

Effect of use of antifungal (clotrimazole) drug in reducing the number of colonies of *Candida albicans* and its correlation with Clinical candidiasis in the patients undergoing progressive radiotherapy for the cervicofacial region

ABSTRACT

Aims: To evaluate the effect of topical antifungal Clotrimazole on candida colonies and its correlation with clinical candidiasis in patients undergoing radiotherapy.

Study design: Randomised Clinical Trial

Place and Duration of Study: Rashtrasant Tukdoji Maharaj Cancer Institute, Nagpur between June 2020 and July 2021.

Methodology: 64 patients (52 males and 12 females) undergoing Co⁶⁰ teletherapy for cervicofacial malignancies were randomly divided in two groups. 32 patients referred to as study group were put on antifungal treatment (1% Clotrimazole) for topical application and other group was the control group and was not given antifungal medication. During the radiotherapy and 6 weeks after the completion of radiotherapy, patients were examined every week for possible oral changes for clinical candidiasis, and swabs were taken at every end of the week for determining candidal colonies.

Results: The overall incidence of clinical oral candidiasis was 46.9% throughout RT in the control group as there was no incidence of clinical candidiasis in the study group. Patients with clinical candidiasis 6-week post-radiation therapy showed continuous symptoms of clinical candidiasis but the reduction in candidal colonies. 22% of patients were oral carriers for candidal colonies. Also, the study group showed not a single patient with clinical candidiasis.

Conclusion: During radiotherapy, although with the use of clotrimazole some patients with negative culture may also become positive for *Candida albicans* and there may be some increase in the several colonies of *Candida albicans* (very less in number as compared to the control group), but its use prevents the development of clinical candidiasis. Antifungal prophylaxis is useful in combating clinical candidiasis.

Keywords: Radiotherapy, Candidal colonies, candidiasis, antifungal, clotrimazole

1. INTRODUCTION

In recent years, significant progress has been made in identifying the proposed hallmarks of cancer growth and management. However, with its growing prevalence, cancer clinical management remains a problem for the twenty-first century. Radiation therapy, surgery, chemotherapy, immunotherapy, and hormone therapy are all treatments available.¹ Along

with surgery and chemotherapy, radiation therapy or radiotherapy is a significant modality used in cancer treatment since it is a relatively cost-efficient single modality treatment that accounts for just around 5% of all cancer care costs.²

Within a short time after the discovery of X-ray and radioactive substances, it was recognized that the radiations were a potential health hazard. Although radiotherapy assured a better quality of life for cancer patients, it was thought to be responsible for various other complications. Radiotherapy produces many undesirable side effects like xerostomia, mucositis, dysgeusia, edema, and fibrosis of soft tissues, decreased resistance to infections, ulcers in the oral cavity, and candidiasis. Xerostomia, mucositis, and infections were the major complications of cervicofacial radiation.³

Candidiasis is the most common infection of oropharynx in patients receiving radiation. Radiation frequently causes alteration of the oral environment predisposing to colonization of the oral mucosa by candida causing opportunistic superficial candidiasis. Radiation induced fragility of oral mucosa leads to occurrence of specific lesions like erythematous lesions, ulceration, and dryness of mouth shows statistically significant role in making oral mucosa more susceptible to oral.⁴ Many antifungal medications, such as nystatin, Amphotericin B, and Gentian Violet, have been proposed and tested for the treatment of candidiasis, with differing degrees of efficacy. Although effective, Nystatin is not palatable due to its bitter taste and hence it is not well accepted by patients for oral application. Clotrimazole was the first Imidazole derivative developed for the treatment of human mycotic infection. It is a potent antifungal agent with no toxic effect on skin or mucous membrane when applied locally. The cure rate with oral torches for oropharyngeal candidiasis is reported to be as high as 100 percent.⁵

The main objectives behind this study are to know the effect of progressive dose of cervicofacial radiotherapy on the changes in the candidal population. It is also to be seen that whether there is some correlation between the changing candidal colonization by radiotherapy and the effect of antifungal (clotrimazole) drug in reducing the number of colonies of *Candida albicans* and the incidence of clinical candidiasis.

2. MATERIAL AND METHODS

2.1. Study Design and Participants. The current Double Blind randomized study was conducted at malignancies at Govt. Medical College and Hosp. Nagpur and Rashtra Sant Tukdoji Cancer Hospital, Nagpur. The study was approved by Institutional Ethical Committee. Written informed consent was obtained from each patient before enrollment and after providing all the required details on the nature of the study according to the principles of the Declaration of Helsinki. Patients were planned to be given a total 60 Gray radiation dose over a period of 6 weeks, with a daily dose of 2 Gray. Inclusion criteria was patients diagnosed with oral cancer with >16 yrs of age and prepared to receive Radiotherapy for the first time. Exclusion criteria was patients with previous Chemo or Radiotherapy, presence of other malignancy within the last 5 years, presence of serious infection, Patients under antibiotic therapy, cancer chemotherapy, Diabetes Mellitus patients and endocrinal disturbances patients, which are known predisposing factors to cause the candidiasis in oral cavity.

2.2. Recruitment and Randomization. A total of 64 patients (52 males and 12 females) undergoing Co60 teletherapy for cervicofacial were selected. Of which all 64 provided written informed consent forms were evaluated and were randomly divided into two groups. A group of 32 patients (28 males and 4 females) were not put on any antifungal drugs and was referred as control group. Other 32 patients (24 males and 8 females) referred as study

group were put on antifungal treatment from 1st week of radiotherapy and was continued till 6 weeks after the completion of radiotherapy. The allocation sequence is performed by subject not involved in the recruitment, data collection, and analysis. Each patient in study group was supplied with 5 gm. collapsible tubes of Surfaz ointment, containing 1% clotrimazole cream U.S.P. manufactured by franco-Indian Remedies Pvt. Ltd., Patients were asked to apply the ointment thrice daily on all the mucosae of oral cavity. Patients were advised not to eat or drink anything at least for an hour after the application of the ointment. During the radiotherapy and 6 weeks after the completion of radiotherapy, patients were examined every week for possible oral changes for clinical candidiasis. In addition to clinical examination swabs were taken at every end of the week to determine number of candidal colonies in oral cavity at that time.

2.3. Outcome Measurement and Data Collection

2.3.1. Assessment of Clinical Candidiasis. To control for confounding variables, the recruited patients were rigorously assessed before to the study's commencement, and those with oral cavity disorders were eliminated. It was assessed based on following criteria Absent, Mild-white patch (C1), Moderate - Thick Candidal plaques (C2), Severe - Candidal granuloma or nodule (C3)

2.3.2. Assessment of Clinical Candidiasis. Both the groups were evaluated for presence of candidal colonies for which plain, sterilized, cotton wool swabs were used to sample the mucosa of the palate, dorsum of the tongue, floor of the mouth, upper and lower buccal as well as labial sulci. The patients were examined clinically and regular weekly oral swabs were taken during the same period. The most frequently used primary isolation medium for Candida is Sabouraud Dextrose Agar (SDA) ⁵ which, along with permitting the growth of Candida, suppresses the growth of many species of oral bacteria due to its low pH. Round, raised, moist, smooth, creasy and white colonies were considered to be of the genus Candida. For the identification of the species of Candida albicans two more tests were carried out: - i) Germ tube test and (ii) Sugar fermentation test.

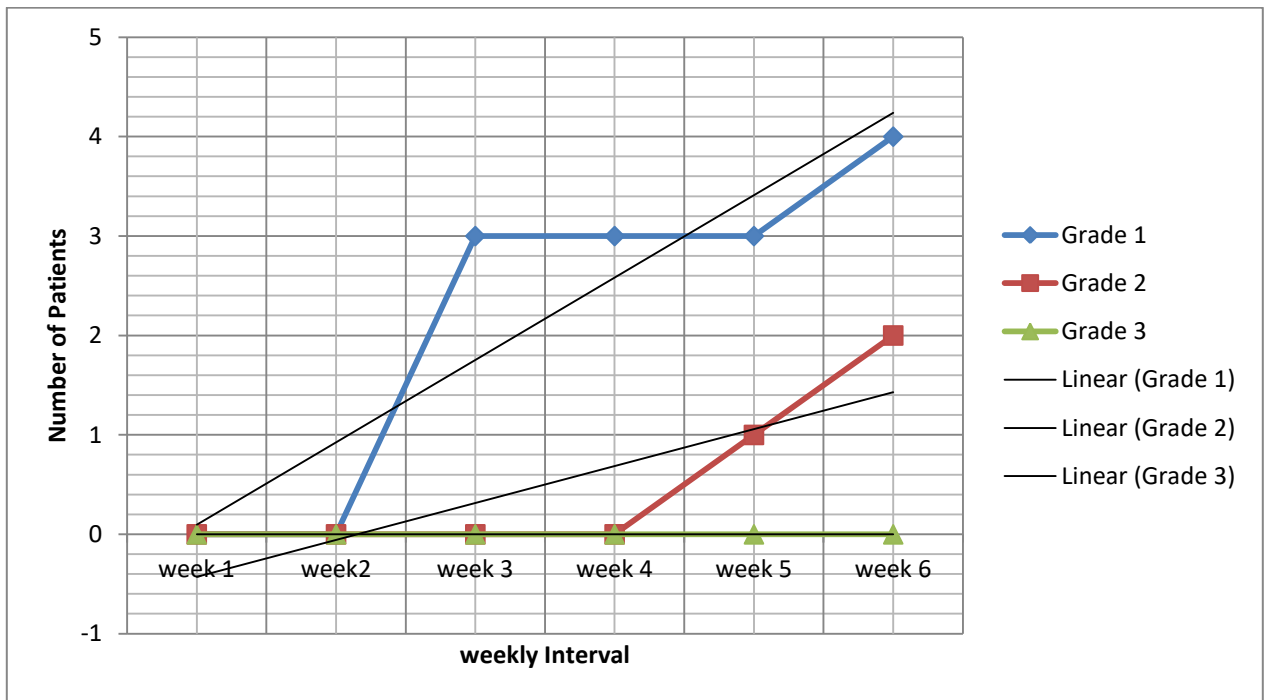
3. RESULTS AND DISCUSSION

Investigations have quantified oral carriage of candida albicans at 300-500 colony forming units in healthy persons.⁶ though in our study 14 patients were positive for candida colonies, they were considered as oral carriers of candida and was not pathological.

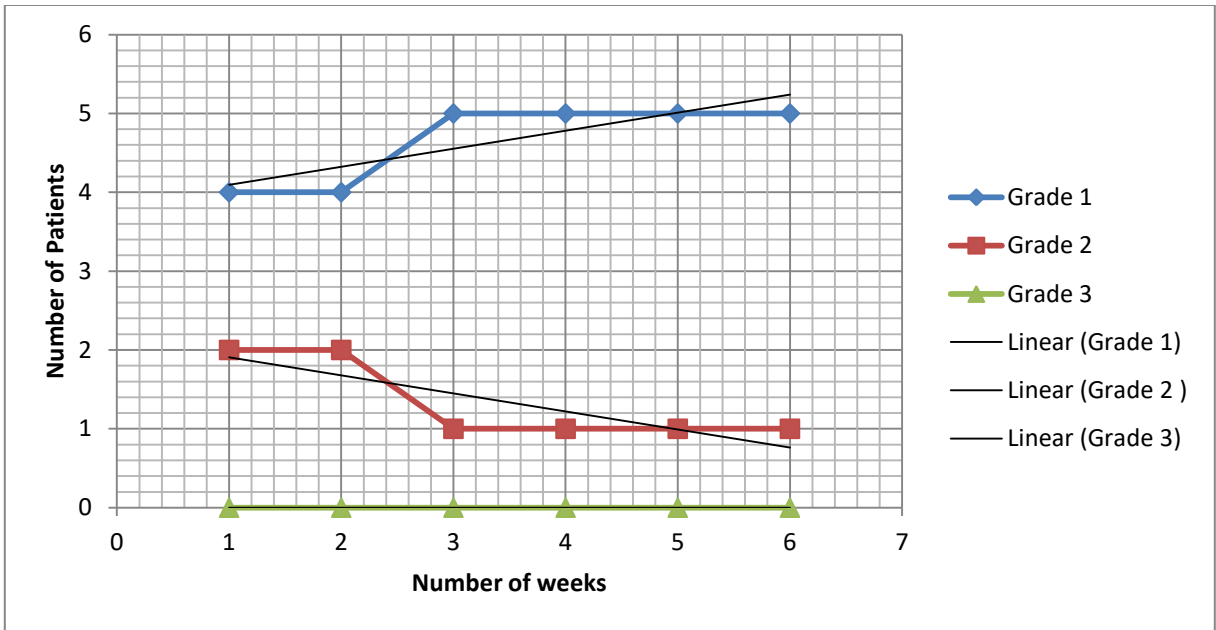
Out of 64 patients included in this study 52 were males and 12 were females, with the age range of 16 years to 80 years. Most of the patients belonged to 4th to 6th decades of life. In control group, at the end of 6th week of radiotherapy 6 patients developed clinical candidiasis, and colony counts were found to be ranging from 264 to 643 (av. 416.12), whereas in study group, none of the patients developed clinical candidiasis (av. 63.08).

The clinical candidiasis was completely absent in study group. Whereas there was sudden rise in candidiasis in study group from 3rd week during radiotherapy which was quiet persistent even after 6 weeks of radiotherapy. At the end of 6th week of post-radiotherapy period in control group, all the 6 patients continued to have clinical candidiasis and number of colonies were found to be ranging from 201 to 376 (av. 319.5) whereas in study group, none of the patients developed clinical candidiasis (av. 26.08).

Graph 1: Occurrence of clinical candidiasis in Control group During Radiotherapy.

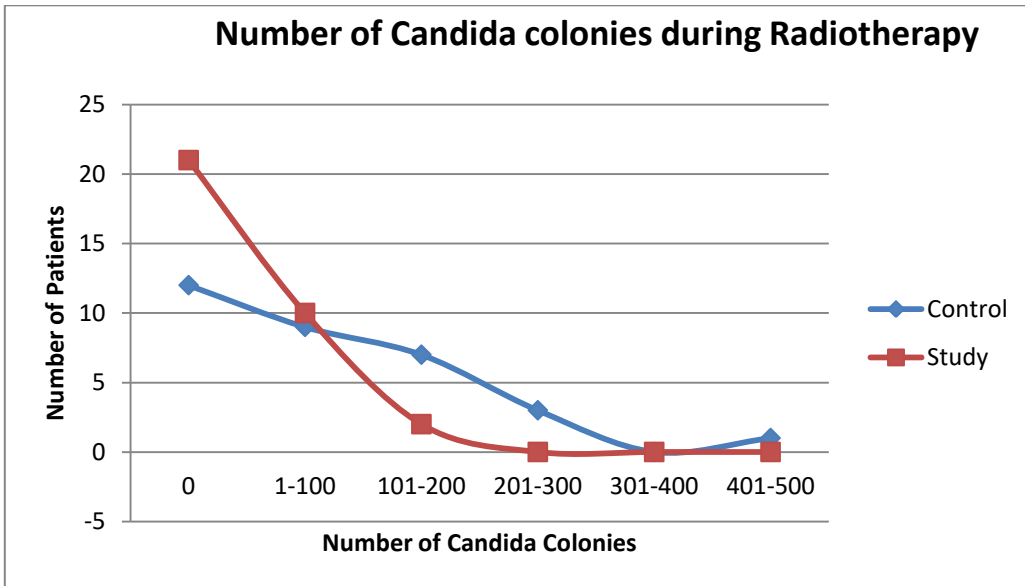


Graph 2: Occurrence of Clinical candidiasis After Radiotherapy in Control Group



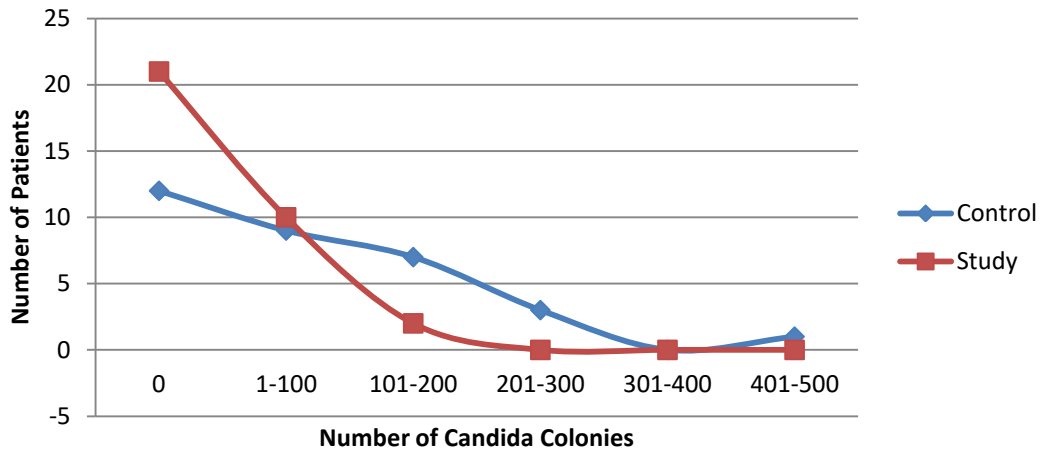
After applying Independent sample test the number of candida colonies had statistically significantly reduced (17.06 ± 51.06) at the end of clotrimazole medication after radiation therapy in Study Group as compared to that in Control Group (-82.25 ± 94.08), $t(62) = -5.2.33$, $p = 0.004$. As the grading of clinical candidiasis is qualitative data whereas the number of candidal colonies is quantitative data, thus statistical analysis could not be performed.

Graph 3: Number of Candida colonies during Radiotherapy



Graph 4: Number of Candida colonies during Radiotherapy

Number of Candida colonies during Radiotherapy



According to a systematic review published by the multinational association of supportive care in Cancer (MASCC)/International Society of Oral Oncology (ISOO) in 2010, oral candidiasis is present in 7.5 % of cancer patients before they begin therapy.⁷ Which was quiet on higher side in our study where, before the start of radiotherapy, 7 subjects each (21.87%) had positive cultures for *Candida albicans* in control as well as in study group. These findings are similar to the findings of Singh GK et al⁸, who observed 6 subjects were positive for candida before therapy with 22 % carrier rate. However, findings of Chen and Webster⁹ were slightly different and they observed 29.60 per cent of the patients with positive cultures for *Candida albicans* before the start of radiotherapy. Clinicians must keep in mind that oral candidiasis develops even before HNC therapy begins, especially in patients who have major risk factors for fungal colonization at baseline, such as ex-or current smoking, rising age, and a history of betel nut chewing.

In the present study, not a single subject with positive cultures for *Candida albicans* demonstrated any signs and symptom of candidiasis, before the start of radiotherapy and the number of colonies in the *Candida* carriers of the control and study groups were found to be ranging from 2 to 330 (Av. 104) and 68 to 211 (Av. 131.71) respectively. According to Chitapanarux et al.¹⁰ in this study the clinically diagnosed incidence of oral candidiasis throughout therapy was as low as 50% and 52%, after the completion of RT. Radiation-induced mucositis may disguise some clinical manifestations of erythematous and angular cheilitis types of candidiasis. This might explain why clinically diagnosed oral candidiasis was underestimated during RT.

At the end of 6th week of radiotherapy, the numbers of colonies of *Candida albicans* were found to be considerably high in the control group (79 to 355 and Av. 181.42) as compared to the colony counts in the study group (32 to 98, Av. 63.08). In fact, as compared to the control group, study subjects had significantly less colony counts of *Candida albicans*, throughout the 6 weeks of radiotherapy. It can be concluded that application of antifungal (clotrimazole) ointment in study group resulted in reduction of number of colonies of *Candida albicans*. Martin MV, Al-Tikriti U, Bramley PA¹¹, in their study on the yeast flora of the mouth during radiotherapy observed that, most of the patients had high colony counts even after 4-6 months after radiotherapy.

The increase in the number of subjects with positive cultures for *Candida albicans* during and at the end of 6th week of radiotherapy from 1 to 20 patients (62.5%) in the control group where no antifungal (1% clotrimazole) ointment was used, is quite significant. It may be due to lowered general resistance of the patients and local causes like xerostomia, changed bacterial flora or less tissue vitality etc. These findings are similar to the findings of Chen and Webster⁹, who also observed 63.30 % subjects with positive cultures for *Candida albicans*, at the end of the 6th week of radiotherapy.

In the control group subjects at the end of the 6th week of radiotherapy 6 subjects developed clinical candidiasis and the number of colonies ranged between 264 to 563 (Av. 416.12). Whereas the average number of colonies in the patients who did not develop clinical candidiasis was only 181.42 which is very less as compared to the number of colonies in patients who developed clinical candidiasis. Even the average number of colonies was much higher (329.33) in those patients when they were first diagnosed as having clinical candidiasis. Although at the end of the 6th week of the post-radiotherapy period, all the 6 subjects continued to have clinical candidiasis, there was a progressive reduction in the number of colonies of *Candida albicans* (201 to 378, Av. 319.5).

In the study group, none of the patients developed clinical candidiasis even at the end of the 6th week of radiotherapy and the number of colonies was found to be ranging from 32 to 90 (Av. 63.08). Even at the end. of 6th week of the post-radiotherapy period, none of the patients developed clinical candidiasis, and the number of colonies, during, the same period was found to be very low (17 to 39, Av. 26.08). It shows that clotrimazole in the present drug regime, is useful in the prevention of clinical candidiasis, in patients undergoing cervicofacial radiation. **The limitation of the study was the small sample size, hence study with larger sample size and with multicentric study is recommended. Though there was significant reduction in colonies of candida and clinical presentation in study group dose dependent relation needs to be evaluated.**

4. CONCLUSION

During the radiotherapy when clotrimazole is not used, there is a progressive increase in the number of subjects with positive culture for candida Albicans, and after the radiotherapy is completed patient's number does not decrease. Moreover, patients with negative cultures may also become positive for Candida albicans. During radiotherapy, although with the use of clotrimazole some patients with negative culture may also become positive for Candida albicans and there may be some increase in the number of colonies of Candida albicans (very less in number as compared to the control group), but its use prevents the development of clinical candidiasis. The clotrimazole ointment application regime of three times a day is found to be effective in preventing the development of clinical candidiasis and is recommended for patients undergoing radiotherapy for relief from adverse symptoms of it. Further studies with different concentration of antifungal medication are recommended for definitive results.

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AUTHORS' CONTRIBUTIONS

Dr. Ashish Lanjekar designed the study, wrote the protocol, Dr. Devendra Palve conducted the evaluation of colonies and statistical analysis, Dr. Pranada Deshmukh wrote the first draft of the manuscript. Dr. Pranada Deshmukh and Dr. Isha Madne managed the analyses of the study. Dr. Pranali Thakare managed the literature searches. All authors read and approved the final manuscript.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Institutional Ethics Committee and have therefore been performed by the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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