when compared to the healthy animal (64%).

The differentiation of T-lymphocytes from B-lymphocytes and enumeration of T-lymphocytes was done by using E-rosette formation where results are given in Table-3 (Fig. 1). Normally, the ratio of T-lymphocytes to B-lymphocytes is 80:20 in the peripheral blood. In the present study T-lymphocytes (E-rosette) count showed 89% on an average to 11% B-lymphocytes.

These findings showed that there was enormous increase in T-lymphocyte population.

Table 1.
Total Leukocyte Count (TLC)

<table>
<thead>
<tr>
<th>Blood parameter</th>
<th>Neoplastic condition</th>
<th>Healthy animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC (average)/mm$^3$</td>
<td>$2.23 \times 10^4$</td>
<td>$1.08 \times 10^4$</td>
</tr>
</tbody>
</table>

Table 2.
Differential Leucocyte Count (DLC)

<table>
<thead>
<tr>
<th>Leukocyte condition</th>
<th>Lymphocytes (average)</th>
<th>Monocytes (average)</th>
<th>Neutrophils (average)</th>
<th>Eosinophils (average)</th>
<th>Basophils (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoplastic condition (%)</td>
<td>74</td>
<td>4</td>
<td>18</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Healthy animal (%)</td>
<td>64</td>
<td>4</td>
<td>26</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3.
Average E-rosette count

<table>
<thead>
<tr>
<th>Condition</th>
<th>Neoplastic condition</th>
<th>Average</th>
<th>Healthy condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells</td>
<td>Eye tumor (average)</td>
<td>Leg tumor (average)</td>
<td></td>
</tr>
<tr>
<td>E-rosettes (T-lymphocytes) (%)</td>
<td>88</td>
<td>90</td>
<td>89</td>
</tr>
<tr>
<td>B-lymphocytes (%)</td>
<td>12</td>
<td>10</td>
<td>11</td>
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indicating the possibility of the neoplastic / cancerous condition in the animal. And this increase can easily be found out by using the E-rosette formation by t-lymphocytes with sheep RBCs.

There are many techniques for the diagnosis for the diagnosis of neoplasms like routine blood studies, routine X-rays, special X-rays/ endoscopic studies, radio nucleic scans. Still the use of biological surface markers is found to be most important, reliable and accurate for the diagnosis of neoplastic conditions at the early stages. E-rosette formation is one of the technique employing biological surface markers to differentiate and enumerate T-lymphocytes which could be used for the early diagnosis of neoplastic conditions.

REFERENCES


