RANDOMIZED, DOUBLE-BLIND STUDY ON ROLE OF LOW LEVEL NITROGEN LASER THERAPY IN TREATMENT FAILURE TUBERCULAR LYMPHADENOPATHY, SINUSES AND COLD ABSCESS

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Summary

Background: Effectiveness of low level nitrogen laser therapy along with antitubercular treatment (ATT) in cases of treatment failure and drug resistant tubercular lymphadenopathy, sinuses and cold abscess.

Methods: In a double-blind randomized controlled trial of LLLT, 104 patients assigned to either the low level nitrogen laser therapy along with ATT (LLLT group) (n = 54) or ATT only (Chemotherapy group) (n = 50). Both groups were treated two times per week for five weeks. Those in the treatment group received pulse nitrogen laser with a pulse duration of seven nanosecond, wave length 337 nanometer and average power output of 5 mW whereas those in the control group were treated with sham laser. The primary outcome measure was bacteriological conversion and the secondary outcome measures were decrease in size of lesion and the clinical improvement.

Results: Acid Fast Bacilli (AFB) smear, AFB culture and Polymerase Chain Reaction (PCR) conversion rate at five weeks (after 10 sittings of laser) were 49.15% (Fishers P exact test, p = 0.015), 60%, 44.44% (Fishers P exact test, p = 0.048) in LLLT group as compared to 11.86%, 20%, 17.77% in chemotherapy group. Average percentage reduction in the size of gland at 5 weeks was 70.67% (p value 0.01) as compared to 54.81 in chemotherapy group. Average time taken for closure of sinuses was 11.03 weeks in LLLT group as compared to 26 weeks in chemotherapy group. The follow up was conducted for two years.

Conclusion: Low level nitrogen laser therapy can be used as an adjunctive therapy along with antitubercular drugs in cases not responding and drug resistant tubercular lymphadenopathy, sinuses and cold abscess.

Key words: Laser, Lymph node tuberculosis, Drug resistant

INTRODUCTION

Tuberculosis affects more than eight million people every year and has serious repercussions on economy, as well as the psychologic and social status of the affected individuals. It has therefore been declared a global emergency in 1993 by World Health Organization (WHO). Since then, significant developments have taken place in the treatment and control of tuberculosis. One notable advancement has been the implementation of the Directly Observed Treatment, Short course (DOTS) along with fixed dose combination of existing drugs. However, the currently available therapeutic regimens have inherent disadvantage of long treatment duration, which often leads to patient non-compliance and the risk of drug resistance. Hence, new modalities of potent treatment which reduce the treatment period and also active against resistant strain are needed to combat this disease.

The present study has been carried out to study efficacy and safety of 10 sittings of Low Level Nitrogen Laser Therapy (LLLT) in the management of treatment failure tubercular lymphadenopathy, sinuses and cold abscess.

MATERIAL AND METHODS

Double-blind randomized controlled trial study on role of low level nitrogen laser therapy in treatment failure tubercular lymphadenopathy,
sinuses and cold abscess has been studied for a period of three years from January 2005 to December 2007 and follow up was done for a period of two years. After getting approval from the Ethical Committee of the institution, the study was started.

All patients gave a detailed medical history and received a physical examination. All patients gave an informed consent to participate in the study.

The criteria for inclusion of patients in the present study were: 1) Antitubercular treatment (ATT) for more than six months showing no response 2) Acid fast bacilli (AFB) grown in culture after six months of ATT 3) Abscess formation showing no response to treatment 4) Sinus tract formation showing no response to treatment 5) Age group more than 15 years but less than 65 years with a diagnosis of drug resistant tubercular lymphadenopathy and 6) DOTS (Directly Observed Treatment, Short Course) Category-II failure.

Exclusion criteria included patients of 1) Human immunodeficiency virus (HIV) positive 2) Hepatitis B surface antigen positive 3) Diabetes Mellitus and 4) Renal disease.

Routine tests included complete haemogram, blood sugar, screening test for HIV, hepatitis B surface antigen, Mantoux test and sputum examination for AFB.

In patients of lymph node abscess, sinus and cold abscess, we have used microbiological analysis for the presence of *Mycobacteria* in the lymph node tissue. AFB smear microscopy was done by flourescent technique, AFB culture was done on Lowenstein Jensen slopes (L-J), Polymerase Chain Reaction (PCR) by professional biotech kit using RNA probe. In all patients, Fine Needle Aspiration Cytology (FNAC) was done.

In both groups, size of lymph node was measured, vertical plus horizontal, by measuring scale at baseline and after 1st and 5th weeks, during laser therapy and follow up measurement was done at 10th and 24th weeks of treatment by investigator.

Similarly, in both groups, aspirate from the lymph node was subjected to AFB smear, AFB culture sensitivity, PCR at baseline, after 1st and 5th weeks of laser therapy. Microbiologist was unaware of the nature of treatment patient was receiving. Follow up AFB smear and AFB culture sensitivity was done at 10th week. In 19 patients, microbiological analysis was not done because of solid nature of lesion.

In both groups, antitubercular treatment was given according to AFB culture and sensitivity report where culture was positive, empiric therapy was given where AFB was not grown in culture. In both groups, ATT was given for two years beyond AFB conversion.

Eligible patients were randomly assigned to the LLLT group and chemotherapy group by a study coordinator who also turned off the machine in the sham treatment arm. To ensure a double-blind study noise of laser machine was inaudible in presence of noise of vacuum pump. In LLLT group, both the laser machine and vacuum pump were started while in case of chemotherapy group only vacuum pump was started (Fig. 1).

The Jelco canula (16 gauge, 50 mm length) was first introduced inside the lesion, pus was aspirated and through the canula, the fiber of the laser equipment was introduced inside the lesion. At each session, in cases of LLLT group, laser was delivered for 780 seconds, while in cases of chemotherapy group sham irradiation was done. This was performed twice per week, for a total of ten sessions. No anesthesia was given.

Laser used in this study was a pulse laser with a pulse duration of seven nanosecond wave length 337 nanometer and average power output of 5 mW at the tip of the fiber. The laser device was manufactured by Raja Ramanna Centre for Advanced Technology, Indore, India.

Primary outcome measure was frequency of AFB smear conversion, culture conversion, PCR for *M. TB* complex conversion in aspirated pus at 1st and 5th weeks of laser therapy. Secondary Average
outcome measure was 1) Average time taken for resolution of fever and 2) Average percentage reduction in size of lesion.

Statistical analyses were based on the intention-to-treat principle.

RESULTS

Epidemiology

A final total of 104 cases were included in the study: Males 33 (31.73%) and females 71 (68.26%). Male to female ratio was 1:2.1. Mean age of the patients was 25.2 years (Range 15-65) in LLLT group as compared to 26.9 years in chemotherapy group. Average duration of illness, before starting treatment, was 17.65 months as compared to 11.82 months in chemotherapy group. Both groups were randomly distributed according to type of lesion (Table 1).

Diagnostic findings

In 75 (72.11%) patients, the tissue samples showed chronic granulomatous inflammation with caseating necrosis. Of the 85 patients, 59 (69.41%) had AFB smear positive, in 11 (12.94%) patients, AFB was grown in culture and in 45 patients, PCR for \( M.TB \) complex detected. Out of 104 patients, in 90 (86.53%) patients, Mantoux Test was more than 10mm in size (Table 2).

Laser therapy

Average time taken for disappearance of fever in LLLT group was 6.25 weeks as compared to 8.07 weeks in chemotherapy group.

Fig. 1: Laser Machine with Fiber and Vacuum pump
Table 1: Distribution of patients according to lesion among LLLT and Chemotherapy group (n=104)

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>No. of patients (LLLT group)</th>
<th>No. of patients (Chemotherapy group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphnode</td>
<td>9 (17.33)</td>
<td>10 (20.00)</td>
</tr>
<tr>
<td>Lymphnode abscess</td>
<td>6 (11.55)</td>
<td>6 (12.48)</td>
</tr>
<tr>
<td>Lymphnode abscess with discharging sinus</td>
<td>25 (48.14)</td>
<td>23 (47.84)</td>
</tr>
<tr>
<td>Cold abscess</td>
<td>14 (26.96)</td>
<td>11 (22.88)</td>
</tr>
</tbody>
</table>

Table 2: Diagnostic criteria of the 104 patients included in the study

<table>
<thead>
<tr>
<th>Diagnostic criteria</th>
<th>Total no of patients in which test was done</th>
<th>No. of pts. showing positive results</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB Smear</td>
<td>85</td>
<td>59</td>
<td>69.41</td>
</tr>
<tr>
<td>AFB Culture</td>
<td>85</td>
<td>11</td>
<td>12.94</td>
</tr>
<tr>
<td>PCR for M.TB Complex</td>
<td>85</td>
<td>45</td>
<td>52.94</td>
</tr>
<tr>
<td>FNAC</td>
<td>104</td>
<td>75</td>
<td>72.11</td>
</tr>
<tr>
<td>Mantoux Test</td>
<td>104</td>
<td>90</td>
<td>86.53</td>
</tr>
</tbody>
</table>

Table 3: Average percentage decrease in the size of lymph node and abscess according to duration in weeks among LLLT and Chemotherapy group (n=104)

<table>
<thead>
<tr>
<th>Group</th>
<th>1st wk</th>
<th>5th wks</th>
<th>10th wks</th>
<th>24th wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLLT</td>
<td>33.77</td>
<td>70.67</td>
<td>86.65</td>
<td>97.12</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>22.98</td>
<td>54.81</td>
<td>69.62</td>
<td>86.51</td>
</tr>
</tbody>
</table>

At 5 weeks t: 2.646, p value 0.01

Table 4: Number of patients showing AFB smear conversion according to duration in weeks among LLLT and Chemotherapy group (n=59)

<table>
<thead>
<tr>
<th>Group</th>
<th>1st wk</th>
<th>5th wks</th>
<th>10th wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLLT</td>
<td>16 (27.11%)</td>
<td>13(22.03%)</td>
<td>1(1.69%)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>2(3.38%)</td>
<td>5(8.47%)</td>
<td>7(11.8%)</td>
</tr>
</tbody>
</table>

Fishers exact test at 5th week p= 0.015

Table 5: Number of patients showing AFB culture conversion according to duration in weeks among LLLT and Chemotherapy group (n=11)

<table>
<thead>
<tr>
<th>Group</th>
<th>1st wk</th>
<th>5th wks</th>
<th>10th wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLLT</td>
<td>3(30%)</td>
<td>3(30%)</td>
<td>1 (9.09%)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>1(10%)</td>
<td>1(10%)</td>
<td>2(20%)</td>
</tr>
</tbody>
</table>
weight gain in patients of LLLT group was 4.51 kg as compared to 3.14 kg in chemotherapy group.

Average percentage reduction in the size of lymphnode, abscess (Fig. 1) and cold abscess at five weeks (after 10 sittings of laser) was 70.67 in cases of LLLT group as compared to 54.81 in chemotherapy group (Table 3). In LLLT group, lymph node and abscess disappeared completely in 23.4 weeks and sinus was closed in 11.03 weeks as compared to 48.1 and 26 weeks in chemotherapy group.

AFB smear, AFB culture and PCR conversion rate at five weeks were 29 (49.15%), six (60%) and 20 (44.44%) in LLLT group as compared to seven (11.86%), two (20%) and eight (17.77%) in chemotherapy group (Tables 4, 5 and 6).

In LLLT group (after 10 sessions of laser therapy), greater reduction in the size of lesion (p value 0.01) and early bacteriological conservation (p value 0.01) was seen as compared to chemotherapy group.

**DISCUSSION**

The standard therapy of treatment failure tubercular lymphadenopathy, abscess, sinus, and cold abscess is antitubercular drugs along with surgery. According to Dharma Kanta et al, treatment of tubercular cervical lymphadenopathy leading to ulceration / sinus formation, excision of ulcer / sinus along with excision of underlying caseating lymphnodes were followed by short course of antitubercular chemotherapy 1. Similarly, results were observed by Siu et al where they suggested that all easily assessable tuberculous lymph nodes should be removed and that persistent discharging sinuses should be treated by surgery 2.

Peripheral lymph node tuberculosis is the most common form of extra pulmonary tuberculosis. Cervical tubercular lymphadenopathy, lymph node abscess with discharging sinus is still the most common cause of persistent cervical lymph node enlargement in the developing countries.

In the present study, we have taken those cases who already had taken ATT for more than six months along with surgical excision, showing no response to treatment. In our knowledge, we have not found any study in which simultaneous monitoring of decrease in the size of lesion along with microbiological study has been carried out.

Low proportion of positive cultures in our study is due to the presence of bacteriostatic substances in tubercular lymph node, which inhibit the growth of bacilli in vitro, bacilli in the glands being scanty. The low rate could also be due to patients having already taken ATT for more than six months. Among culture positives, most common drug resistance was found to be of Isoniazid followed by Pyrazinamide and Rifampicin.

Three patients of lymphadenopathy did not respond because lymphnodes were multiple and deep seated. One patient of sinuses had recurrence because of multiple sinuses. All four patients required surgical excision. In rest of the patients, no recurrence of the lymphnode and no discharge from the sinus were seen during two years of follow up.

The exact mechanism how LLLT works is not known, possibly LLLT enhances immune system; inhibits growth of the tubercular bacilli,
increases circulation to the site of lesion to deliver more drugs.

Influence of laser on the immune system has been evidenced in medical literature. Immunological effects on leukocytes, T & B cells and NK lymphocytes, macrophages result in local and systemic effects through a complex mechanism of action, which is not yet definitively elucidated. *In vitro* experiments have also provided some evidence as possible influence of nitrogen laser irradiation on the immune system, for example nitrogen laser irradiation, was seen to enhance the intracellular killing of internalized bacteria in human neutrophil\(^1\).

The high intensity focused nitrogen laser irradiation has been shown to lead to direct inhibition of bacteria\(^2\). *In vitro* experiment of UVA radiation from Nitrogen laser irradiation on tubercle bacilli at 337 nm, average power 2.0 mW was observed to cause a dose-dependent decrease in cell viability due to significant change in fluidity of lipid regions in the cell wall of laser exposed cells\(^3\).

Twice per week was based on *in vitro* report of effect of nitrogen laser irradiation (337 nm) on viability of clinical isolates of *Mycobacterium tuberculosis*. Bacteria were exposed to a nitrogen laser (average power 2.0 mW) *in vitro* at power density of 70 +/- 0.7 W/m\(^2\) for 0-30 min, and the cell viability was determined by luciferase reporter phage (LRP) assay. Immediately after laser exposure, all the clinical isolates investigated showed a dose-dependent decrease in cell viability. However, when the laser-exposed isolates were incubated in broth medium for three days, most of these showed significant recovery from laser-induced damage\(^4\). Previous study on effect of LLLT (337 nm, average power 2.0 mW twice per week) on treatment failure tubercular lymphadenopathy\(^5\) and drug resistant pulmonary tuberculosis\(^6\) showed significant results in laser treated group.

In continuation of the pioneering work of Finsen on treatment of skin TB by Ultra Violet (UV) light\(^7\) and *in vitro* reports on bactericidal effect of UV light on tubercular bacilli\(^8\), Eshanchanov *et al* reported the use of UVA radiation from nitrogen laser (337-nm) for the treatment of patients with Pulmonary Tuberculosis\(^9\). Ethne L *et al* and J. Stephen Guffey *et al* also observed bactericidal effects of LLLT on bacteria\(^10\,11\). *In vitro* exposure of UVA radiation has been reported to lead to alteration in cell membrane properties via damage to membrane lipids in Escherichia coli\(^12\,13\).

Only a few studies and isolated case reports describing the role of low level laser therapy in pulmonary Tuberculosis\(^14\-18\) and tubercular lymphadenopathy\(^19\,20\) are available in the literature.

**CONCLUSION**

In our study, LLLT has given encouraging results both in the faster healing of the lesion and clearance of tubercle bacilli among LLLT group as compared to chemotherapy group. More studies are needed to determine exact dose and duration of treatment.

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