Role of levamisole immunotherapy as an adjuvant to radiotherapy in oral cancer. I. A three-year clinical follow up

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Eighty-two patients with squamous cell carcinoma of the oral cavity belonging to stages $T_1N_0M_0$ and $T_2N_0M_0$ were randomized to receive either levamisole or placebo therapy following conventional radiotherapy. Oral levamisole, at 150 mg daily doses for three consecutive days, was given once every two weeks. The patients were followed-up for three years and the results reported. Levamisole appears to have some beneficial effect in prolonging the disease-free interval of these patients (44% in the levamisole group compared to 32% in the placebo group after 30 months of therapy). This, however, did not have any effect of the metastatic potential of the tumors. The effects of levamisole on peripheral blood leukocytes and lymphocytes were more promising. The restoration of leukopenia and lymphopenia observed after radiotherapy was faster in the levamisole group when compared to the placebo group.

Key words: Oral cancer, levamisole, immunotherapy, clinical follow up.

Cancer of the oral cavity is the predominant type of cancer representing almost 30% of all cancers registered at this center [12]. Several workers have reported a defect in immune responses and disturbances in the cellular composition in these patients [2]. This impairment has been considered to be one of the factors predisposing the patient to the uncontrolled growth and recurrences after conventional therapy. It is generally assumed that immunological factors may influence the prognosis of cancer patients and that nonspecific immune stimulation may improve survival [4]. Several biological modifiers are being tried in cancer patients as adjuvants to accepted modalities of treatment in order to improve specific as well as nonspecific immunocompetence [1, 2, 7, 11, 23]. Levamisole, one among the various immune modulators has been reported to be a drug with a broad-spectrum immunorestorative effect [9] in a number of cancer patients and immunodeficient patients [2, 5, 10, 16, 18, 19]. Reports using levamisole as an immunotherapeutic agent in cancers are encouraging [3, 5]. Cancers of the head and neck constitute a heterogeneous group and hence the response in each group is unknown. The present study deals with the effect of levamisole as an immunostimulant in patients with squamous cell carcinoma

of the oral cavity treated by radiotherapy, with regard to the disease-free status of the patient.

Materials and methods

Selection of patients. Patients attending the clinics of the Regional Cancer Center, Trivandrum, India, with squamous cell carcinoma of the oral cavity were selected for the study. Selection was restricted to those with early lesions (UICC staging $T_1N_0M_0$ and $T_2N_0M_0$) falling in the ECOG score group 0—2. None of the selected patients gave a history of any other systemic or immune disease. All subjects were informed of the study and gave their consent following a clearance by the ethical committee of the institution. All the selected patients were randomized into two groups — one receiving radiotherapy alone (control group) and the other receiving radiotherapy followed by levamisole immunotherapy (experimental group). Fifteen apparently healthy volunteers were selected for comparison of parameters such as total leukocyte and lymphocyte numbers.

Treatment schedule. Radiotherapy. All the patients (both control and experimental) were given curative radiotherapy. If the lesions were suitable for interstitial implant, radium implant was done giving a dose of 60—65 Gy in 6 days. For cases unsuitable for implant, external irradiation with telecobalt giving a tumor dose of 50—52.5 Gy in 15 sittings over three weeks was administered. Patients with residual tumor (less than 80% regression of the tumor) after radiotherapy were excluded from the study. This applied to both control and experimental groups.

Immunotherapy. Levamisole was administered to the patients of the experimental group one month after the completion of radiotherapy. They received 150 mg oral levamisole daily for three consecutive days and this regimen was repeated every 14 days for 30 months. The control group was given placebo tablets containing lactose at the same schedule.

Follow up. The patients were followed-up every six weeks for three years. Clinical evaluation and assessment of immune competence (manuscript submitted for publication) was carried out before the start of radiotherapy and at every follow up.

Analysis of data. The actuarial (life table) method was used to compute the recurrence rate and the statistical significance of the effect of levamisole on the disease-free period. Student's t-test was used to analyze the difference in total leukocyte and lymphocyte counts.

Results

Total leukocytes and lymphocytes. Radiotherapy depressed the leukocyte and lymphocyte counts significantly ($p < 0.05$). These cells were repopulated into the blood stream and increased in number with time after therapy. The process of repopulation was hastened by administration of levamisole. In the group receiving levamisole, the leukocyte and lymphocyte numbers were comparable to that in the normal healthy controls by 6 months and 18 months of therapy, respectively, while in the placebo group the leukocyte number was significantly lower than the control values at periods of 12 months. Lymphocyte values were not comparable to normal values even at 30 months after therapy (Tab. 1).

Disease-free interval. Of the 105 patients selected for the study, 22 exhibited residual lesions following radiotherapy and one patient could not tolerate levamisole administration, and hence they were removed from the study. Thirteen patients were lost to follow up. At 12 months of immunotherapy, 57% of the patients in the levamisole group were free of disease while only 44% of the patients in the placebo group were free of disease (Table 2). At 30 months, the percentage of recurrence-free patients was 44 in the levamisole group, while it was 32 in the placebo group. This difference was however not statistically significant at the 5% level. Nodal metastasis was observed in 5% (2/40) of the patients in the levamisole group and 7% (3/42) of the patients in the placebo group. This again was not statistically significant. Three patients out of 82 died during the period of observation. All these belonged to the placebo group. None of the patients in this study developed distant metastases.

Side effects. Radiotherapy was well tolerated by all the patients. Levamisole administration had no side effects in most patients (80%). Eight patients out of 40

Table 1. Total leukocytes and lymphocytes in oral cancer patients given levamisole (L) and placebo (P) at various intervals following radiotherapy

	Treatment	Before RT	Immediately after RT	Time interval after RT (months) 6	12	18	24	30	Normal controls
Total leukocytes/ /mm³ (No. of individuals)	L	7020 ± 2329 (52)	5020 ± 3512 (52)	6860 ± 3612 (40)	7010 ± 4011 (30)	7412 ± 4121 (24)	8210 ± 4000 (18)	8192 ± 3984 (16)	8010 ± 3010 (15)
	p		< 0.05	< 0.001	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	
	P	6850 ± 2550 (53)	5101 ± 3496 (53)	5801 ± 3648 (42)	6101 ± 3891 (27)	6680 ± 3960 (22)	7106 ± 4110 (15)	7503 ± 4010 (13)	
	p		< 0.01	< 0.001	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
Total lymphocytes/mm³ (%)	L	2860 ± 1080 (41)	1260 ± 875 (25)	2201 ± 1206 (32)	2612 ± 1517 (37)	3337 ± 1860 (45)	3650 ± 1870 (45)	3975 ± 1900 (49)	3681 ± 1620 (15)
	p		< 0.001	< 0.001	< 0.001	< 0.01	> 0.05	> 0.05	> 0.05	
	P	2848 ± 1045 (41)	1330 ± 908 (26)	1750 ± 1070 (30)	2013 ± 1290 (33)	2414 ± 1452 (36)	2585 ± 1749 (36)	2745 ± 1332 (33)	
	p		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

Values are mean ± SD compared to controls. L — levamisole treated group, P — placebo group. $p < 0.05$ considered significant.

Table 2. Calculation of recurrence rate by the actuarial (life table) method

Period	Total	No. showing recurrence	No. of deaths	No. with no disesae	Lost to follow up	Proportion with no disease	Z
Levamisole group							
0—6	40	6	0	30	4	84	1.41
6—12	30	5	0	24	1	70	1.25
12—18	24	4	0	18	2	57	1.78
18—24	18	2	0	16	0	51	1.96
24—30	16	2	0	14	0	44	1.09
Placebo group							
0—6	42	12	0	27	3	71	
6—12	27	5	0	22	0	58	
12—18	22	4	1	15	2	44	
18—24	15	0	2	13	1	38	
24—30	13	2	0	11	0	32	

Value < 1.96 considered significant at 0.5% levels.

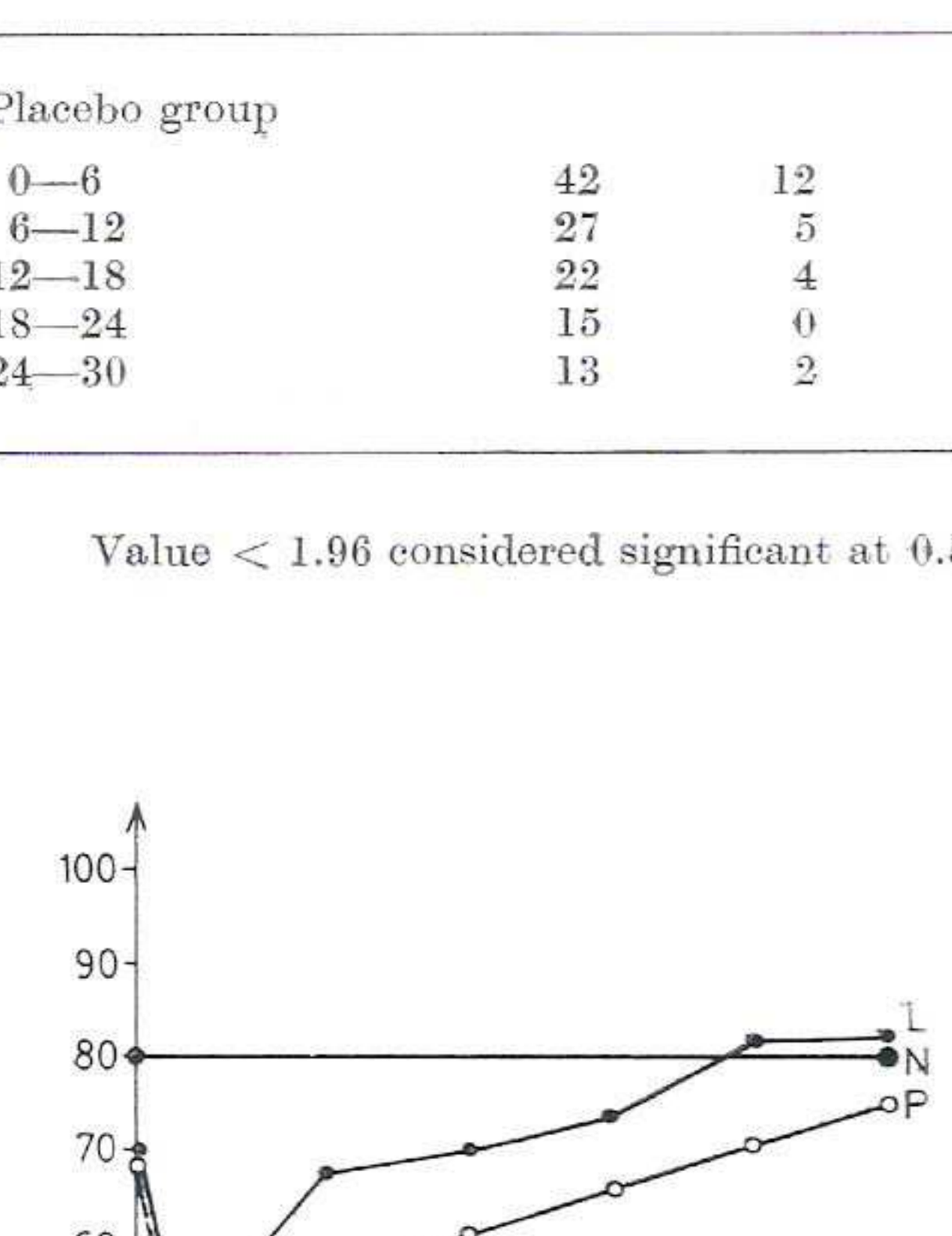

Fig. 1. Total leukocytes (10^{-2}/mm^3) in peripheral blood at different intervals of therapy. N — normal, P — placebo, L — levamisole.

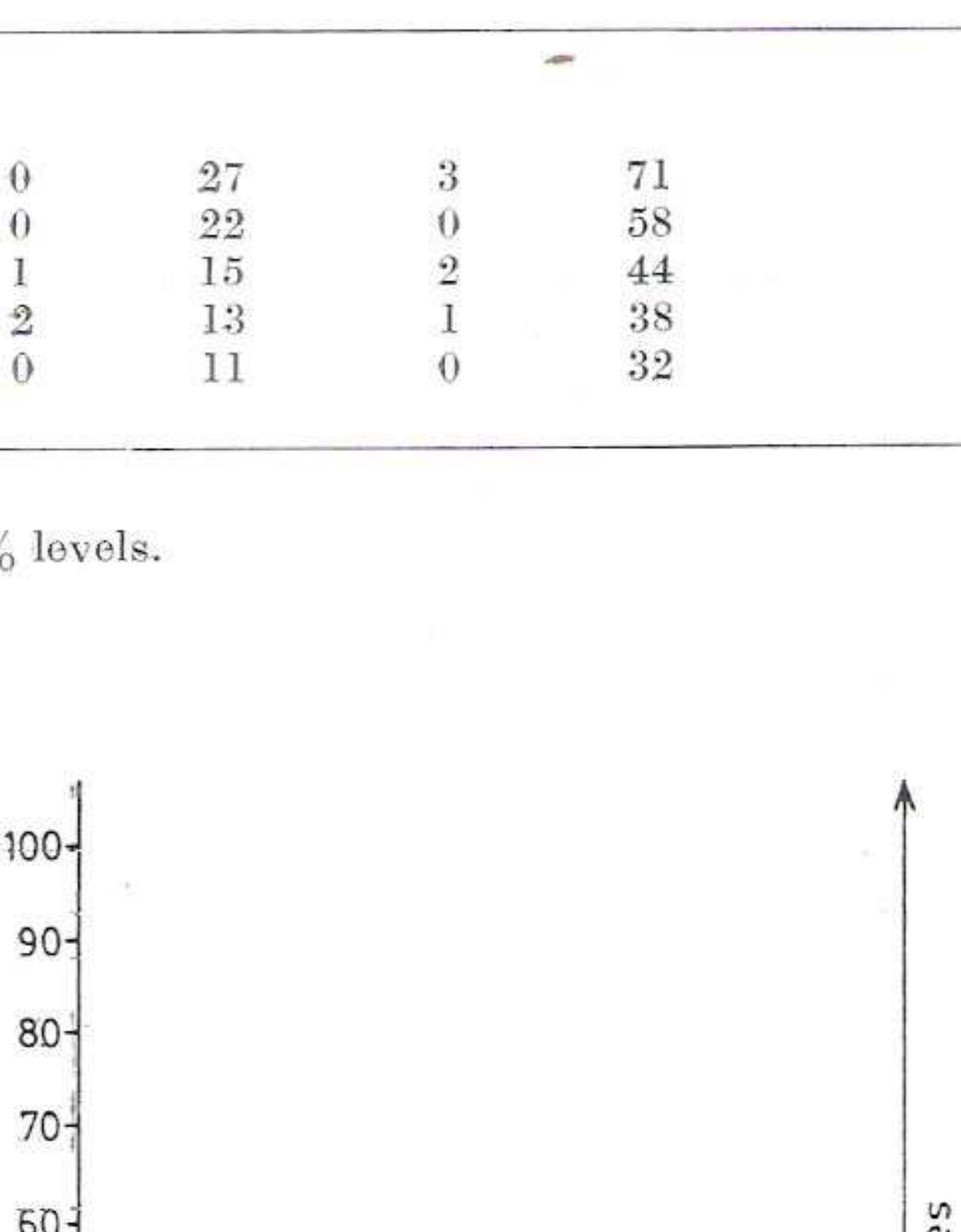

Fig. 2. Total lymphocytes (10^{-2}/mm^3) in peripheral blood at different intervals of therapy. N — normal, P — placebo, L — levamisole.

complained of nausea, vomiting and headache and gastrointestinal upsets. One patient, however, could not tolerate levamisole due to severe vomiting and hence was excluded from the study.

Discussion

Cancer of the oral cavity is a disease which is curable to a large extent with radiotherapy. Still in a number of cases recurrences and nodal metastases occur. It has been observed from various reports that radiotherapy has a debilitating effect on the immune status of the patients [13, 17, 22, 24—26]. This is also evident from the gross reduction of leukocytes and lymphocytes seen in our patients after radiotherapy. From our study it is also clear that these cells which play an important role in the immunosurveillance mechanism take a long time to repopulate themselves after radiotherapy. This interval in which the numbers of these effector cells are reduced in circulation could give a chance for the residual tumor cells to reestablish themselves giving rise to recurrences and metastases at a later stage. Opportunistic infections also tend to set in at this time. A stimulation of the immune system at this stage would be beneficial to the host in control of the malignancy. Our results show that levamisole does hasten the restoration of the leukocyte and lymphocyte numbers in circulation and brings it up to normal levels much earlier than in the control group. The lymphocyte numbers which have been shown to play a very important role in the control of malignancies remained at a low level in the placebo group even 30 months after therapy and this could lead to impairment of immune function. Levamisole does seem to have some beneficial effect in prolonging the recurrence-free intervals, though this was not statistically significant. This finding is in accordance with that of other workers. However, the rate of metastasis formation does not show significant reduction in patients administered levamisole.

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