A detailed description of materials used and methods employed for conducting this trial is explained in this chapter to give a clear picture how this study was conducted.
3.1 ETHICAL CLEARANCE

Manipal University Ethical Committee approved the study protocol before beginning the trial (University Ethical Clearance No. UEC/11/2009). All patients gave written informed consent for willingly participate in the trial. This Clinical trial is registered under clinical trial registry India, reference number CTRI/2012/05/002674.

3.2 STUDY CENTER

Sirdi Sai Baba Cancer Hospital and Research Center, Manipal, Karnataka, India

3.3 DURATION

June 2009 until January 2012

3.4 PATIENTS

3.4a Inclusion Criteria

This trial enrolled patients if they

1. were newly diagnosed with locally advanced unresectable primary Head and Neck Cancer
2. were ≥18 years (up to 65 years) of age
3. had Eastern Cooperative Oncology Group (ECOG) performance score ≤ 2
4. were scheduled to undergo concurrent chemoradiotherapy for primary

3.4b Exclusion criteria

Patients were excluded if they

1. had Trismus, because difficulty in delivering laser beam to the selected sites in oral cavity
2. had any medically compromised conditions which impair wound healing (e.g. diabetes)
3. had any distant metastasis, because of issue in palliative treatment regimen
4. had any history of prior surgery or radiation or neo-adjuvant chemotherapy for HNC
5. were not willing to participate in the study
3.4c **Sample size calculation**

An initial pilot study was conducted involving 15 HNC patients undergoing CCRT to standardize the LLLT dosages. Sample size was calculated from results of this pilot study using formula for comparison of two sample means of OM grades. Power of the study was kept at 90%, and taking this into consideration $Z_\alpha$ and $Z_\beta$ were substituted. We used standard deviation ($\sigma$) of the variable from the results of previous studies by Maiya et al\textsuperscript{28} and Arora et al\textsuperscript{29} in the same center. Acceptable clinical difference ($\delta$) of the variable was decided on the basis of previous studies on similar patients population\textsuperscript{28,29} and opinion of clinical experts.

$$n = \frac{2(Z_\alpha + Z_\beta)^2\sigma^2}{\delta^2} + 20\% \text{ Attrition}$$

$$n = \frac{2(1.96 + 1.282)^2(3.0)^2}{(1.7)^2} = \frac{189.190}{2.89} = 65.464 \approx 66$$

$n \approx 80$ (in each group with 20% attrition)

Where

Type I error ($\alpha$) = 0.05, Type II error ($\beta$) = 0.20, Power = (1-$\beta$) = 0.80

$n$ = sample size

$Z_\alpha = 1.96$ (at 95% level of confidence)

$Z_\beta = 1.282$ (for Power = 90%)

$\sigma = 3.0$ (pooled Standard Deviation of the observations of the two samples)

$\delta = 1.7$ (anticipated smallest difference in the estimated mean of OM grades of two groups)
3.5 CONCURRENT CHEMORADIOThERAPY REGIMEN

All patients received concurrent chemotherapy. The patients were treated with single agent platinum, Cisplatin at the dose of 100 mg/m² (body surface area) administered three weekly (Day 1, 22, 43). Radiation dosage of 66 Grays (2Grays/session) was given in 33 fractions, five days a week over the period of 45 days (6.5 weeks). Patients with residual disease were eligible for higher doses of radiation; however no patient received more than 72 Gy. All patients were treated on a six MV linear accelerator using 3D-Conformal Radiotherapy. (Figure 1) The portals were designed to treat the primary tumor, involved lymph nodes, and the relevant areas of lymphatic drainage. Parallel-opposed radiation fields, with a matching lower neck field, were the technique used in all cases. After 40 Gy, Field Size Reduction (FSR) was done to limit the dose to the spinal cord. Matching posterior electrons were added for patients who required further treatment to the posterior cervical region.

Figure 1: Linear accelerator employed for radiotherapy (ELECKTA: Precise Treatment System 1500) {Machine parameters: Photons – 6 & 15 MV for deep seated tumors, Electrons – 4,6,8,10,12 & 18 MeV for superficial tumors}
3.6 STUDY DESIGN

3.6a Settings and Randomization

This was a prospective, single centered, triple blinded, randomized controlled trial carried out in Sirdi Sai Baba Cancer Hospital, Manipal, India. Three hundred thirty five HNC patients were screened for inclusion and exclusion criteria. Among them 240 met the inclusion criteria, were block randomized into laser (116) and control (124) group. Randomization of patients to the groups was performed using computer generated random number table. Patients were site and stage of HNC matched in each block and there were total of 12 blocks. At the end of the study 19 patients were lost to follow up (Dropouts), 221 patients completed the trial 111 in Laser group and 110 in control group. Flow of participants through the study is shown in Figure 5.

3.6b Blinding

Blinding was done at three levels, i.e., patients, assessor and data analyzing statistician. Patients were advised to keep their eyes closed during LLLT session. They were only able to hear the beep sound produced by the laser instrument. Assessor was blinded to the intervention group; only lists of patients were given to him for assessing the desired outcomes in the patient’s daily record. Once the data sheet were filled with the assessment records of the patient, the patient’s group were coded as A and B and sent for analysis. Hence, the data analyzing statistician was also blinded to the trial groups.
3.7 STUDY INTERVENTION REGIMENS

Both group patients received the standard oral care and oral hygiene protocol as mentioned in best medical practice guidelines.\textsuperscript{106} Dental consultation was sought, and necessary oral treatment administered before the start of radiation. The oral hygiene measures included frequent mouth washes with Sodium Bicarbonate. Bland, soft diet was prescribed for all patients. Whenever a patient was found to develop oro-pahryngeal candidiasis, topical and/or systemic antifungal measures were promptly started. The Control group received sham treatment whereas Laser group patients received LLLT.

3.7a Instruments Used:

   
   \( a. \) Helium Neon Gas Laser
   
   \( b. \) Wavelength = 632.8nm (Visible Red)
   
   \( c. \) Power Output = 24mW = 0.024W
   
   \( d. \) External Beam Diameter = 0.6cm\(^2\)

2. Wavelength specific goggles.

3. Sterile Gloves, Mask, Tongue Depressor, etc.

4. Torch.

3.7b Equipment Calibration

Calibration of the Laser therapy equipment was done daily using Newport Hand Held Power Meter Model 840, (Newport Corporation, Irvine, California) to know the exact power output of the laser instrument. (\textit{Figure 2})
Methodology

Laser Instrument with HNC patients

Laser probes and Protective Goggles

Other Instruments used

Calibration Equipment

Figure 2: Instruments used for delivering Low Level Laser therapy
3.7c Low level Laser Therapy Protocol

A single operator experienced in the LLLT field treated all the patients. Patients in Laser group were treated with Low-Level Helium-Neon Laser at six anatomical sites in the oral cavity excluding cancer site daily before radiation session for 45 days. (Figure 3-4) These six sites, i.e., buccal mucosa, lateral and ventral tongue, labial mucosa, floor of the mouth, soft palate and oropharynx are considered hot points for developing acute mucosal reactions. All hygienic measures were used while delivering the LLLT. Non-contact method was used with minimum distance between the laser probe and tissue irradiated was about 1-5 cm. The treatment time (t) for each application point was calculated by the equation \( t = \frac{\text{energy density (J)} \times \text{surface area (cm}^2\)}{\text{Power (W)}} \). A constant spot of 1cm\(^2\) was irradiated for varying length of time to achieve the desired dose of 1.8-5 J[using formula Dosage/ Energy Density (J) = Power output (W) x irradiation time (seconds)/ Area (cm\(^2\)]], which was standardized at the initial phase of the study in our institution. Total duration of each session varied from about 15-30 minutes for each patient depending on the area of laser irradiation. A reason for delivering the LLLT just before radiation session was to give stabilizing dosages to the oral mucosa so that it is better prepared for the radiation exposure. To achieve beneficial effects of laser on cellular level it has to be given within 20-30 minutes before radiation because enhanced cellular activity was found to be optimal during this period after the laser irradiation.\(^{265,270,274}\)

3.7d Safety during Laser Session

During the treatment, the patient and the therapist wore wavelength specific protective eye goggles.
3.7e LLLT Dose Standardization

We standardized the LLLT dosages depending on the tissue response at the initial pilot phase of our study. Variable irradiation time was used to deliver the desired energy density/dose to the tissue area of 1cm². During initial 6-8 sessions an energy density of 1.8J was delivered which might have acted as a basement cells membrane stabilizer for oral mucosa. Once grade 1 mucositis was evident, an energy density of 1.8-3J was delivered which might have acted on both basement cells membrane stabilizer as well as pain gate mechanism. When grade 2-4 mucositis appeared an energy density of 1.8-3J was delivered at the periphery and 4-5J was delivered at the center of the lesion. (Table 1)

**Table 1:** Describing LLLT Treatment parameters used during the study

<table>
<thead>
<tr>
<th>Beam Used</th>
<th>Power Density (mW/ cm²)</th>
<th>Spot size (cm²)</th>
<th>Energy Dose/ point (J)</th>
<th>Irradiation time/ Point (s)</th>
<th>Sessions/ week during CCRT</th>
<th>Total Energy Dose/ Session (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous mode</td>
<td>24</td>
<td>1.0</td>
<td>Initially 1.8, Later 3-5</td>
<td>75-208</td>
<td>5</td>
<td>28.8-90</td>
</tr>
</tbody>
</table>

3.7e Control Group: Sham Therapy Protocol

The Control group patients received sham treatment. Sham effect was created by keeping the laser probe inactive (i.e. probe was off). Only beep sound was produced by the laser machine after the completion of similar duration as of LLLT group.
3.8 Dropouts

There were 19 dropouts, most of the dropouts happened in the early phase of CCRT. Eight patients took discharge against medical advice due to financial constraints and poor general health, seven patients died during the course of CCRT because of respiratory complications (2), cardiac failure (2), and poor general health (3) and four patients required change of treatment plan for HNC.
**Figure 4:** Images of sites treated with LLLT
3.9 EVALUATION (OUTCOME MEASURES)

An experienced radiation oncologist who was unaware of the trial intervention group did the clinical assessment of all the patients. Assessor was trained before the initiation of trial for assessing the outcomes. His efficacy in assessing the outcomes was assessed on a pilot trial basis. Following outcomes were assessed

1.) **Severity of Mucositis** was assessed each day using the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer (RTOG/ EORTC) scoring system. Where Grade 0-4, 0= none, 1= erythema of oral mucosa, 2= patchy mucositis, 3= confluent mucositis, 4= Ulceration, necrosis or hemorrhage.

In addition, **time of onset** and **total duration** (i.e. number of days) of severe mucositis experienced were also recorded.

2.) **Severity of Oral Pain** was assessed each day of treatment using:

   **A. The Numeric Rating Scale (NRS):** Patients were asked to rate their oral pain on a Visual Analog Scale of 0 to 10. Where 0 = No pain, 10 = Maximum pain imagined by them.

   **B. Verbal scale (VS):** Patients were also asked to orally describe their pain in terms of various levels, i.e., worst possible to no pain and these values were later coded for statistical analysis as (Worst possible = 4, severe = 3, moderate = 2, low = 1 and none = 0).

In addition, **time of onset** and total duration (number of days) of severe pain experienced were also recorded.

3.) **Need for supplemental analgesics** medication to treat pain was recorded using World Health Organization analgesic ladder. The number of days step I (NSAID), step II(weak opioid like Tramadol), and step III (strong opioid like Morphine) analgesic required for pain relief were
recorded. Also, the **time point at which various steps of analgesia required** and **total duration of step III analgesia** required were recorded.

4.) **Severity of Dysphagia** was assessed using Functional Impairment Scale (FIS). Patients were asked for the type of food they were able to take through mouth and related difficulty in swallowing. This was rated as 1-4, where able to- eat solid foods =1, eat soft foods = 2, drink liquids = 3, no oral alimentation possible = 4.

In addition, **time of onset** and **total duration** (number of days) of total parenteral nutrition (tube feeding) required were also recorded.

5.) **Weight Loss**, to know the impact of mucositis on the nutritional status of the patients over the course of treatment was assessed each week using weighing machine.

6.) **Patients reported measures of Oral Mucositis** were recorded by each patient at the end of every week using the **Oral Mucositis Weekly Questionnaire-Head and Neck**.\(^{259}\)

**Oral Mucositis Weekly Questionnaire- Head and Neck Cancer (OMWQ-HN)**

The OMWQ-HN is a mucositis-specific questionnaire. It consists of 12 items that assess impact of OM on patient well-being and oral functions. The time frame for reference is the past one week. Initial four questions (9 items) use a Likert-type response format whereas last three questions use a 0-10 rating scale. Question 3 describes Mouth and Throat Soreness (MTS) and question 4 describes limitations of oral function associated with MTS. First two questions value were reversed (i.e. 1=Excellent instead of 7, 7=Very poor instead of 1) while doing the analysis so that overall higher scores showed more difficulty. A prospective multi-center observational study by Epstein et al, established the validity, reliability, feasibility of this 12 item OMWQ-HN questionnaire for internal and test retest consistency, compliance with the program, and against the FACT-HN, which served as a benchmark tool for validity measurements.\(^{259}\)
7.) **Quality of life** was assessed at three times point, i.e. at the baseline, in the end of CCRT and after one month follow up using the **Functional Assessment of Cancer Treatment- Head and Neck Questionnaire (Version 4)**.\textsuperscript{251,252} This questionnaire includes generic specific and disease specific components. This questionnaire was given to the patients to record their experience and after completion was requested to return to the assessor.

**The Functional Assessment of Cancer Therapy-Head and Neck Cancer (Version 4)**\textsuperscript{6}

The Functional Assessment of Cancer Therapy - Head and Neck (Version 4) consists of the FACT-G, to which a 12-item site-specific, head and neck cancer sub-scale is added. The head and neck sub-scale evaluates the unique concerns of patients with HNC (e.g., swallowing, chewing). The FACT-G has four sub-scales: physical well-being (PWB) (7 items), social/family well-being (SWB) (7 items), emotional well-being (EWB) (6 items), and functional well-being (FWB) (7 items). All items have a Likert-type response scale (ranging from 0 [not at all] to 4 [very much]). Higher scores in physical and emotional domains show poor QOL while higher scores in social and functional domains show better QOL. In head and neck specific component higher score (i.e. very much=4) shows better QOL in six items while in other six items it shows poor QOL. During data analysis six items showing poor QOL scores were reversed (i.e. 4=0 and 0=4), so that overall higher score shows better QOL. Item responses on sub-scale and total scores were calculated as described in the FACT-HN scoring guidelines.\textsuperscript{252} Reliability and validity of FACT-HN questionnaire in various Indian regional languages has already been established.\textsuperscript{253}

8.) Any **Unscheduled Radiation interruption** that happened due to OM was also recorded in terms of **number of days and after how many Radiation fractions** interruption happened.
3.10 DATA ANALYSIS

Various methods of the analyses were used for the final analysis of outcomes. Descriptive analysis was done for the age, gender, region, site, staging and histo-pathological status of head and neck cancer. Man Whitney U test was used to compare between the laser and Control group for time point at which severe outcomes started and total duration (number of days) severe outcomes were present. Oral mucositis (RTOG/EORTC), and its associated pain (VAS) and swallowing difficulty (FIS) scores were summarized using frequency and percentage for each group and different time points. Generalized estimating equation (GEE) was used to test significance difference in proportion of observed outcomes between treatments. GEE for binary data was used for testing difference. Odds ratio was used to summarize risk of developing severe oral mucositis over time as compared to its first appearance. Third week scores were taken as the reference value for making comparison of rate of progression of various outcome grades between the two groups. For Oral Mucositis, Verbal Scale and Functional impairment Scores random effects logistic regression to see difference in developing severe outcome between groups was used. For Oral Mucositis, Functional impairment and Verbal Scale we had used random effects linear regression to see difference in developing severe outcome between groups. For OMWQ-HN and FACT-HN scores were analyzed using repeated measures analysis of variance (ANOVA) through general linear model. Level of significance was kept at $p<0.05$. Besides per protocol analysis, a separate intention to treat analysis of whole data was done using analytical approach by carrying forward last observation of the patient by including the drop outs patients. Microsoft Office Excel 2010, SPSS 18.0 and SAS software were used for analyzing the data.
Ethical Approval granted by Hospital Ethical Committee before starting the trial

335 Head and neck cancer patients scheduled to undergo CCRT were screened for inclusion and exclusion criteria

95 Excluded: 28 Split # RT +15 Trismus +18 Diabetes +9 cancer recurrence +6 distant metastasis +11 ECOG score >2 +8 Refused

240 met inclusion criteria, consent and baseline assessment was done

Block randomization (Site and Stage matched) by Computer Program

Patients-Blinded

Laser group (116)
Low-level Laser therapy + general oral care

Control group (124)
Sham Light therapy + general oral care

Tester-blinded

Daily
OM: RTOG/EORTC
Pain: VAS
Dysphagia: FIS

Weekly
OMWQ-HN
Weight Loss
Analgesics Use

Pre- Post- 1 Month
QOL: FACT-HN
End of CCRT
Radiation Break

Laser: 5 Dropouts =2 expired, 2 = discharged against medical advice, 1= change of treatment plan

Control: 14 Dropouts=5 expired, 6 = discharged against medical advice, 3= change of treatment plan

221 completed (Laser=111 and Control=110 group)

Statistician-blinded

Data Analysis: Descriptive, Generalized Estimation Equation and Odds Ratio

Figure 5: Flow of participants through the study