ALTERED LYMPHOCYTE SUB-POPULATIONS IN CHILDREN SUFFERING FROM RECURRENT MULTIPLE INFECTIONS

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SUMMARY: Children suffering from a variety of chronic and recurrent infections are suspected to be deficient in one or more than one compartment of their immune system. Peripheral blood lymphocytes of a group of 30 such children were bioassayed through E-rosetting. Thirty healthy children were also included as controls. Significantly lower means of total leukocytes, total lymphocytes and rosetting lymphocytes were obtained with sick children as compared to the respective values for the healthy children. However, the mean non-rosetting cells of the diseased children was 20% higher than that of the controls. The phenomenon is discussed. Plausible association of altered lymphocyte population, autoimmune disorders and malignancies is discussed as well. A general lymphocytopenia even in the healthy children is also being reported.

Key Words: Atypical infections, immunologic status, e-rosette assay.

INTRODUCTION

Child mortality is a major threat especially in a third world country like Pakistan. While the use of antibiotics and timely preventive measures such as vaccination has brought down the death incidence due to bacterial and viral infections to a great extent, the occurrence of cancers, tumors and immunologic disorders is increasing. Cases with symptoms of unknown etiology and pathological complications are being brought more frequently.

Chronic and atypical infections are usually secondary to immunodeficiency. Deficiency in either one or more than one compartment of immune system may be present. The extent of immunodeficiency is measured by frequency and function of lymphocytes and granulocytes. A number of screening methods are available to evaluate the immune status of an individual. One such bioassay utilizes the ability of population of normal peripheral lymphocytes to form rosettes with the unsensitized sheep red blood cells (SRBCs) (5,9,17). Rosettes comprise of a lymphocyte to which three or more SRBCs are attached. Evidence is at hand that the E-Rosette forming lymphocytes are derived from thymus, known as T-cells (25), and are the main cells of immune response.

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E-Rosette assay is a convenient method employed for identification and enumeration of this immunologically important lymphocyte population.

The lymphocytes that do not directly bind with the SRBCs are the bursa derived B-cells. They require the anti SRBCs IgG which is attached to Fc receptor present on these cells (18).

Alterations in the numbers of peripheral lymphocyte population have been reported to be associated with a variety of conditions, such as leukemias, malignancies, autoimmunity, rheumatic disease of heart, rheumatic fever, chromosomal aberrations and a variety of infections (3,10,12,15, 20-23, 25).

Present study was conducted to analyze the immune status of children with a history of chronic or recurrent atypical infections. The interest was initiated due to known associations of such infections with immunodeficiencies which in turn are one of the predisposing factors for malignancies.

MATERIALS AND METHODS

A total of thirty children with recurrent multiple infections showing symptoms of various malignancies and/or immune disorders, ranging from age of eight months to eleven years were the subject of this study. A similar number of healthy

Table 1: Summary of statistics of diseased and healthy children.

	Diseased	Control
Age in years*	4.91±0.58	6.41±0.39
Body weight (kg)*	13.21±0.73	17.0±1.01
Ratio Male/Female	19/11	21/9
Age at onset of symptoms in months*	4.63±0.37	-
Associated malignancies	17/30	-
Under radiotherapy	11/30	-

* Values are means of 30 readings±S.E.

children was also included as controls. All of the patients were either admitted in or brought to National Institute of Child Health for treatment. The healthy children were either visiting the OPD of the hospital due to minor accidental injuries or were selected at random from various localities of the city. Summary of personal data of both the groups is provided in Table 1.

The test subjects had a history of high fever, septicemia, gastrointestinal tract, urinary tract or respiratory tract infections since five months after birth. Their general health was very poor and symptoms were acute. The hospital authorities had diagnosed seventeen of them to be suffering from various malignancies and immune disorders listed in Table 2. However, immune status of these individuals was not evaluated. Even out of seventeen children with some kind of malignancy were under radiotherapy.

Single venous blood sample was drawn from each subject and collected in heparinized vials. A small aliquot of blood was saved for enumeration of total WBCs and differential blood count. Rest of it was layered on Ficoll I (Histopaque 1077; Sigma Chemical Co.) and subjected to density gradient centrifugation as described by Boyum (7). The separated lymphocytes were washed three times with RPMI-1640 (Flow Laboratories) and resuspended in the same.

Table 2: Incidence of various diseases/disorders among the test subjects.

Diseases/Disorders	Frequency
Hodgkin' disease	8
Non-Hodgkins Lymphoma	4
Burrkit's Lymphoma	2
Acute lymphocytic Leukemia	3
Systemic Lupus Erythematosus	2
Rheumatoid Heart Disease	3
Rheumatic Fever	2
Meningititis	1
Encephalitis	1
Hepatitis	2
Pneumonia	1
Glomerulonephritis	1

Absolute numbers of lymphocytes were estimated using Neubar ruling chambers while the relative values for the same were obtained by observing blood smears through Field's Staining. T-lymphocytes were identified by rosetting with SRCB. Sheep red blood cells were collected from sheep's heparinized blood, washed three times with phosphate Buffered Saline and made upto 3% suspention in PBS. Separated lymphocytes were mixed with equal volumes of 3% SRBSs and Fetal Calf Serum (Flow Laboratories). The mixture was centrifuged at 200 g for five minutes at room temperature and incubated in ice bath for six minutes. The tubes were then gently agitated to resuspend the cells in the pellet and then loaded on heamocytometer rosetting lymphocytes generally surrounded by five to eight SRBSs, were counted. Eosin Y was used to distinguish living cells from the lysted ones. Values obtained were transformed to number of cells/ml of undiluted blood.

Data obtained from the assay of T-cells were analyzed employing t-test. A chi-square test of the independence was also used to check association between total lymphocytes and the rosette forming cell counts and incidence of recurrent multiple infection in children.

RESULTS

Statistics of various cell counts are provided in Table 3. Individual counts of the four types of cells in all 60 subjects included in this study are plotted in scatter diagrams (Figures 1 and 2). Respective means and standard errors are depicted through vertical bars.

The diseased children had significantly lower means for the total leukocyte, total lymphocytes and rosetting lymphocyte counts as compared to the respective variables of control sample. Probability being <0.001 in each case.

Total lymphocytes in the sick children as compared to the controls were depleted to 46.28%. A similar comparison of the rosetting cells revealed that lymphocytopenia in the diseased children was even more marked in this compartment of immune system, the number decreased to 17.0%. A reverse situation existed between the non-rosetting cells of the control and test subjects, the means for the being 0.34x10.6 cells/ml and 0.41x10.6 cells/ml, respectively. The difference was significant with $p<0.05$.

Comparison of numbers of rosetting and non-rosetting cells of both groups of children with that of the total lymphocytes of the healthy children (1.21x10.6 cells/ml)

Table 3: Frequencies and ratios of lymphocytes and their sub-populations in children suffering from atypica infections and healthy controls.

	Diseased	Control
Total leukocytes *	5.27±0.09	8.14±0.124
Total lymphocytes *	0.56±0.031	1.21±0.04
Lymphocytes percent of leukocytes	10.62	14.81
Rosetting lymphocytes*	0.15±0.012	0.88±0.32
Non-Rosetting lymphocytes*	0.41±0.028	0.34±0.0027
Rosetting lymphocytes percent of total lymphocytes	26.78	72.72
Non-Rosetting lymphocytes percent of total lymphocytes	73.21	28.09

* Numbers are mean of 30 readings x 10^6 cells/ml±S.E.

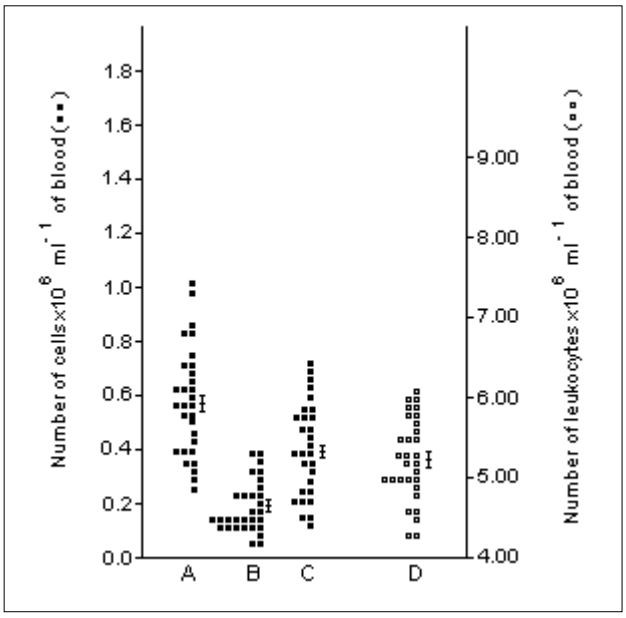

Figure 1: Numbers, means and standard error of the four cell types studied in the diseased children;
A: Total lymphocytes
B: Rosetting lymphocytes
C: Non-Rosetting lymphocytes
D: Total leukocytes

also supported this observation. Rosetting cells of healthy children constituted 72.72% of the total lymphocytes while those of the diseased children constituted only 12.39%. Percent-ages of non-rosetting cells were 28.09% and 33.80%, respectively. This inverse relationship of non-rosetting cells in healthy and diseased children is discussed later.

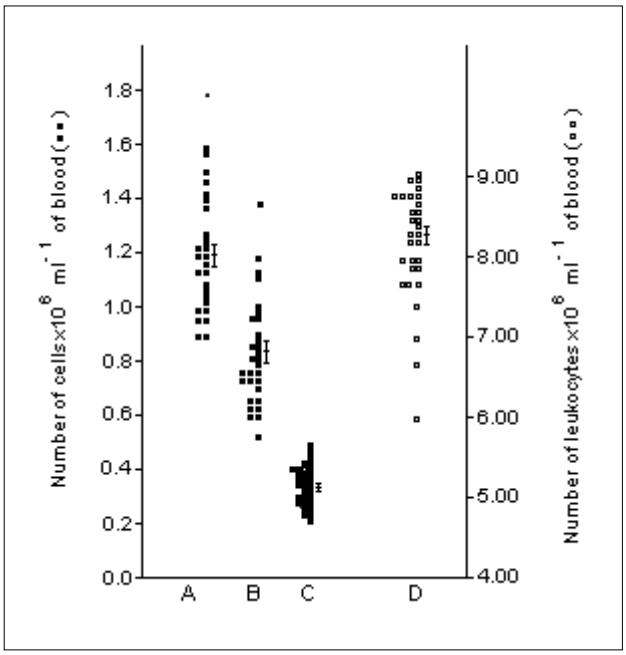

Figure 2: Numbers, means and standard error of the four cell types studied in the control subjects;
A: Total lymphocytes
B: Rosetting lymphocytes
C: Non-Rosetting lymphocytes
D: Total leukocytes

Values obtained for lymphocytes in both groups of children studied here were below the normal expected range. Lymphocytes constituted 14.86% of leukocytes in healthy children and 10.62% in the test subjects.

The results of chi-square test of independence revealed that there is very strong association between the two types of lymphocyte counts (total and rosetting) and the health status (sick and controls) of the subjects.

DISCUSSION

In the present study we have attempted to show imbalance in total lymphocyte population in peripheral blood of young children suffering from a variety of infections and pathological conditions. We are also reporting imbalances in the proportion of the two sub-populations of lymphocytes identified by sheep erythrocyte rosette forming ability of the T-cells.

A significant ($p<0.001$) depletion in total and the rosetting lymphocytes as compared to controls is being reported by us. 17 out of 30 children included in this study were suffering from one or the other kind of malignancy. As malignancies could be secondary to immune deficiency, lower than normal values obtained for various cell

types were expected and are in agreement with similar findings of other workers. Chiorazzi *et al.* (8) have reported alteration in T-lymphocyte sub-sets in patients with chronic lymphocytic leukemia. A positive correlation between the depletion in leukocytes specially the lymphocytes and malignancy may be attributed toradiotheraphy some of these patients were receiving. Peripheral T-lymphocytes were temporarily sequestered in bone marrow when patients of acute lymphocytic leukemia were treated with long term radiotherapy (6).

Depletion in T-rosette forming cells has been reported in 60% of human subjects suffering from a variety of conditions like rheumatoid arthritis, rheumatic fever, myocarditis and wound infections (2). This depletion was shown to be due to anti-lymphocytic antibodies presents in the sera of these individuals. The antibodies were reported to be specific against the T-cell and not the bone marrow precursor of the T-cell.

Our finding that test subjects depletion of total lymphocytes and T-lymphocytes but not of the non-rosetting lymphocytes are parallel with the results of Averbakh *et al.* (1). These workers have reported T-cell numbers to vary in patients suffering from sarcoidosis at various stages of the disease while the number of B-cells did not show any alteration.

Further more in the present study the non-rosetting cells were found to be significantly higher ($p<0.05$) in test subjects as compared to the controls. Although the mean values being reported by us (0.41x106 cells/ml and 0.34x10.6 cells/ml respectively) contain the Null cells as well, but they are indicative of alterations in counts. Increase in the number of non-rosetting cell may be secondary to imbalance in T-cell sub-sets. Defects in T-suppressor cells lead to exaggerated functions of B-cells. Risk of autoimmune diseases increases in this situation. A greater deficiency in Suppressor/Cytotoxic T-Sub-set may allow expression of a clone of helper T-cell specific for auto antigens. The possibility is discussed by Geffner *et al.* (14) in their report involving patients of autoimmune thyroiditis.

Alteration in T-lymphocytes and their sub-sets in peripheral blood of patients of multiple sclerosis is demonstrated by Merrill *et al.* (19). Significant difference in OKT 1+ OKT4+ cells and non-significant difference in OKT 8+ cells has been reported in patients suffering from rheumatic fever, rheumatic disease of heart and acute glomerulo-nephritis (4). The diseased children included in our studies also suffered from similar syndromes.

We also report here a lower than normal value of lymphocytes even in healthy children mean percentage being 14.86. Normally one ml of undiluted blood of a healthy individual contains approximately 6-9x10.6 lymphocytes. Lymphocyte percent of leukocytes ranges from 20-25 (13). In a separate study, being reported elsewhere we have compared values of lymphocyte sub populations in heroin addicts and normal healthy adults. Lymphocytes in the normal adult population constituted 27.30% of the total leukocytes which was within the expected range. A substantially lower value of lymphocytes in healthy children obtained in the present study is indicative of the fact that there is a general lymphocytopenia in them but not in the adult populations. However larger samples of population need to be investigated to derive decisive conclusions.

The practical significance of such studies lies in the diagnosis, prognosis and specific therapy of patients suffering from multiple recurrent infections and are at the risk of autoimmune disorders. In many cases immunodeficiency was corrected after successful treatment of the infection (11). On the basis of data obtained upon various parameters of immune system a prognostic table may be constructed which shall enable us to predict a relapse in these individuals. This, however shall be possible with the use of monoclonal antibodies for the identification of various T sub-sets. We plan to utilize them in future studies.

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