

R E S U L T S

RESULTS

GAMMA-GLUTAMYL TRANSPEPTIDASE (GGT) ACTIVITY IN SERUM

In Alcoholics The GGT activity in the serum of alcoholics ranged between 41-66 IU/l which was significantly higher than the average normal value of 11 IU/l (Table 1, Fig. 1).

After Therapy To begin with the mean GGT value in the group of alcoholics selected for ascorbic acid supplementation was 57.3 IU/l which was reduced significantly to 32.5 IU/l after the therapy.

Similarly there was a reduction in GGT activity in the group of alcoholic selected for zinc supplementation from a mean value of 49.5 IU/l to 35.8 IU/l after the supplementation.

Thus both ascorbic acid and zinc supplementation could reduce the elevated activity of serum GGT in alcoholics significantly towards normalcy. The mean values of GGT in the alcoholics both before and after ascorbic acid and zinc supplementation have been presented in Table 4 , Table 6, Fig.3 and Fig.4.

LEVEL OF SERUM TOTAL PROTEIN AND ALBUMIN

In Alcoholics The values of serum total protein in the alcoholics ranged between 6.5-9.3 gm% which was similar to the normal range of 6.2-9.3 gm% . However there was good variation in the albumin-globulin ratio in almost all the alcoholics under study. The mean serum albumin value as estimated by the dye binding method in the alcoholics was 3.7 gm % which is significantly lower than the mean normal value of 4.5 gm % . Simultaneously there was an increase in the serum globulin percentage (Table 1, Fig. 1). In figures 1, 3 and 4 the gm % values of serum albumin have been expressed in terms of percentage of total serum protein. A comparison of the albumin-globulin ratio between infective hepatitis patients and chronic alcoholic subjects was made (Table 2, Fig. 2). The percentage of serum albumin was much lower in the infective hepatitis patients than the alcoholics.

After Therapy Ascorbic acid and zinc therapy could change the altered albumin-globulin ratio in the alcoholic subjects towards normalcy. The level of serum albumin increased from a mean value of 3.6 gm% to 4.2 gm% (Table 4, Fig. 3) with ascorbic acid and 3.9 gm% to 4.3 gm% (Table 6, Fig.4) with zinc therapy. Any significant change in the level of total protein has not been observed after the therapies. The constant level of total protein in both the cases indicate that there was decrease in the level of certain serum proteins to compensate the increase in serum albumin level.

ELECTROPHORETIC ANALYSIS OF SERUM PROTEINS

In Alcoholics Electrophoretic analysis of serum protein quantitates the different types of proteins present in the serum. The data of electrophoretic analysis of serum proteins (Table 1, Fig.5) indicate a significant reduction in the level of serum albumin from 58% in normal controls to 48.7% in the alcoholics. The level of significance was calculated at $p < .005$. There was increase in some subclasses of serum globulin such as β and γ globulin. The amount of α_1 and α_2 globulin however remained within the normal range. The increment in β and γ globulin indicate overall increased production of immunoglobulin in the alcoholics.

After Therapy The results of electrophoretic analysis after ascorbic acid and zinc supplementation to two separate groups of alcoholics have been presented in Table 4, Table 6 and Fig.6, Fig.7 respectively. In the group supplemented with ascorbic acid there was an increase in the percentage of serum albumin from a mean value of 46.3% to 52% and slight decrease in the percentages of β and γ globulins after the therapy. The values of α_1 and α_2 globulins however remained almost unchanged (Table 4).

A similar picture was observed in the group supplemented with oral zinc sulphate. Here also the percentage of serum albumin increased from a mean value of 49.6% to 55.1% and

there was slight decrease in the percentages of β and γ globulins. No significant change in the mean values of α_1 and α_2 globulin was observed either (Table 6). The increase in the levels of β and γ globulin in the alcoholics seems to revert towards normalcy after ascorbic acid and zinc therapy. This possibly indicate a reduction in the level of serum immunoglobulin after the therapies.

The subjectwise data for changes in different parameters after the therapies have been presented in the appendix.

QUANTITATION OF IMMUNOGLOBULINS

In Alcoholics Corroboration for the increased level of immunoglobulins in alcoholics can also be found when the immunoglobulins were quantitated through the immuno diffusion technique. Furthermore, both IgG and IgM have been found to contribute for higher level of immunoglobulins in the alcoholics (Table 1, Fig. 8a and Fig. 8b), IgG being the major contributor. The serum IgG values in the alcoholics ranged between 1400-2800 mg/100 ml which is significantly higher than normal range of 950-1500 mg/100 ml. Whereas that of serum IgM in the alcoholics ranged between 153-369 mg/100 ml which is also higher than the normal range of 100-195 mg/100 ml.

The quantitation of IgA was not in the agenda of the

present study as because its increment in the alcoholics has been reported by other workers (Ittarigua, 1977 ; Stites et al, 1984).

After Therapy Quantitation of immunoglobulins carried out in the alcoholics after ascorbic acid supplementation showed increase of serum IgG values and simultaneous decrease in the IgM values in all cases (Table 4).

PERCENTAGE OF B AND T CELLS IN PERIPHERAL BLOOD

In Alcoholics It is considered that there are around 30% B cells and the rest are the T cells in the circulating population of lymphocyte in a normal person. The nylon wool column has been used to differentiate these two cell types in the peripheral circulation of normal and alcoholic persons. While the normal persons have 35-40% of B cells and 60-65% of T cells, the alcoholics have 20-30% of B cells and 70-80% of T cells (Table 1, Fig. 9).

Another interesting point has been noted; while T cell count increases in the alcoholics the rosette forming T cells actually decreases. The mean value of the rosette forming cells in alcoholics is 47% while the corresponding value for normal person is 57% (Table 1, Fig. 9).

After Therapy The profound effect of ascorbic acid therapy has also been noted in restoration of the percentage of B and T cells in peripheral circulation towards normalcy (Table 4, Fig.10). But this has no significant effect on the rosette forming cells which remains at the same level as in the alcoholics before therapy (Table 4, Fig.10). Whereas zinc supplementation causes increase in the percentage of rosette forming cells without much effect on the peripheral B and T cell percentage (Table 6, Fig.11).

PHA INDUCED BLAST TRANSFORMATION

Antigenic or mitogenic stimulus activates the lymphoid cells by initiating a series of biochemical events; then the lymphocytes gradually differentiates into blasts. Instead of measuring the different biochemical activities often the degree of blast transformation of a lymphoid cell population is considered as the measure of activation of lymphocytes.

In Alcoholics Blastogenesis with two different doses of PHA have been studied in both normal and alcoholic subjects in the present investigation (Table 3, Fig. 12). The mean value of blast transformation with 5 $\mu\text{g/ml}$ PHA in the alcoholics was more or less the same as the normal value of 26.3%. On the otherhand in the alcoholics the mean value of blastogenesis with 10 $\mu\text{g/ml}$ PHA was 44% which was

significantly lower than the mean value of normal controls. In the normal controls the mean value of blastogenesis was 55.2% .

After Therapy The blastogenic indices with 5 μ g and 10 μ g/ml PHA doses were also measured after ascorbic acid therapy and were found not to differ much from the indices before therapy (Table 5, Fig.13). Whereas oral zinc sulphate supplementation caused significant increase in the percentage of blast forming cells. In average there was increment of 10% in the blastogenic index with each dose of the PHA (Table 6 and Fig. 14).

PHA INDUCED DNA SYNTHESIS

Blast transformation of stimulated lymphocytes is usually accompanied by the synthesis of DNA. Thus the DNA synthesis by the T lymphocytes of the alcoholics before and after therapies were studied to test the degree of activation of the lymphocyte population.

In Alcoholics The T cells from the alcoholics as such, without any stimulation incorporates quite a significant level of radioactive thymidine. When the cells were stimulated with 5 μ g/ml and 10 μ g/ml PHA there was only marginal increase in the radioactivity count (Table 3, Fig.15).

After Therapy The ascorbic acid supplementation did not influence much the DNA synthesis. The incorporation of $^3\text{H-TdR}$ by the lymphocytes remains almost at the same level as in the alcoholics (Table 5, Fig.16). Whereas the zinc supplementation causes an overall increase in the incorporation of $^3\text{H-TdR}$ by the lymphocytes even without PHA stimulation. PHA stimulation after zinc therapy causes furthermore increase in DNA synthesis (Table 7, Fig. 17).

Consistently much higher level of incorporation of $^3\text{H-TdR}$ was observed with lymphocytes from two particular patients. The data have been presented in the last two lines of the Table 7. The indices from these two subjects corroborates the trend obtained with other subjects in the group, the data of which have been represented in Fig.17.

CYTOTOXICITY ASSAY

The activity of T lymphocytes in terms of cytotoxic ability was measured by the ^{51}Cr release assay. The T lymphocytes were from normal, alcoholics and alcoholics after ascorbic acid and zinc therapy.

In Alcoholics Blastogenesis and DNA synthesis studies indicated a higher state of activity in the T lymphocytes from alcoholics without PHA stimulation. So the cytotoxic activity of the peripheral T lymphocytes from alcoholics

before and after ascorbic acid and zinc therapy were studied without any stimulation.

The normal cells as control did not show any cytotoxicity towards allogeneic target cells at any effector : target ratio. The indices were of negative value which is customarily shown as zero. The cytotoxic activity of the T lymphocytes of alcoholics was at significantly higher state, around 32% (Fig.18).

After Therapy Interestingly ascorbic acid supplementation reduced the cytotoxic activity of the T lymphocytes of the alcoholics. Zinc therapy caused further reduction in the cytotoxic activity (Fig.18).

The reduction in the cytotoxicity of lymphocytes after two types of therapies has a strong implication in reference to blastogenic and DNA synthesis ability of the cells and hepatotoxicity in general. This has been discussed.

PLASMA AND LEUCOCYTE ASCORBIC ACID AND DEHYDROASCORBIC ACID LEVEL

In the present investigation the level of ascorbic acid and dehydroascorbic acid has been assayed to get an idea about the metabolic utilization of ascorbic acid in the alcoholics before and after therapies. It has been indicated

that ascorbic acid play a role in inactivating free-radicals and oxidants that might otherwise denature cell constituents. Dehydroascorbic acid is the reversibly oxidised form of ascorbic acid and its accumulation in blood and tissues under varying physiological state cause pathological conditions. Both serum and leucocyte ascorbic acid and dehydroascorbic acid level was estimated to get a proper reflection of tissue status of ascorbic acid.

In Alcoholics The mean values of plasma and leucocyte ascorbic acid level in the alcoholics was measured at 0.75 mg/100 ml and 21.2 $\mu\text{g}/10^8$ leucocyte respectively. The corresponding values for the control were 1mg/100 ml and 26 $\mu\text{g}/10^8$ leucocytes. Simultaneously an elevation in the level of dehydroascorbic acid both in plasma and leucocytes of alcoholics were noted. In the normal controls, the blood level of dehydroascorbic acid was almost negligible (Table 8).

After Therapy After supplementation of ascorbic acid for 1 month, the level of ascorbic acid and dehydroascorbic acid both in plasma and leucocytes returned towards normal values (Fig.19 and Fig.20).

It is interesting to note that although there was no significant change in the level of ascorbic acid, the level of dehydroascorbic acid decreased to a good extent both in plasma and leucocyte (Fig.21 and Fig.22) in the group receiving zinc supplementation.