

# In vitro Assessment of 'T' Lymphocyte Functioning in Vitiligo

## Support for Autoimmune Hypothesis Concerning the Disease

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Ten patients with vitiligo in the active state of the disease and an equal number of age- and sex-matched controls were selected for certain immunological markers. A significantly high production of leukocyte migration inhibition factor was observed in these patients when compared with controls. Levels of immunoglobulin G were found to be significantly elevated. Eighty percent of the cases showed elevated levels of circulating immune complexes. Our investigation supports the autoimmune hypothesis for the disease, showing an increased release of LMIF with a subsequent activation of B-cells which might have led to the observed hypergammaglobulinaemia and elevated levels of circulating immune complexes in these patients. **Key words:** Leukocyte Migration Inhibition Factor; T-cells; Circulating Immune Complexes; Immunoglobulins.

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The cause of vitiligo is unknown. Several hypotheses have been proposed, among which autoimmunity is generally favoured (1). The clinical association between vitiligo and organ-specific autoimmune diseases such as Hashimoto's thyroiditis, Grave's disease and pernicious anaemia also suggests that vitiligo may have an autoimmune etiology (1-3). Although humoral antibodies to normal melanocytes are studied in the sera of patients with active vitiligo (4), no report is available on the functional activities of T-lymphocytes.

We report here our investigation on the release of Leukocyte Migration Inhibition Factor (LMIF) by activated 'T' cells as a marker of their function. The levels of immunoglobulins (Igs) G, A and M and the circulating immune complexes (CICs) in sera of these patients were also estimated.

### METHODS AND MATERIAL

Patients visiting the clinic of Central Research Institute in Unani Medicine, Hyderabad, were selected for the study. Ten patients with active disease (presenting continued appearance of new lesions or extension of old lesions) and an equal number of age- and sex-matched controls comprised the subject material for the present study. None of the patients nor controls presented any symptoms of the known autoimmune disorders listed above, including insulin-dependent diabetes mellitus. Assessment of LMIF was done *ad modum* Hamblin & Mani (5). Briefly, 5 ml of anticoagulated blood was collected in a tube and kept for sedimentation. The leukocyte-rich plasma was harvested and washed twice in the Eagle's MEM (HI-Media, India). The leukocytes were pelleted in microcapillaries and cultured in polystyrene chambers (Laxbro, India) in 0.5 ml of RPMI 1640 (Difco, USA) containing 1%

penicillin/streptomycin, 10% human AB serum and 1 µg/ml PHA. The control chambers did not receive PHA. The experiment was set up in duplicate. The area of migration was measured by micrometer and the average of two sets was used. The percentage Migration Inhibition was calculated as shown below:

$$\text{Migration Index} = \frac{\text{Area of migration in test}}{\text{Area of migration in control}}$$

$$\text{Percentage Inhibition} = 1 - \text{Migration Index} \times 100$$

Serum Ig concentrations were estimated on Tripartigen plates (Hoechst (India) Pharmaceuticals Ltd.) by the single radial immunodiffusion method of Mancine et al. (6) and are expressed as mg/dl of blood. Estimation of CICs was performed in sera *ad modum* Digeon et al. (7). The results are expressed as percentage PEG index, as follows:

$$\text{PEG Index (\%)} = \frac{\text{Test O.D.} - \text{Control O.D.}}{\text{Control O.D.}} \times 1000$$

A serum sample is considered as positive for CICs by this method only when it has a value exceeding the mean + 2 S.D. of controls.

### RESULTS AND DISCUSSION

The results of the investigation are given in Fig. 1. Statistical analysis using Student's *t*-test for equal samples revealed a significant increase in release of LMIF in patients, compared with controls ( $p < 0.05$ ). Seventy-five percent of the cases had percentage inhibition values exceeding the mean + 1 S.D. of controls. Although a generalized increase in serum immunoglobulins (G, M and A) is observed in these patients, only the rise in IgG is statistically significant ( $p < 0.05$ ). Circulating immune complex levels were also found to be elevated in patients vis-à-vis controls ( $p < 0.001$ ).

The concept of vitiligo as being an autoimmune disease is growing ever since the detection of auto-antibodies to normal

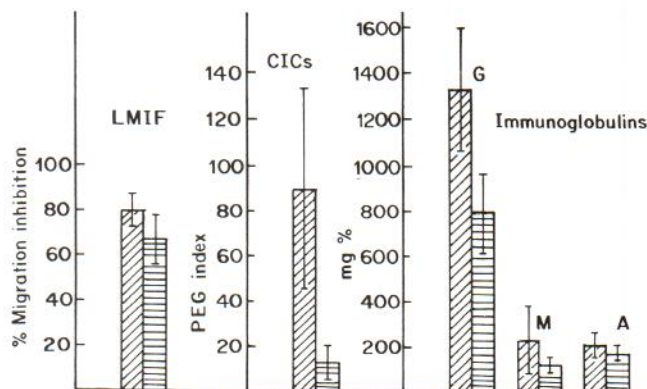


Fig. 1. Certain immunological markers in vitiligo patients (▨) and controls (■): LMIF = Leukocyte Migration Inhibition Factor; CICs = Circulating Immune Complexes.

melanocytes in these patients. The imbalance in the T-cell population has also been reported by Soubiran et al. (8), using monoclonal antibodies to these cells. However, the functional behaviour of these lymphocytes in relation to antibody production is not reported in the literature. LMIF is one of the lymphokines released from activated T-lymphocytes and is a marker of their function. The significant increase in LMIF release from patients' lymphocytes observed in our investigation indicates a hyperfunctioning of these cells. A simultaneous increase in antibody levels in sera of these patients suggests a T-cell mediated B-cell activation. The presence of significantly large amounts of CICs indicates an active state of the disease and the role of such complexes in tissue destruction in autoimmune diseases is well established (9). It was also interesting to note an IgG antibody involvement in CIC formation in all these patients, in addition to hypergammaglobulinaemia, suggesting a probable production of anti-idiotypic antibodies to IgA and IgM.

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