LYMPHOCYTE SUB-POPULATIONS IN A GROUP OF HEROIN ADDICTS IN PAKISTAN

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SUMMARY: A study on a group of fifty heroin abusers was conducted to analyze their immunocompetence. Absolute numbers of lymphocytes and their sub-populations in the peripheral blood were used as the parameter. Sub-populations of lymphocytes were distinguished on the basis of their ability to form rosettes with speep's erythrocytes. Another group of fifty individuals comprising of non-abusers was studied as control. The mean value for total lymphocytes and the T-cells in non-abusers were within the expected normal range, while those obtained for the heroin abusers were below normal value. Difference between the cell counts of the two groups were statistically significant. The mean of non-rosetting cells of the two groups of population studied did not differ significantly. Results indicate a marked deficiency in cellular immune compartment of the subjects with not much impairment of the humoral immune system. The chi-square test for independence between lymphocyte counts and drug abuse revealed a degree of association with 88% confidence.

Key Words: Immunomodulation, heroin addiction, T-cells, E-Rosettes.

INTRODUCTION

Modulation of lymphocyte sub-populations resulting from various clinical and physiological conditions is being reported since about last two decades. Such modulated lymphocyte counts have been associated with bacterial infections (9,16,20,25), Mycotic infections (2,8,11) and viral infections (3,4). Lymphocyte abnormalities have also been found to be associated with factors other than infections, for example aging (5), nutrition (21), consumption of ethanol (22), use of antibiotics (15), various immunodeficiencies (28, 29), homosexuality (14) and addiction to narcotic drugs (10,18). Profound immune deficiency particularly in the specific cellular responses has been reported in the above mentioned cases. Humoral immune responses are, however, not impaired most of the time. Non-specific cellular immune responses as measured by the phagocytic activities of polymorphonuclear leukocytes are also adversally affected by agents like apomorphine (12, 13) and ethanol drinking (17).

Separation of lymphocytes from red blood cells and polymorphonuclear leukocytes of human peripheral blood and their enumeration have been described by Aiuti *et al.* (1). Estimation of absolute values of lymphocyte sub-populations and their respective rations have been utilized as a tool for quantitation of immune response.

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Drug abuse in Pakistan has become a major socioeconomic problem and needs immediate attention and serious considerations. Clinical data upon the drug abusers that might help in their management or might evoke awareness against drug abuse in Pakistan is scanty at present. This study was initiated with the aim to establish immune status of drug abusers in Pakistan employing counts of lymphocytes and their sub-populations in peripheral blood.

MATERIALS AND METHODS

In all hundred human subjects were included in this study. Fifty of these were drug abusers who had been taking heroin for a period of varied lengths. Another group of fifty individuals mainly volunteered university students and employees, was included to serve as control. Clinical features of the drug abusers and non-abusers are provided in Table 1.

On the day of study 5 ml of venous blood was collected in heparinized bottles. The blood was kept in ice chest during transportation to the laboratory. There it was handled to give total white blood cell counts and lymphocyte counts, using hemocytometer and Neubar ruling chambers, and differential blood count using Wright's stain.

Prior to count, lymphocytes were separated from the red blood cells and polymorphonuclear leucocytes by density gradient centrifugation using Ficoll (Histopaque 1077 of Sigma). The procedure for separation of lymphocytes using Ficoll was first described by Boyum (6). The isolated lymphocytes were divided in two aliquots.

One was used to count the total number of lymphocytes, while the second was used for rosetting with sheep's erythrocytes.

Fresh 4% sheep's red blood cell (SRBCs) suspension was prepared in phosphate buffered saline. Lymphocytes were mixed in equal quantities with the SRBCs in the presence of Fetal Calf Serum (flow laboratories) at room temperature, centrifuged, transferred to an ice bath and allowed to react for one hour, as described by Lay *et al.* (19). Resulting rosettes were counted in Neubar counting chambers. Lymphocytes to which three or more red blood cells were adhered, were considered as rosetting cells. Number of rosetting cells per ml of undiluted blood was then calculated. Vital staining with Eosin Y was performed to distinguish living cells from the dead cells.

A t-test was performed to compare the means of rosetting and non-rosetting cells in drug abusers and non-abusers. A chi-square test of independence was also carried out to check association between drug abuse and the counts of total lymphocytes and rosetting lymphocytes.

RESULTS

Total white blood cell counts, lymphocytes percent of total leukocytes, total lymphocyte and T-lymphocyte counts of the two groups studied are given in Table 2. Values for non-rosetting cells have been obtained through subtraction. Clinical features revealed that subjects belonging to both groups has similar means age. While no female case was studied as drug abuser, the group of non-abusers included eighteen females. Among the drug abusers large variation existed in the duration of heroin usage ranging from four months to twenty years. T-cell counts of these individuals, however, were not strikingly different, being 0.22 and 0.14 x 10^6 cells/ml, respectively. While reports for alcohol consumption are nil, a good number used sedatives, chewed beatle nut and leaves and/or smoked tobacco.

Values obtained from various cell counts in abusers and non-abusers and their ratios are given in Table 2. It is evident from these figures that while most cell counts for abusers differed from that of non-abusers, the counts for non-rosetting cells were similar. The mean value for total lymphocyte counts obtained for non-abusers was 1.7 x 10^6 cells/ml as compared to that of abusers, 0.59 x 10^6 cells/ml. The means of T-lymphocyte counts obained for the two groups of individuals were 1.25x10^6 cells/ml and 0.16 x 10^6 cells/ml respectively. T-cell percent of total lymphocytes calculated for non-abusers was 73.52 and for the drug abuser 27.11.

A comparison of total and T-lymphocytes of drug addicts with respective values for normal individuals revealed a marked depression in abusers. The depression was more marked in T-cell counts as compared to total lymphocyte counts, being 34.7% for the total lymphocytes and 12.8% for the T-lymphocytes. Ratios of rosetting cells of abusers and non-abusers to the total lymphocyte count of the non-abusers were also calculated. They are 0.735 and 0.094 respectively.

The mean of absolute number of lymphocytes that did not rosette with SRBCs was 0.45x10^6 cells/ml for non-abusers and 0.43x10^6 cells/ml for the drug abusers. Their ratios with the number of total lymphocytes in non-abusers were 0.264 and 0.252 respectively.

DISCUSSION

Drug addiction can modulate immunocompetence of the abuser. It has been shown that altered lymphocyte sub-population ratios are associated with addiction to substances like alcohol, cocaine, heroin etc. Absolute values for lymphocytes in normal human peripheral blood range from 1-2x10^6 cells/ml of undiluted blood (7). T-lym-

Table 1: Clinical features of heroin abusers and non abusers.

	Abusers	Non-abusers
No. of Individuals	50	50
Age in years	28.7 ± 7.249*	29.5 ± 6.673*
Sex (Male/Female)	50/0	32/18
Duration of addiction	4 months to 20 years	Nil
Quantity of heroin used (gms/day)	0.65 ± 0.07*	Nil
Parenteral/Non-parenteral	39/11	Nil
Current usage of		
a. alcohol	Nil	Nil
b.Sedatives	31	3
c. Beathe nuts and leaves	27	21
d. Tobacco	19	24

* Numbers are mean values for 50 individuals ± SD.

Table 2: Frequencies and ratios of lymphocytes and their sub-populations in heroin abusers and non abusers.

	Abusers	Non-abusers
Total WBC count ($X10^6$ cells/ml)	3.02 ± 0.38	7.21 ± 0.40
Total lymphocytes ($X10^6$ cells/ml)	0.59 ± 0.17	1.70 ± 0.35
Lymphocytes percent of WBCs	15.03 ± 0.98	27.30 ± 1.20
Rosetting lymphocytes ($X10^6$ cells/ml)	0.16 ± 0.07	1.25 ± 0.27
Non-rosetting lymphocytes ($X10^6$ cells/ml)	0.43 ± 0.12	0.45 ± 0.20
Rosetting lymphocytes percent of total lymphocytes	27.11 ± 0.28	73.52 ± 0.13
Non-rosetting lymphocytes percent of total lymphocytes	72.88 ± 0.10	26.40 ± 0.09
Ratios of rosetting cells to total lymphocyte count of non-abusers	0.094	0.735
Ratios of non-rosetting cells to total lymphocyte count of non-abusers	0.252	0.264

The figures are means of 50 individuals ± SD.

phocytes constitute 70-75 percnet of the total lymphocytes. In our studies mean of absolute number of total lymphocytes in non-abusers is within this range while the value obtained for the drug abusers is much below the lower figure of the range, showing to a depletion of 34.7%. T-cell population was still markedly reduced in drug abuser as compared to non-abusers, depletion being 12.8%. An approximate three-fold decrease in the T-cell population is indicative of selective T-cell deficiency.

In another study E-rosette forming ability of lymphocytes has been shown to be modulated by simultaneous and independent use of alcohol, cocaine and heroin (10). Percentage of rosetting lymphocytes was depressed following heroin addiction. However, they have suggested that this depression was not due to lymphocytopenia but because of alterations in the sheep red blood cell receptors on the T-cells. This suggestion was based on the lack of T-cell depletion when they were estimated using Lyt-3 (anti-E-receptor) and OKT-3 (anti-total T-cell) monoclonal antibodies.

We have calculated the number of non-rosetting cells which include B-cells and Null cells in almost equal proportions. Comparison of values obtained for non-rosetting cells in abusers and non-abusers (0.43 and 0.45 x 10^6 cells/ml respectively) with the corresponding values of total lymphocytes (0.59 and 1.7x10^6 cells/ml revealed that while in non-abusers, non-rosetting cells constituted about 26.4% of the total lymphocytes, in drug abusers, their proportion was increased to 72.88%. This observation is in agreement with an earlier study carried out by McDonough *et al.* (24). They have reported that while use of opiate in human beings resulted in depletion in E-rosetting cells (T-cells) the number of non-rosetting cells increased. It was further shown that when lymphocytes growing *in vitro* were supplied with nalaxane, null-cells were converted to T-cells to an extent that normal sub-population ratios were reached. In another study natural killer activity (T8) has been shown to be enhanced when normal human leukocytes were incubated with endogenous opioid *in vitro* (23) resulting in deficiency in cell mediated immunity. Such findings may reveal very interesting and intimate cell interactions and need to be studied further.

Modification in lymphocyte sub-populations in drug addicts has also been reported from Spain by Lattore *et al.* (18). Fifty five percent of the drug abusers included in their study gave a helper-inducer/suppressor-cytotoxic ratio less than one, which is indicative of impairment of immune response. This was accompanied by a significant reduction in E-rosette forming cells however B-cells and phagocytic cells were in normal range.

When T4/T8 ratios of a group of parenteral and non-parenteral heroin abusers and were compared (27), the differences were found to be non-significant, ratios being 2.41 for parenteral abusers and 1.84 for the non-parenteral abusers. Same workers in another study (26) had reported the mean T4/T8 ratio for normal subjects to be 2.0. T4/T8 ratios for the two groups of abusers with that of the normal subjects were also statistically analyzed. The differences were still non-significant. They have suggested that neither narcotic drugs nor repeated use of needles for injecting these substances are directly responsible for low T4/T8 ratios in drug abusers with AIDS. The low T4/T8 ratios are probably due to the virus itself i.e., LA/HTLV-III.

In the present study to establish the plausible association between drug abuser and counts of lymphocytes, we performed a chi-square test of independence. Value of 2.5269 was obtained after employing Yate's correction factor. Though for one degree of freedom it was non-significant yet degree of association is evident with 88 percent confidence. Study of larger population may yield conclusive results.

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