"EFFICACY OF CURCUMIN AS A RADIO-SENSITISER AND IN MINIMISING MUCOSAL DAMAGE IN PATIENTS RECEIVING RADIOTHERAPY FOR HEAD AND NECK SQUAMOUS CELL CANCERS"

By

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Under the Guidance of

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Dr. ARUN P.

LIST OF ABBREVIATIONS

HNSCC	⇨	Head and neck Squamous Cell Carcinoma
5-FU	⇨	5-fluorouracil
HPV	⇨	Human Papilloma Virus
DNA	⇨	Deoxyribonucleic Acid
PKA	⇨	Protein Kinase A
PKB	⇨	Protein Kinase B
TP53/p53	⇨	Tumor protein 53
RB1	⇨	Retinoblastoma 1
p53	⇨	Tumor protein 53
BAX	⇨	BCL2 associated X protein
CDK	⇨	Cyclin Dependent Kinase
GSTM1	⇨	Glutathione S-transferase mu 1 protein-coding gene
RNA	⇨	Ribonucleic Acid
MDM2/4	⇨	Mouse double minute protein
RNA	⇨	Ribonucleic Acid
TNFα	⇨	Tumour necrosis factor alpha
EGF	⇨	Epidermal growth factor
PI3-kinase	⇨	Phosphoinositide 3-kinase
PDK1	⇨	Phosphoinositide-dependent kinase-1
PTEN	⇨	phosphatase and tensin homologue gene
VEGF	⇨	Vascular endothelial growth factor
FGF	⇨	Fibroblast growth factor
IL	⇨	Interleukin
RT	⇨	Radiotherapy
CECT	⇨	Contrast enhanced computerized tomography
SDR	⇨	Split dose repair
EBRT	⇨	External Beam Radiation Therapy
MSKCC	⇨	Memorial Sloan-Kettering Cancer Center

MACH-NC	⇨	Meta-analysis of chemotherapy in head and neck cancer
HR	⇨	Hazard ratio
SLDR	⇨	Sublethal damage repair
PLDR	⇨	Potentially lethal damage repair
EORTC	⇨	European Organisation for Research and Treatment of Cancer
RTOG	⇨	Radiation Therapy Oncology Group
LRC	⇨	Locoregional control
DFS	⇨	Disease free survival
RECIST	⇨	Response Evaluation Criteria in Solid Tumors
CR	⇨	Complete response
PR	⇨	Partial response
SD	⇨	Stable disease
dCK	⇨	deoxycytidine kinase
ROS	⇨	Reactive oxygen species
NF-κB	⇨	Nuclear factor kappa B
FSSAI	⇨	Food Safety and Standards Authority of India
FDA	⇨	Food and Drug Administration
GM-CSF	⇨	Granulocyte-monocyte colony stimulating factor
HSV	⇨	Herpes simplex virus
NCCTG	⇨	North Central Cancer Treatment Group
iNOS	⇨	Inducible nitric oxide synthase

ABSTRACT

Background:

Malignancy of head and neck region is common in Kolar district. Surgery, radiotherapy and chemotherapy form the mainline of treatment for these cancers. They are used alone or in combination. Radiation or chemoradiation are often used in patients unfit for surgery. The chemotherapeutic agents usually act as radiosensitiser, but their benefit is at the expense of various toxicities they are known to produce. Curcumin has demonstrated promising results in in vivo and in vitro studies as a radiosensitiser, and also to reduce oral mucositis which is one of the dose-limiting toxicity of radiation and chemotherapy. The anti-inflammatory property of curcumin may hold promise in reducing radiation induced mucositis. So it may be an ideal drug to be tried in patients undergoing radiotherapy or concurrent chemo radiation with head and neck cancers

Objectives:

- To find out the efficacy of curcumin as an adjuvant with regards to reduction in tumor size and volume in patients receiving radiotherapy or concurrent chemo radiotherapy (radiosensitisation) for head and neck cancers squamous cell cancers compared to their stage matched controls.
- To find out whether administration of curcumin to patients undergoing radiotherapy or concurrent chemo radiotherapy for head and neck squamous cell cancer reduces the mucositis and ulceration as compared to their controls .

Methods:

Ours was a single blinded randomized study involving 64 patients. Three patients were dropped from the study. 61 patients who underwent radiotherapy were included in the study and randomized into group A and B based on 4x4 block randomisation. Group A received 500mg of curcumin thrice daily and group B received placebo till the completion of radiotherapy. 21 patients who underwent chemoradiation were included to study the radiosensitisation potential of curcumin, with 12 patients in group A and 9 in group B. The tumor response was assessed

using RECIST criteria at the end of three months post treatment using Contrast enhanced computerized tomography (CECT) scan. All 61 patients were assessed for the effect of curcumin on oral mucositis on weekly basis during treatment and 2 months post treatment using NCI-CTAE and WHO criteria.

Results:

58% of patients in group A had partial response in comparison to 33.3% in group B. The difference was not statistically significant due to inadequate number of cases. Both the groups had similar grade of mucositis in first two weeks of treatment. The severity of mucositis was progressive with 4 patients developing grade III mucositis in control group in comparison to group A, where the majority of patients were having grade I mucositis (73.3%). The difference was statistically significant from 3^{rd} week onwards. (p value<0.001).

Conclusion:

Curcumin reduces the incidence and severity of radiation induced mucositis. No systemic toxicity was noticed with intake of curcumin. Curcumin has a role in reducing the severity of mucositis, which can benefit patients undergoing radiation/ chemoradiation. Further studies are required to validate the role of curcumin as a radiosensitiser in the treatment of head and neck squamous cell carcinomas.

KEYWORDS:

Oral carcinoma, Radiation, Chemoradiation, Radiosensitiser, Curcumin, Mucositis

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INTRODUCTION

Malignancy of head and neck accounts for 12% of all the cancers in India.[1] In Kolar district, squamous cell carcinoma of Head and Neck is the commonest type of malignancy.[2]

Various modalities of treatment like surgery, radiotherapy and chemotherapy are used to treat squamous cell carcinoma of head and neck region. Each modality of treatment gives rise to various complications which at times causes discontinuation of treatment. Radiation and chemotherapy forms the major line of treatment in patients unfit for surgery. The chemotherapeutic agents are often used as radiosensitiser, but their benefit is at the expense of various toxicities they are known to produce. This resulted in the need for alternative agents as an adjuvant with low toxicity profile. This created a special interest in finding out the efficacy of phytochemical agent Curcumin as radiosensitiser, which was found to have radiosensitisation potential both in in vitro and in vivo studies. One of the dose limiting factor in patients undergoing radiation is the radiation induced mucositis, which is aggravated by chemotherapy drugs like Cisplatin, 5-flurouracil (5-FU), Taxanes. Since Curcumin is known as an anti-inflammatory agent down the ages, it may hold promise in limiting radiation induced mucositis. So it may be an ideal drug to be tried in patients undergoing radiotherapy or concurrent chemo radiation with head and neck cancers.

OBJECTIVES OF THE STUDY

1) To find out the efficacy of curcumin as an adjuvant with regards to reduction in tumor size and volume (radiosensitisation) in patients receiving radiotherapy or concurrent chemo radiotherapy for head and neck squamous cell cancers compared to their stage matched controls.

2) To find out whether administration of curcumin to patients undergoing radiotherapy or concurrent chemo radiotherapy for head and neck squamous cell cancers reduces the mucositis and ulceration as compared to their controls.

REVIEW OF LITERATURE

Cancer is considered as a genetic disease and accumulation of alteration at molecular level in genome of somatic cell (somatic mutations) results in the progression to cancer. The molecular alteration can be spontaneous or can be the result of external carcinogens.

Head and Neck cancer is the most common cancer in developing countries. It is the most common cancer seen in males in India and the fifth most common in females.[3] People from India and Southeast Asian countries suffer from oral cavity cancers while in western population, oropharyngeal cancers are more common. [3]

Etiology of Head and Neck Squamous Cell Carcinoma

Head and neck Squamous Cell Carcinoma (HNSCC) are commonly associated with the habit of the individual. Consumption of tobacco in various forms, chewing of arecanut, alcohol intake and Human Papilloma Virus (HPV) infection are the commonest risk factors for the development of various forms of HNSCC. These will contribute to the accumulation of genetic aberration which will lead to progression to HNSCC.

Tobacco:

Smoking in all its forms and smokeless tobacco are the two commonest forms of consumption of tobacco. Tobacco contains nearly 60 chemicals which are proven carcinogens, majority of which are polycyclic aromatic hydrocarbons. Other known carcinogens include lactones, coumarin, ethyl carbamate, volatile N-nitrosamines, nitrosamino acids, tobacco specific

N-nitrosamines, inorganic compounds, radioactive Polonium 210, and Uranium 235 and 238. Smoking tobacco has been implicated in cancers of all sites of upper aerodigestive tract. [4] Oropharyngeal cancers have been associated with increased tar consumption in a dose dependent manner.

Form of tobacco consumption has a bearing on the site of cancer in the head and neck region. Dark tobacco used in cigars and pipe blends is known to affect lower respiratory mucosa. Cigar and pipe smoking have been associated with a relative risk of 3.3 in the development of oral and oropharyngeal cancers, whereas the use of filtered cigarettes increases the risk of endolaryngeal cancers.[5]

Smokeless tobacco: There is a 4-6 fold increased risk of development of oral cancers in people using oral tobacco. Use of smokeless tobacco and arecanut is implicated as a most common cause of oral and oropharyngeal cancer in Southeast Asian countries.[4] Smokeless tobacco is consumed in various forms in different parts of the country. These include "Khaini" (Tobacco lime mixture), guthka (Ready-to-eat tobacco product containing areca nut, slaked lime, catechu, and tobacco and flavoring agents and sweeteners), betel quid (contains four main ingredients, betel leaf (*Piper betel*), areca nut, catechu, slaked lime, and tobacco), mishri (made at home by roasting tobacco flakes on a hot griddle until it turns brown or black. It is applied to gums and teeth and retained in the mouth for variable time period), gul or gudakhu (paste prepared from powdered tobacco and molasses, which is applied to the gums and teeth with a finger). Betel quid chewing is the commonest form of smokeless tobacco consumption. There is a 59% attributable risk of development of oral cancers in people consuming tobacco in this form.[6]

The smokeless form of tobacco contains approximately 4200 chemicals. Highest amount of carcinogenicity in smoke less form of tobacco can be attributed to N-Nitrosonornicotine (NNN), 4-(methylnitrosamino)-1 (3-pyridyl)-1 butanone (NNK), and N-nitrosamino acids.[7]

In India, tobacco consumption is responsible for half of all the cancers in men and a quarter of all cancers in women[3]. Poorer prognosis in smokers has been attributed to smoking-related comorbidity and a high probability of second primaries in the head and neck and lung. This can be interpreted in terms of „„field cancerization"""that is, widespread presence of genetic alterations and precancerous lesions in the head and neck epithelium of smokers. The mechanism how smoking causes cancer is depicted in the figure 1.

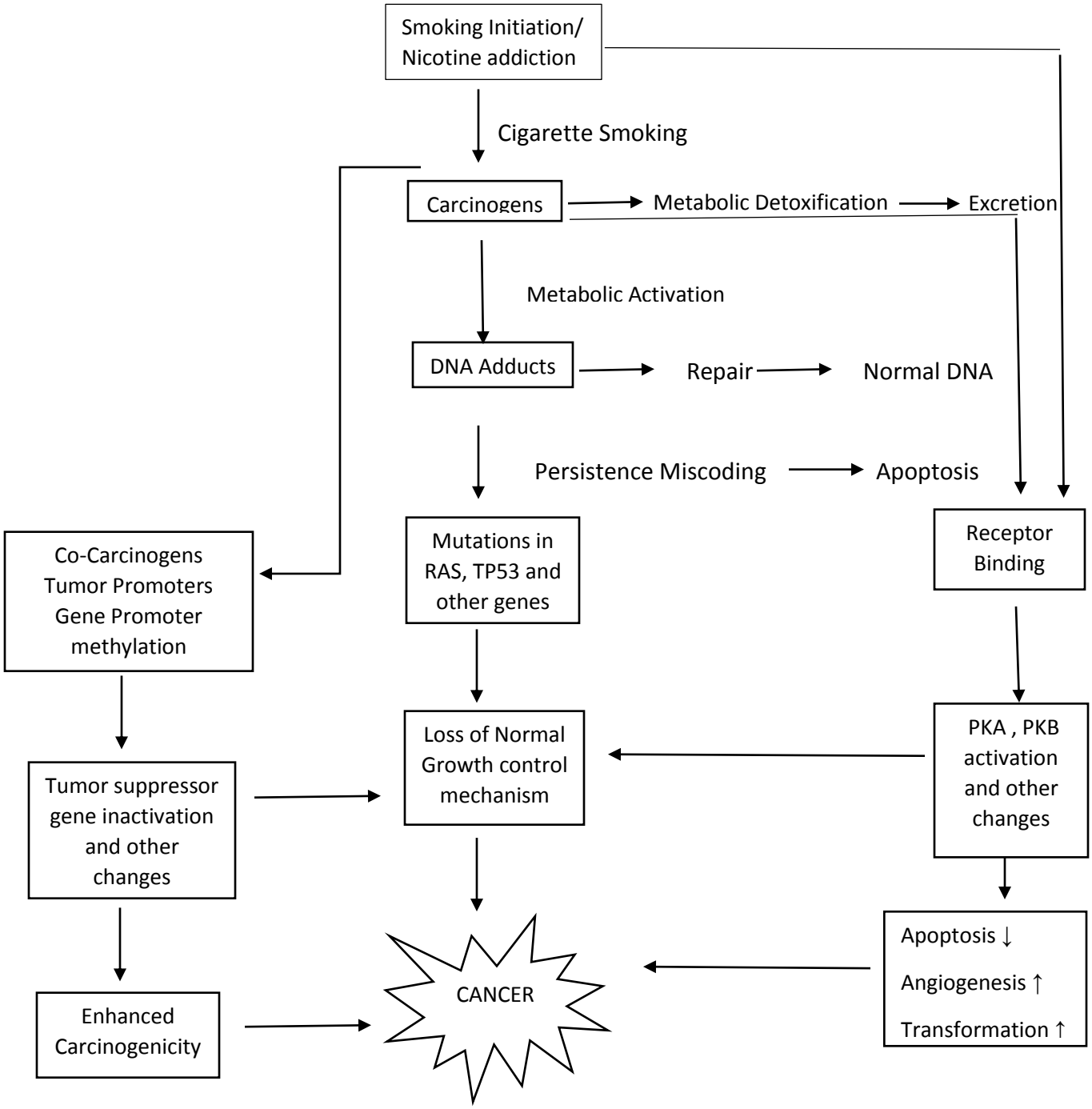

Fig 1 Mechanism of cancerogenesis due to smoking.

Areca nut:

It is a confirmed carcinogen and is causally associated with oral submucous fibrosis.[1] Areca nut contains polyphenols, alkaloids such as arecoline, arecaidine, guvacine, guvacoline and minerals. Arecoline, has been found to stimulate synthesis of collagen in fibroblasts, and other chemicals such as catechin, flavonoid, and tannin compounds in areca nut causes cross linking of collagen fibers, making them less susceptible to degradation by enzyme collagenase, which results in increased formation of cross linkages and accumulation of collagen in patients having oral submucosal fibrosis, which is a premalignant condition.

Alcohol:

Chronic exposure to alcohol will result in carcinogenic change in cells and tissues. Similar to tobacco, the carcinogens in alcohol also requires to be activated into their active intermediate that is acetaldehyde. Acetaldehyde brings about the carcinogenic change primarily by binding to DNA. During alcohol metabolism there is production of reactive oxygen radicals, which are mutagenic. Alcohol promotes cytochrome P450 activity which increases the activation of procarcinogens in both tobacco and alcohol and in addition alcohol when consumed along with tobacco acts as a solvent for the carcinogens, thereby facilitating their entry to cells, especially in the upper aerodigestive tract. The combined use of tobacco and alcohol increases the risk of laryngeal cancers by about 50% over the estimated risk.[8]

Human Papilloma virus:

HPV prevalence in HNSCC is around 50%, and highest is seen in malignancies of oropharynx. HPV 16 is the commonest type identified in 30.9% of oropharyngeal and 16% of laryngeal cancer. Prevalence of HPV in oral cancer are highest in Asia (33%) compared to Europe (16%) and North America (16.1%).[9]

HPV is a double stranded DNA papovavirus that primarily infects human epithelial cells. They are classified as high risk and low risk types on the basis of their ability to promote tumorogenesis. The high risk types are 16 and 18 and are associated with cervical and anogenital cancers. The low risk types 6 and 11, causes non-cancerous pathologies like papilloma and condyloma. The HPV attributed for HNSCC is HPV 16.[10]

The infection with high risk HPV subtypes will result in transformation in human keratinocytes with the help of early viral proteins namely E6 and E7. The HPV protein E6 blocks the action of TP53 tumor suppressor gene, and E7 blocks RB1 tumor suppressor gene without causing gene mutations. The predominant site of HPV-associated tumors is in the oropharynx, and has a predilection for non-smokers (about 50%). The HPV positive HNSCC tumors are found to be more radiosensitive, this is attributed to the stable transfection of HPV16 protein E6 into a human cancer cell line has been found to increase in vitro radiation sensitivity.[10] In addition, HPV-positive carcinomas tend to maintain functional non mutated TP53. Similar to cervical cancers, detection of HPV in HNSCC is associated with sexual history, thereby indicating direct exposure as a cause for infection.

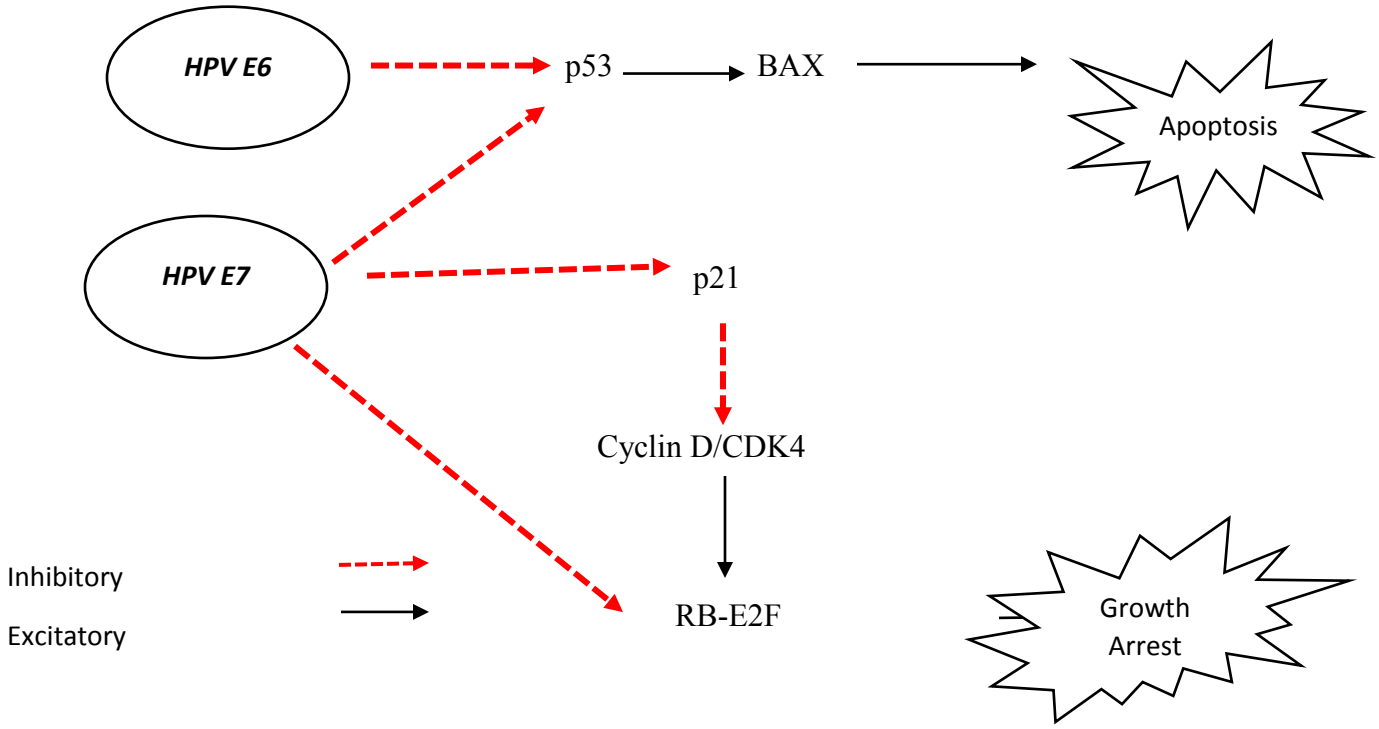

Fig 2 Viral proteins E6 and E7 causing Apoptosis and growth arrest

Molecular biology of Head and Neck Squamous Cell Carcinoma

The susceptibility of an individual to the carcinogenic effects of tobacco, alcohol and HPV varies widely, and is dependent on various hereditary factors. It has been found that there is 2 to 14 fold increased incidence of HNSCC in first degree relatives of patients with HNSCC.[11] There are certain inherited genetic polymorphisms also which can increase the risk of HNSCC by altering the function of carcinogen activating enzymes group and detoxifying enzymes group namely the cytochrome P450 and GSTM1 respectively. Increased susceptibility for HNSCC is also seen with polymorphisms of prominent cell cycle regulators such as Cyclin D1, p53 and p21. P53 mutation is common in West whereas in India and South East Asia there is a preponderance of Ha-ras mutations (35%), loss of heterozygosity of Ha-ras (30%), N-ras amplification (28%), and N-myc amplification (29%). [12]

Genetic Progression Model for Head and Neck Squamous Cell Cancer

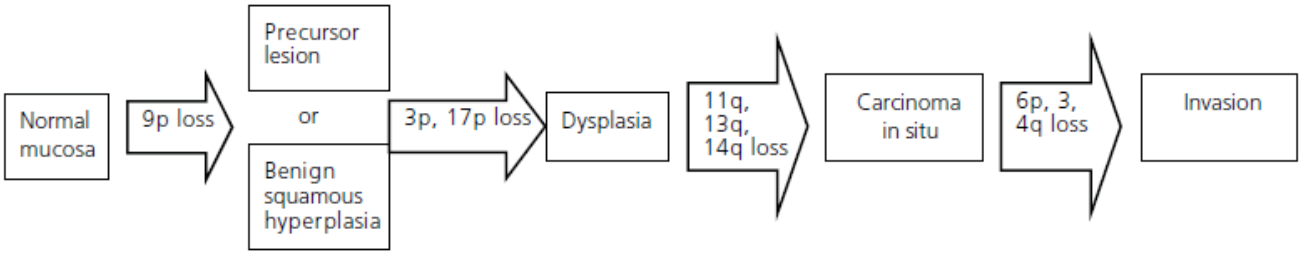

Fig 3 Genetic progression of Head and Neck Cancer

Critical genetic aberration occur in an arbitrary manner. Accumulation of such genetic aberration allows keratinocyte to progress through various stages from simple hyperplasia to dysplasia to carcinoma in situ to invasive carcinoma. Deletion of Chromosomal 9p21 region is a commonly detectable event in hyperplastic lesions. Deletion in chromosome 3p is also an early event seen in squamous cell carcinoma. Progression of hyperplasia to dysplasia, is found to be associated with amplification of 3-q23p and p15 mutation. The transformation from dysplasia to malignancy involves 11q13 amplification and gains of the chromosomal regions 7q11.2, 8q23-24 and deletions of 13q21, 14q23. Process of metastasis is usually associated with gain of 1q21, 17q, 19q, 20q and deletions of 5q33-34, 8p, 10p12, 10q, 18q, 4q, 11p14 and PTEN inactivation.[13]

There is a correlation between the cumulative exposure to carcinogens and host susceptibility factors, which drives the cancer pathogenesis by induction of various somatic genomic mutations. The cancer causing somatic genetic aberration can be divided into two broad categories namely mutations affecting Proto-oncogenes and those affecting tumor suppressor genes. A Proto-oncogene is a normal cellular gene that encodes a protein which is usually

involved in regulation of cell growth or proliferation. Mutations in proto-oncogene will convert them into cancer-promoting oncogene, and they are characterized by the ability to promote cell growth in the absence of normal growth-promoting signals. Their products, the oncoproteins resemble the normal products of proto-oncogenes except that they are often devoid of important internal regulatory elements, and their production in the transformed cells does not depend on growth factors or other external signals. In this way cell growth becomes autonomous, freed from checkpoints and dependence upon external signals. Proto-oncogenes are activated by various genetic events such as chromosomal gain or amplification that increase gene dosage, activating mutations that result in changes or increases in gene activity, or translocation/ rearrangement in chromosomes that produce new genes. Most common abnormality of proto-oncogenes seen in human tumors is the point mutation of RAS gene.

Tumor-suppressor genes are genes which encode proteins that in one way or another inhibit cell proliferation. When oncogenes drive the proliferation of cells, the products of tumor suppressor genes help in controlling the cell proliferation. The most important tumor suppressor gene is the RB and p53 gene.[13] The tumor suppressor genes limit the effects of cancer causing events to such an extent that they induce programmed cell death. The loss or mutation of these tumor suppressor genes will give a green signal for cancerogenesis. The tumor suppressor genes activating mutations such as missense or non-sense mutations, decreased protein production due to mutation or hypermethylation of gene promotor or increased activity of micro-RNAs, increase in protein turnover by means of ubiquitin-based proteasome degradation.

Important molecular signaling pathways affected in HNSCC

Apart from the alterations occurring at genomic level, HNSCC are characterized by multiple alterations in the biochemical signaling pathways, which control the oncogenic properties, such as balance between cell survival and apoptosis, angiogenesis, invasion and metastasis. The most common signaling pathways affected in HNSCC are as follows.

1. The p53 pathway

The p53 protein is a transcription factor that plays an essential role in pathogenesis of human cancers, including HNSCC.[13] When there is a cellular stress, it causes activation of p53 pathway, which will either result in arresting of cell cycle so as to allow repair or if the insult is too much it may cause apoptosis of cell. P53 plays an important role in cancerogenesis as it is seen mutated in more than 60 per cent of HNSCC. In 10-15% of HNSCC, there is an overexpression of human variant of mouse double minute proteins 2 and 4 (MDM2 and MDM4), which promotes proteasome-based degradation of p53 by ubiquitination.

2. The retinoblastoma pathway

The retinoblastoma (Rb) pathway plays an important role in the regulation of cell cycle progression from the G1 phase into the S phase, which is the commitment step in the cell cycle. Gross alteration in the Rb pathway has been proved to result in the development of cancer including HNSCC.[14]

Activation of proto-oncogene cyclin D1, results in cell cycle progression by promoting phosphorylation of pRB by cdk4. This is another important mechanism for the activation of Rb pathway seen in 30% of HNSCC.[14]

3. Epidermal growth factor receptor pathway

The ErbB/HER family of tyrosine kinase receptors, including epidermal growth factor receptor (EGFR/ErbB1), Her2Neu (ErbB 2), ErbB3 and ErbB4, are important activators of

mitogenic signalling.[15] These receptors are activated by various ligands, including tumor necrosis factor alpha (TNFα) and EGF. The activation of receptor recruits intracellular signaling complexes which will in turn activate mitogenic signaling pathways, such as the RAS/MEK/ERK cascade, STAT cascade, PI3K/AKT cascade, and several angiogenic, cell adhesion and cell cycle regulatory pathways. In 40-95% of HNSCC and premalignant mucosa lesions, there is an overexpression of EGFR and its ligands. EGFR overexpression in HNSCC is a result of several factors, including transcriptional induction and genetic amplification.

4. The PI3-kinase pathway

The PI3-kinase pathway is an important downstream effector of the EGFR and many other membrane-based receptors and plays a central role in cancer pathogenesis.[16] Phosphoinositol triphosphate (PIP3) activates PDK1, which results in phosphorylation of AkT (Protein kinase B). AkT is the active component of the pathway and promotes the cellular survival by affecting the function of many proteins by phosphorylation and promotes cell survival. The tumor gene phosphatase and tensin homologue gene (PTEN) is an important negative regulator of the PI3K-AKT pathway. It causes inhibition of this pathway by regulatng PIP3 dephosphorylation, which decreases the phosphorylated AKT fraction and causes arrest of cell cycle in G1 phase.[16]

The activation of components of the PI3k cascade is common in HNSCC, and occurs through several mechanisms including chromosomal amplification of the PIK3CA locus, activating mutations in PI3K, amplification of AkT, somatic mutation, homozygous deletion or methylation of the PTEN locus in HNSCC.

5. DNA repair pathways and genetic instability

There is plenty of evidence suggesting genomic instability is a cardinal feature of progression to HNSCC.[17, 18] Factors that promote genomic instability may include deficiencies in various mechanisms such as DNA repair, chromosome cohesion and condensation, mitotic progression, spindle assembly and regulation of chromosomal telomere length. An important method by which genome integrity is altered in HNSCC is through abnormalities in the p53 pathway. Inherited p53 mutation leads to many different cancers, as seen in patients with Li–Fraumeni syndrome.

6. Angiogenesis

Any tumors to grow to sizes beyond 5–10mm depends on the circulatory system for nutrients and for release of their metabolic waste products. Neoangiogenesis is a key step for progression of cancers. Tumors secrete various factors, such as vascular endothelial growth factor (VEGF), acidic and basic fibroblast growth factor (FGF1/2), interleukin 8 (IL-8), along with cell adhesion molecules such as integrins and cadherins to promote angiogenesis. There has been various histopathological studies showing increased microvessel density associated with progression of tumor.[19] It has been found that there is up regulation of VEGF family members and FGF proteins and down regulation of thrombospondin-1 in a high percentage of HNSCC and some of these factors have also been detected in serum of HNSCC patients.[19]

There are several inherited mutations which are associated with increased risk for HNSCC development. The heritable syndromes such as Li-Fraumeni syndrome, Fanconi anemia, Bloom's syndrome and Dyskeratosis congenita are found to cause an increased incidence of squamous cell carcinoma of mucosal membranes. The gene or the mutation affected in these syndromes are mentioned below.

Table 1: Mutation involved in various Heritable Syndromes[17]

Heritable Syndrome	Mutation Involved
Fanconi Syndrome	FANCA-A to FANCA-M mutation
Bloom Syndrome	BLM (DNA helicase) mutation
Xeroderma pigmentosum	XP-A to XP-G mutation
Ataxia telangiectasia	ATM mutation
Li–Fraumeni syndrome	p53 mutation

1.2 Treatment modalities

Surgery and radiotherapy are the only curative treatments for head and neck carcinomas. Chemotherapy has no curative role alone but is used as an adjuvant with radiotherapy, so it is routinely used as a part of combined modality of treatment.

Role of Radiotherapy in head and neck cancer

It was in January 1896, Emil Grubbe provided the first example of therapeutic use of radiation by treating an advanced ulcerated breast cancer with x-rays.

Biologic Basis of Radiation Therapy

Radiation can be administered to cells in two forms. It can be either given in the form of photons namely the x-rays and gamma rays or in the form of particles namely the protons, neutrons and electrons. When these photons or particles interact with cell components, they cause ionizations which can have direct or indirect effect.

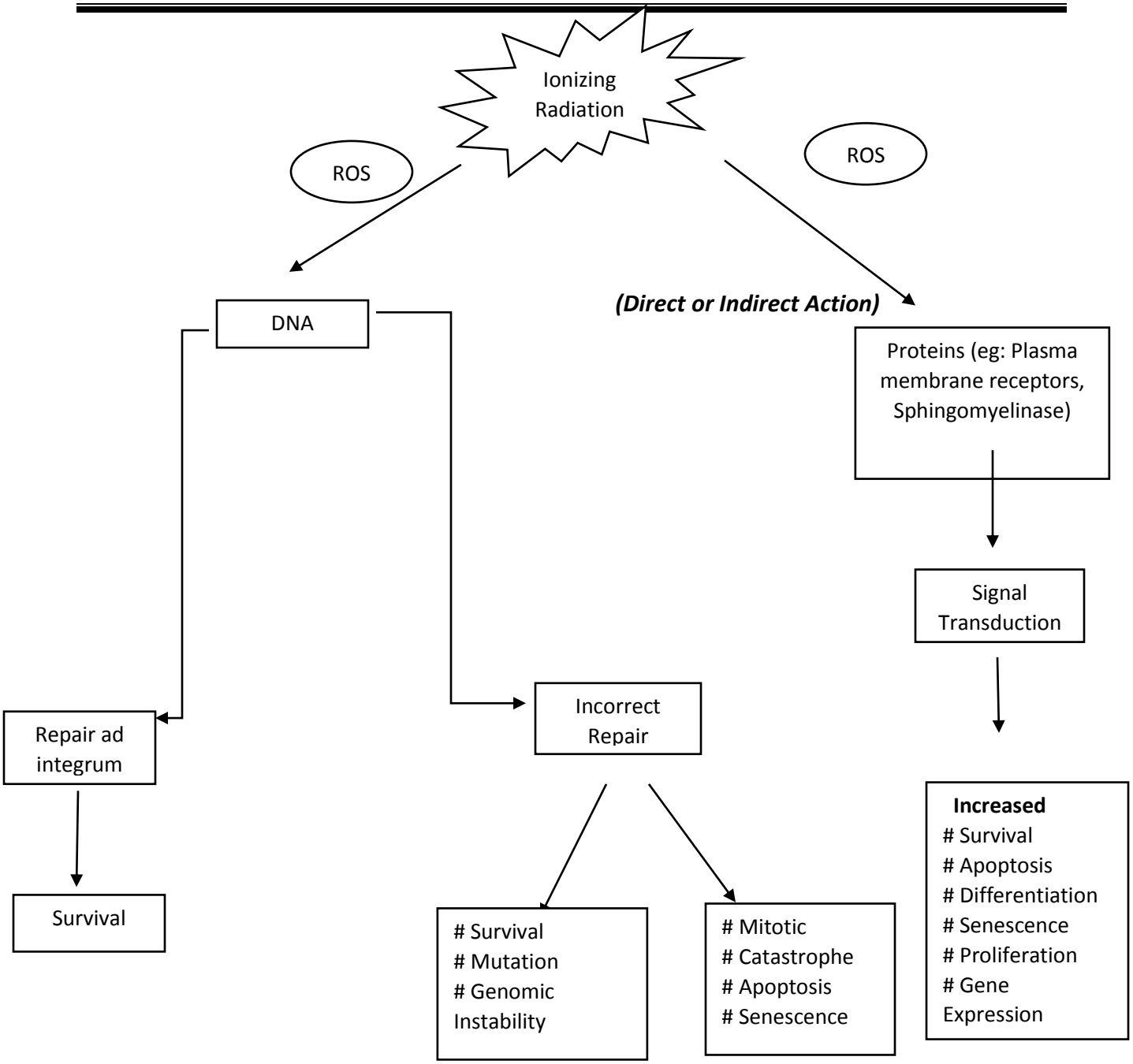

Fig 4 The effect of ionizing radiation on cells

Direct effect:

The direct effect of radiations results in damage of DNA in chromosomes with the help of charged nuclei such as carbon nuclei and neutrons. This is termed as High linear energy transfer. The interaction of photons with other molecules such as water causes production of free radicals like OH^-

which has the ability to diffuse into the nucleus of cell and causes damage to DNA in the chromosomes. This mechanism of DNA damage is called Low linear energy transfer and is the major mechanism of DNA damage induced by X-rays.

The most important cellular effect of radiation is Double-stranded breaks of nuclear DNA. This breakage results in irreversible loss of the reproductive capability of the cell and eventually result in cell death. The earliest response to radiation is activation of ataxia-telangiectasia mutation, which involves a conformational change thereby resulting in the activation of its kinase domain and phosphorylation of serine molecule.[18] This phosphorylation causes the ATM homodimer to split into active monomers that phosphorylate wide range of proteins. This results in downstream activation of p53, which causes degradation of Cdc25 phosphatases, Cdk-cyclin complex inactivation and arrest of cell cycle at G_1, intra-S or G_2 phase. The cells exposed to ionizing radiation can undergo three main transformations; they can either continue normal cell division or it can undergo a state of DNA damage-induced senescence (these cells are metabolically active while reproductively inactive), or they can undergo DNA damage-induced apoptosis or mitotic-linked cell death.

Indirect effect:

The physical interaction of ionizing radiation with the molecular infrastructure of the cell results in chemical reactions, which in turn results in production of variety of short-lived ions and chemically unstable free radicals. The most common radicals are produced from the radiolysis of cellular water and they include hydroxyl radicals like (OH^-), hydrated electrons, hydrogen atoms, and hydrogen peroxide. These free radicals are extremely unstable and they interact immediately with neighboring molecules to produce chemically stable lesions.

The cell cycle progresses in a specific order, this is ensured by various checkpoints genes. There are three main places in cell cycle where the checkpoints induced by DNA damage function:

(1) Junction between G1 phase and S phase

(2) Intra-S phase

(3) Between G2 phase and mitosis.

The cells which have sustained DNA damage will be stopped from progressing through cell cycle and they become arrested at the next check point in cell cycle. If irradiated cells have already passed the restriction point, a tumor suppressor gene called Retinoblastoma Tumor suppressor gene (Rb) causes transient arrest in S phase. The G1 or S and intra-S phase checkpoints inhibit replication of damaged DNA and works in a coordinated manner with the DNA repair mechanisms to allow the restitution of DNA integrity and thereby increases cell survival.

Factors Affecting Radiation Response

The fundamental principles of fractionated radiotherapy include repair, reassortment, repopulation and reoxygenation. Studies on split dose repair (SDR) done by Elkind et al found out that there is tumor growth delay or increased survival if a dose of radiation was split into two fractions compared to the same dose administered in one fraction.[19] Reassortment and repopulation are also dependent on the interval of time between radiation fractions. When cells are given short time intervals between doses, they can progress from a resistant portion of the cell cycle (e.g., S phase) to a sensitive portion of the cell cycle (e.g., G2 phase). This transit between resistant and sensitive phases of the cell cycle is called reassortment. If irradiated cells are given even longer intervals of time between doses, the survival of the population of irradiated cells will increase. This increase in split dose survival after longer peiods of time is due to cell division and is called repopulation.

Dose-rate effects

Lowering the dose rate, and thereby increasing exposure time, reduces the effectiveness of killing by x-rays because of increased Split dose repair (SDR) .Lowering the dose rate, and thereby resulting in increased exposure time, reduces the effectiveness of killing by x-rays due to increased SDR. In some cells, there is a threshold for lowering the dose rate and will paradoxically find an increase instead of decrease in cell killing.This increase in cell killing is due to the accumulation of cells in a radiosensitive portion of the cell cycle.

Cell cycle

The phase of the cell cycle at the time of radiation has an effect how the cell will respond to radiation. Cells which are in late-G1 to early-S and G2 or M phases are most sensitive while cells in G1 and mid- to late-S phases are more resistant to radiation. To utilise this difference in sensitivity we use fractionated radiotherapy and chemotherapeutic agents which will reassort cells into more more sensitive phases of the cell cycle in combination with radiation.

Tumor Oxygenation

The most important microenvironmental influence on tumor response to radiation is molecular oxygen. Hypoxia in tissue will result in decreased killing after radiation, which can be expressed as an oxygen enhancement ratio (OER). OER is defined as the ratio of doses which gives the same killing under hypoxic and normoxic conditions.

At high doses of radiation, the OER is approximately 3, whereas at low doses it is approximately 2. Oxygen must be present within 10 microsec of irradiation to achieve its radiosensitising effect, as under hypoxic conditions the damage to DNA can be repaired more easily than under oxygenic situations where the damage to DNA is fixed due to interaction of oxygen with free radicals generated by radiation.

Although normal tissue and tumors vary in their oxygen concentrations, only tumor cells possess low levels of oxygen enough to influence the effectiveness of radiation killing.

Drugs that affect radiation sensitivity

Drugs that affect radiation sensitivity includes mainly the chemotherapeutic agents ranging from antimetabolites (5-fluorouracil, gemcitabine) to platinum compounds (cisplatin and carboplatin) to taxanes (paclitaxel and docetaxel) to latest agents like molecular targeted agents (cetuximab). These agents are found to enhance the effect of radiation by their indolent cytotoxic effect or due to its cytostatic effect, can reduce the rate of proliferation and increase the effectiveness of radiation.

The two main types of radiation treatment are:

1) External Beam Radiation Therapy

2) Brachytherapy.

External Beam Radiation Therapy (EBRT)

External beam radiation therapy results in generation of energy particles at some distance from the patient. The various equipments used are Linear Accelerator, Telecoblat Unit, Telecaseim Unit etc. Dual-energy linear accelerators allow for the generation of either low-energy megavoltage x-rays (4-6 MeV), high-energy megavoltage x-rays (15-20 MeV) or electrons. Most patients are treated with x-rays or gamma rays because of the skin-sparing properties, penetration and beam uniformity. Due to the typical location of head and neck cancers (7 to 8 cm deep) and regional lymph nodes, 4 to 6 MeV x-rays or cobalt 60 gamma rays are typically used.

Brachytherapy

This is a technique in which radioactive sources are placed directly into the tumor and surrounding tissues (interstitial implants), within body cavities (intracavitary therapy), or onto epithelial surfaces (surface molds). The various radioactive materials used are Iridium-192, Caesium-137, Iodine-125 and Palladium-103. Brachytherapy has the advantage of delivering high dose of radiation in shorter time simultaneously sparing the surrounding normal structures. The tumors where brachytherapy is preferred are cancers of cervix cancer, endometrium, esophagus, head & neck and chest wall tumors.

The radiotherapy can be given in various regimens, they are:

1. Altered Fractionation
2. Hyperfractionation
3. Accelerated fractionation

Altered Fractionation

A conventional course of radiation for HNSCC generally delivers 70-72 Gy in 7- 7.5 weeks, with a once-daily dose of 1.8-2.0 Gy delivered over 36-40 fractions. In the late 1970s, to optimize treatment delivery various efforts were initiated to alter the conventional fractionation regimen and to test new schedules. This was termed altered fractionation. Altered fractionation regimens allow multiple fractions per day that are 21 smaller than the standard once-daily 1.8-2 Gy dose. There are two types of altered fractionation: hyperfractionation and accelerated fractionation.

Hyperfractionation

Hyperfractionation relates to radiotherapy delivered for each fraction rather than the total treatment time, and in this regimen small doses per fraction are delivered in most cases, 1.10-1.25 Gy/fraction for a total of 56 fractions, over a relatively standard period of time (usually 7 weeks).

Accelerated fractionation

Accelerated fractionation relates to the intensity of total dose delivered over time; the fraction size is usually larger (e.g. 1.6-1.8 Gy/fraction) and delivered more than once daily, and to a dose of 10 Gy per week but treatment is delivered over a reduced total period of time (usually 6 weeks or less) compared with hyperfractionation.

Both hyperfractionation and accelerated fractionation cause increased acute morbidity relative to conventional fractionation. Hyperfractionation aims to improve efficacy by increasing the total dose while maintaining the total treatment time and risk of late morbidity relative to standard fractionation. It exploits the difference in fractionation sensitivity between tumors and normal tissues, which lead to late morbidity, and can enhance tumor-cell killing without significantly increasing late toxicity. In contrast, accelerated fractionation relates to the intensity of radiation therapy delivered over time; a schedule that exceeds 10 Gy per week is classified as accelerated. Total dose must be equivalent to or slightly reduced relative to standard radiotherapy regimens in order to prevent increased late morbidity. The aim of accelerated regimens is to target tumor proliferation, which is a major cause of radiotherapy failure. Treatment acceleration helps overcome this problem because it counterbalances tumor-cell repopulation, especially in fast-growing tumors such as head and neck carcinomas.

Indications for Postoperative Radiation Therapy

Postoperative radiation therapy is indicated when the estimated risk of loco-regional recurrence of disease is > 20 percent. A study done at MSKCC (Memorial Sloan-Kettering Cancer Center) showed that postoperative radiation therapy for head and neck cancer in patients with nodal metastases at multiple levels decreased recurrences from 71 percent (surgery alone) down to 13 percent (surgery and postoperative radiation therapy).[20]

Indications for postoperative radiation therapy of the primary tumor bed include:

(1) Advanced T3 or T4 lesions

(2) Positive or close margins of resection

(3) Perineural/ vascular invasion

(4) High-grade histology

(5) Concern of the surgeon with respect to the adequacy of the procedure, irrespective of the status of the surgical margins on final pathology review.

Indications for Postoperative Radiation Therapy for Cervical Node Metastasis include:

(1) Extracapsular extension

(2) Lymph node size > 3 cm (N2a, N3)

(3) Multiple ipsilateral lymph node involvement (N2b)

(4) Bilateral or contralateral lymph node metastasis (N2c)

(5) Massive nodal metastases > 6 cm (N3)

(6) Surgical procedure (excisional or incisional biopsy) prior to definitive surgery

(7) Perineural/vascular invasion.

1.5 Role of Chemotherapeutic Agents in Head and Neck Squamous Cell Carcinoma

In order to increase the efficacy of radiation on tumor cells it is combined with chemotherapy. MACH-NC meta-analysis update published in 2011, showed the results from 87 randomised clinical trials with 16,192 patients revealed a clear benefit with the use of chemotherapy but with a hazard ratio (HR) between 0.87 and 0.88.[21] Steel and Peckham postulated the following four principal mechanisms of interaction between concomitant chemotherapy and radiotherapy.[22]

1. SPATIAL COOPERATION: Where the actions of both the modalities are focused on different anatomical sites (localized tumors for radiotherapy and distant micro metastasis for chemotherapeutic drugs).

2. INDEPENDENT TOXICITY: Whereby, the combination of radiation and drugs are selected such that toxicities to specific tissues do not overlap with, or add to radiation induced toxicities.

3. ENHANCEMENT OF TUMOR RESPONSE: Where the ability of chemotherapeutic drugs to enhance radio-response is exploited, resulting in a greater anti-tumor outcome than would be expected on the basis of additive actions.

4. PROTECTION OF NORMAL TISSUES: By delivering higher doses of radiation to tumor by technological improvements in radiation delivery or through administration of agents that selectively protect normal tissues from damage by radiation or drug.

1.51 Mechanism of Interaction between chemotherapeutic agent and Radiotherapy

Combined modality can effectively improve the tumor response to chemo-radiotherapy by a variety of mechanisms. [23,24]

a) Increasing Radiation Damage

The DNA molecule is the critical cellular target for radiation damage. Although radiation induces many different lesions in the DNA, double strand breaks and chromosome aberrations are the primary lethal lesions. Therefore any agent that makes DNA more susceptible to radiation damage may enhance cell killing. Halogenated pyrimidine compounds incorporate into DNA and make it more susceptible to radiation damage.

b) **Inhibition of Cellular repair**

The radiation induced damage to cells can be Sublethal damage or Potentially lethal damage. Both these can be repaired. Sublethal damage repair (SLDR) occurs when the radiation dose is fractionated and represents the shoulder part of the cell survival curve; while the potentially lethal damage repair (PLDR) denotes the increase in cell survival as a result of post radiation environmental conditions. The repair of SLDR is rapid, with a half time of approximately one hour, and is completed within 4 to 6 hours after irradiation. PLDR occurs when environmental conditions prevent cells from dividing for several hours. PLDR is considered to be responsible for major cause for resistance in some tumor types. Any drug interfering in the cellular repair mechanisms can potentially enhance cell or tissue response to radiation. Many chemotherapeutic agents like cisplatin and nucleoside analogues interact with cellular repair mechanisms and inhibit repair, and hence may enhance cell or tissue response to radiation.

c) Cell Cycle Redistribution

Cell sensitivity to radiation and to most of the chemotherapeutic drugs depends significantly on the phase of cells in the cell cycle. Hence both types of agents are effective against tumors with large growth fractions of clonogenic cells. Cells in G2 and M phase of the cell cycle are about three times more radiosensitive than cells in the S phase. These differences can be therapeutically exploited in chemo-radiotherapy through cell cycle redistribution strategies, either by using drugs that arrest cells in radiosensitive phases of the cell cycle or drugs that eliminate radioresistant S-phase cells. Taxanes and nucleoside analogues are the examples of the above described mechanisms respectively.

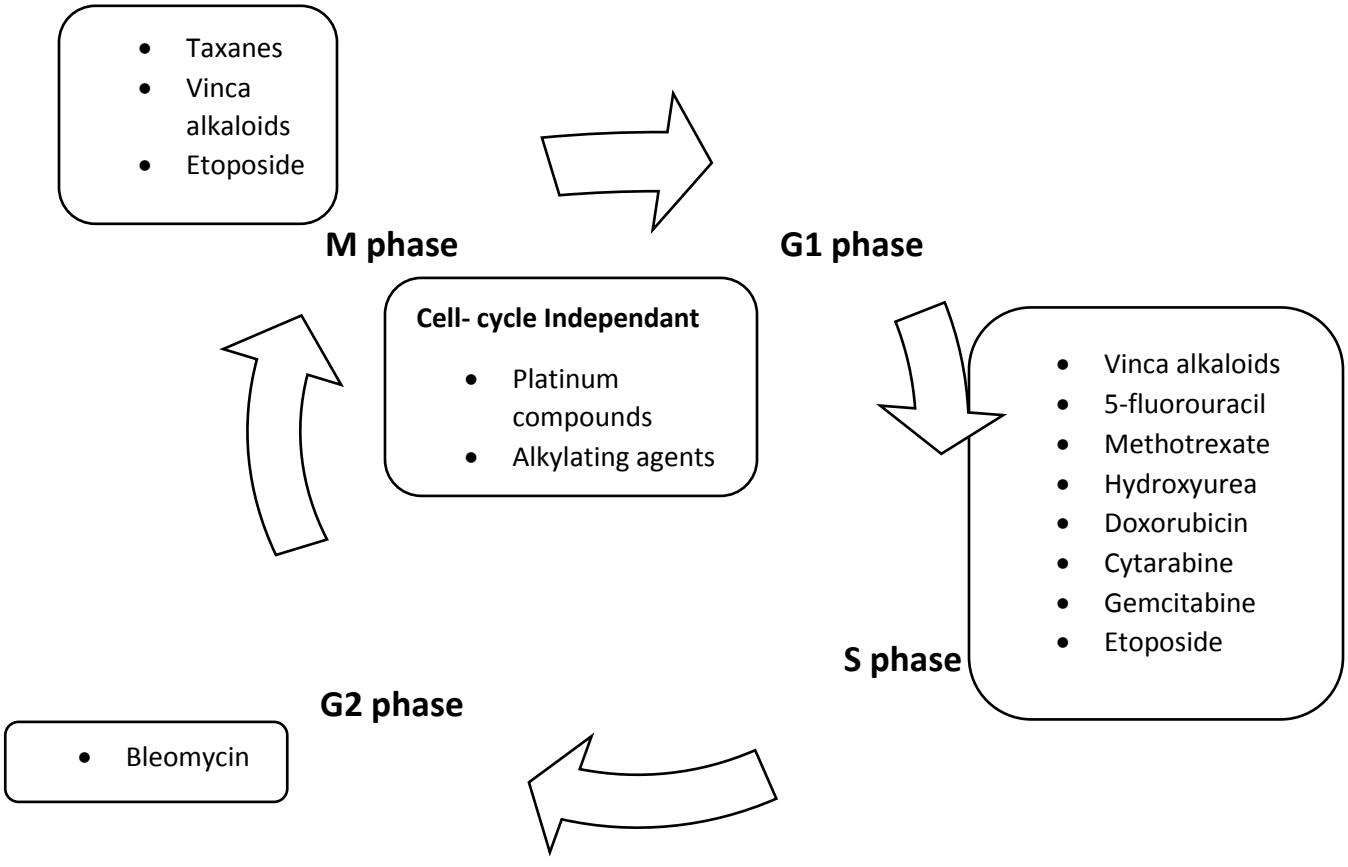

Fig 5 The phase of cell cycle where various chemotherapeutic agents acts

d) Counteracting Hypoxia-Associated Tumor Radioresistance

Solid malignant tumors have characteristic defective vascularization. Hypoxia occurs at distances from blood vessels larger than 100 to 150 micrometers. The hypoxic cell content in a tumor can be variable, more than 50% in some tumors. The presence of hypoxia makes tumors more resistant to radiation as well as to chemotherapeutic agents. Hypoxia is a major treatment limiting factor in radiation therapy. Combining chemotherapeutic agents with radiotherapy can reduce or eliminate hypoxia or its negative influence on radiation response on tumor. Most chemotherapeutic drugs preferentially kill proliferating cells, which are primarily found in well oxygenated regions of the tumor because these regions are located close to blood vessels; they are easily accessible to chemotherapeutic agents, thereby leads to an increased oxygen supply to hypoxic regions and hence re-oxygenates the hypoxic tumor cells. Massive loss of cells after chemotherapy lowers the interstitial pressure, which in turn allows the re-opening of previously closed capillaries and the re-establishment of blood supply. Finally by eliminating oxygenated cells, more oxygen becomes available to cells that survive chemotherapy.

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f) Inhibition of tumor cell repopulation

The rate of cell proliferation in tumors treated by radiation or chemotherapeutic drugs is higher than that in untreated tumors. This phenomenon is termed as accelerated repopulation. The mechanisms of accelerated repopulation is through recruitment of previously quiescent cells and shortening of the cell cycle time. In tumors accelerated repopulation seems to begin several days or weeks after the initiation of radiotherapy. While it has a beneficial sparing effect on acutely responding normal tissues during radiotherapy, it has the opposite adverse impact on tumor control. Chemotherapeutic drugs by their cytotoxic or cytostatic activity can reduce the rate of proliferation when given concurrently with radiotherapy and hence increase the effectiveness of treatment.

Extensive meta-analysis have demonstrated an additional survival benefit of 4-4.5% with the addition of chemotherapeutic agents.[25] The most commonly used chemotherapeutic agent used as radiosensitiser are Cisplatin, Carboplatin, 5FU, Paclitaxel, Gemcitabine and others.

Cisplatin

Barnett Rosenberg and co-worker in 1960 accidently discovered the antitumor activity of cisplatin. Cisplatin given before irradiation causes an increase in slope of radiation dose response curve. The exact mechanism of action is not known. Preliminary experiments show it ability to

inhibit DNA synthesis and to a lesser extent, RNA and Protein synthesis. It binds to DNA and forms inter- and intra-strand DNA adducts. Cisplatin inhibits sub lethal and potentially lethal damage repair. Anti-tumor activity is greater if administered by continuous infusion because it is phase and cycle nonspecific drug with preferential action on G1 phase of cell cycle. The incidence of nephrotoxicity is also decreased by continuous infusion. It has biphasic elimination with initial $t_{1/2}$ of 12- 43 min and terminal $t_{1/2}$ of 36-48 hours. When given as a single agent or in combination with other drugs, cisplatin is usually administered as a single IV dose of 50 to 75 mg/m^2 every 3 to 4 weeks. But outside clinical trials, most popular schedule of concurrent Cisplatin is not the three-weekly regimen, but a weekly schedule of Cisplatin in dose of 30-40mg/m^2.[24] It is usually given in 250 mL of normal saline, as a 1- to 4-hour infusion. Shorter infusion times are associated with greater toxicity.

EORTC 22931 and RTOG 9501 studies using 3 weekly cycles of 100mg/m^2 of Cisplatin with EBRT showed a significant advantage in Loco regional control (LRC) and Disease free survival (DFS) in comparison with exclusive adjuvant RT. The LRC found was 82% and 81% respectively, [24] but these studies also reported a high rate of significant adverse effects and 2% toxicity related deaths. Lower doses of cisplatin (50mg/m^2) on weekly basis with radiotherapy report an overall survival of 36% and a LRC of 70%. Although acute toxicity was less, late toxicity of around in 22% of patients. Even doses as low as 40mg/m^2 have shown 40% incidence of grade III mucositis and 17% hematological toxicities.[24]

Studies have proved that cisplatin along with 5FU has a synergistic effect and led to significant increase in the response rate in locally advanced HNSCC, but at the expense of greater toxicity. [26,27]

Common toxicities from cisplatin include renal insufficiency with cation wasting, nausea and vomiting, peripheral neuropathy, auditory impairment, and myelosuppression with thrombocytopenia prominent. Less common but serious side effects include hypersensitivity, visual impairment, seizures, and late leukemia as a secondary treatment--related condition. Renal damage can be minimized by ensuring vigorous hydration during therapy. Vigorous premedication for nausea and vomiting has to be routinely administered.

OTHER PLATINUM ANALOGUES

Carboplatin

Its mechanism of action is same as cisplatin. It is given as infusion, 20mg x min/ml can be safely administered in 200ml of dextrose 5% in water over two hours. It is administered as a rapid intravenous infusion. It is excreted from body by the kidneys predominantly and cumulative urinary excretion of platinum is 54% to 82%. Studies using 50mg/m^2 or 100mg/m^2 of carboplatin along with EBRT failed to show any advantage in terms of LRC, DFS and overall survival when compared with radiation alone. [28]

Its advantages are:

1) Easier to administer.

2) Extensive hydration not required because of lack of nephrotoxicity at standard doses.

3) Is reconstituted in chloride free solutions.

Its main toxicity is myelosupression, which is its dose limiting toxicity. The drug is most toxic to platelet precursors. Neutropenia and anemia are frequently observed. It also causes nausea and vomiting, which is frequent, lesser severe, shorter in duration and can be easily

controlled with standard anti emetics. Another side effects, is alopecia. Some other side effects which has been reported are neurotoxicity, nephrotoxicity and ototoxicity.

Oxaliplatin

Its mechanism of action is same as cisplatin. It is given as intravenous infusion as a single dose every two weeks (85 mg/m^2) or every three weeks (130mg/m^2) alone or with other active agents. It is mainly excreted from the body through kidneys predominantly with more than 50% of platinum being excreted in the urine at 48 hours. Its toxicities include neurotoxicity, sensory neuropathy is its dose limiting toxicity. Laryngopharyngeal spasm and oropharyngeal dysesthesia often precipitated by exposure to cold have also been reported. This neurotoxicity is reversible unlike cisplatin.

5 Flourouracil

It was synthesized in mid-1950s and to this date it is the most widely used anticancer agent, having its effect in wide range of solid tumors namely, the Head and neck cancer, gastroesophageal cancer, breast cancer, hepatocellular cancer, pancreatic cancer, colorectal cancer, anal cancer.[29]

5-FU enters cells via the facilitated uracil transport mechanism and is then anabolized to various forms of cytotoxic nucleotides by several biochemical pathways. It is considered to exert its cytotoxic effects through various mechanisms, but most important ones are by inhibiting Thymidylate synthase (TS), by incorporating itself into RNA and by incorporating into DNA. Along with this, the stress caused to the cells due to the inhibition of TS may activate the apoptosis pathways in susceptible cells. They act in S phase of cell cycle, so prolonged exposure of tumor cells to 5-FU would increase the fraction of cells being exposed to drug, so it is usually

given as infusion. The studies have shown that infusional 5 FU, when given along with radiotherapy showed complete response rate in 68% and the overall median survival of 33 months compared to 56% complete response rate and an overall survival of 25 months in placebo.[29]

The toxicity of 5-FU is dose- and schedule-dependent. The main side effects are diarrhea, mucositis, and myelosuppression. The hand-foot syndrome is more commonly seen with infusional 5-FU therapy. Acute neurologic symptoms have also been reported, such as somnolence, cerebellar ataxia, and upper motor signs. Treatment with 5-FU can, on rare circumstances, cause coronary vasospasm, resulting in a syndrome of chest pain, cardiac enzyme elevations, and electrocardiographic changes.

Paclitaxel

Paclitaxel belongs to Taxane group of antineoplastic agents. It is a natural product derived from the bark and needles of Pacific Yew Tree, *Taxus brevifolia.*

It is cell cycle specific – M phase specific cytotoxic agent. The M phase arrest causes the accumulation of cells in the G2/M-phase of the cell cycle. It is an antimicrotubule agent that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. It specifically binds to the N-terminal of the amino acid sequence of the beta tubulin subunit of the cellular tubulin polymers, thereby stabilizing the polymers by shifting the dynamic equilibrium that exists between tubulin dimers and microtubules in favour of the polymerised state. This prevents the separation of the chromosomes during metaphase and cells get arrested in mitosis. It is also found to promote apoptosis by inducing Bax expression and decreasing Bcl-2 expression which secondarily induces tumor reoxygenation further

enhancing sensitivity to radiation.[30] For concurrent chemoradiotherapy, the drug is usually given in the dose of 30mg/m^2 as intravenous infusion in 500 ml normal saline over one hour, on weekly basis till radiotherapy completes.

Most common toxicity associated with paclitaxel is neutropenia. The onset is usually on days 8 to 10, and recovery is generally complete by days 15 to 21 with an every-3-week dosing regimen. The most important pharmacologic determinant of the severity of neutropenia is the duration that plasma concentrations are maintained above biologically relevant levels (0.05 to 0.10 μmol). Paclitaxel induces a peripheral neuropathy that presents in a symmetric stocking glove distribution.[31] Severe neurotoxicity is uncommon when paclitaxel is given alone at doses below 200 mg/m^2 on a 3hr or 24 hour schedule every 3 weeks or below 100 mg/m^2 on a continuous weekly schedule. The most common cardiac rhythm disturbance, a transient sinus bradycardia, can be observed in up to 30% of patients. Routine cardiac monitoring during paclitaxel therapy is not necessary but is advisable for patients who may not be able to tolerate bradyarrhythmias. RTOG-97-03 randomised study on 241 patients having stage III and IV squamous cell carcinomas of head and neck studied the use of Paclitaxel along with Cisplatin in comparison to 5FU and hydroxyurea.[32] The Complete Response (CR) rate was 82% in paclitaxel arm. Most of the studies showed toxicities ranging from grade 3/4 mucositis, myelosuppression, and grade 3/4 leucopenia in more than 20% of treated patients.

Gemcitabine:

Gemcitabine is another potent antimetabolite radiosensitiser. It is difluorinated deoxycytidine analogue. Gemcitabine is inactive in its parent form and it requires intracellular activation to exert its cytotoxic effects. It is activated to the active triphosphate metabolite form by the enzyme deoxycytidine kinase (dCK). Gemcitabine triphosphate is then incorporated into

DNA which causes chain termination and inhibition of DNA synthesis and function. Another mechanism by which it acts is by direct inhibition of DNA polymerase α, β, and γ, which in turn interferes with DNA chain elongation, DNA synthesis, and DNA repair. The triphosphate metabolite is also a potent inhibitor of ribonucleotide reductase, which further results in the inhibition of DNA biosynthesis, by reducing the levels of key deoxynucleotide pools.

Studies have proved that a higher therapeutic ratio is achieved by using a twice-weekly regimen of gemcitabine, rather than once-weekly regimen. Weekly administration of gemcitabine along with radiation was found to have a LRC of 60% in patients with unresectable tumors and a median survival of 20 months.[33] Gemcitabine is a relatively well-tolerated drug when used as a single agent, however in some clinical trials, such as those in lung and head and neck cancers, the combination of gemcitabine with radiation has led to increased grade 3/4 mucositis and esophagitis.[34]

Side Effects of Chemoradiation in head and neck squamous cell cancers[35, 36]

The side effects of radiotherapy includes Xerostomia, osteoradionecrosis of mandible, swallowing dysfunction and speech problems. Radiation can also cause late hypothyroidism. Patient can have esophagitis as an acute complication of radiotherapy and esophageal stricture as a late complication.

The important side effect pertaining to our study is mucositis, which is the dose-limiting toxicity of chemoradiation.

Mucositis and its sequelea

The inflammation of the mucous membrane of oral and oropharyngeal region is termed as oral mucositis. It is a common complication of cancer therapy in which patients are receiving head and neck radiotherapy.

Oral Mucositis can lead to pain, discomfort and inability to tolerate food or fluids. It will also result in increase in opportunistic infections in the mouth and worsens the patient‟s quality of life. Poorly managed Oral Mucositis is one of the leading causes for interruptions of treatment and thereby increases the overall treatment time. This prolongation of overall treatment time adversely affects the loco-regional tumor control and also increases the overall cost of the treatment.

In a study conducted by Trotti et al, studied more than 6,000 patients with Head and neck Squamous Cell Carcinoma (HNSCC) who received radiotherapy (RT) with or without chemotherapy (CT). The overall incidence of mucositis in this patient population was 80 to 100%, with 25-45 % of cases being grade 3 or grade 4.[37]

Risk Factors for Oral Mucositis:

The development of Oral Mucositis is predominantly influenced by the type of malignancy and also the cytotoxic therapy administered for its treatment. The patient factors also play an important role in oral mucositis. Younger patients are more susceptible for Oral Mucositis as there is a rapid epithelial mitotic rate and there is a presence of increased epidermal growth factor receptors in the epithelium at the early age. On the other hand, the physiologic decline in renal function with age may result in higher incidence of Oral Mucositis in elderly patients.

A systematic review of the literature identified a vast number of patient and treatment related risk factors.[38]

Table 2: Patient and Treatment related risk factors for developing mucositis[38]

Patient-related Risk Factors	Treatment-related risk Factors
Gender	Radiation therapy: dose, schedule
Age older than 65 years or younger than 20 years	Chemotherapy: agent; dose, schedule
Inadequate oral health and hygiene practices	Myelosuppression
Periodontal diseases	Neutropenia
Microbial flora	Immunosuppression
Chronic low-grade mouth infections	Reduced secretory immunoglobulin A
Salivary gland secretory dysfunction	Inadequate oral care during treatment
Inadequate nutritional status	Infections of bacterial, viral, fungal origin
Herpes simplex virus infection	Impairment of renal and/or hepatic function
Inborn inability to metabolize chemotherapeutic agents effectively	Use of antidepressants, opiates, Anti Hypertensives, antihistamines, diuretics, and sedatives.
Exposure to oral stressors including alcohol and smoking	Protein or calorie malnutrition, and Dehydration
Ill-fitting dental prostheses	Xerostomia

Pathogenesis of Oral Mucositis:

The research in molecular level and cell biology have suggested oral mucositis to be a multistep process. The normal, healthy oropharyngeal mucosa has a very rapid turnover rate of 7-14 days and it acts as a barrier to secondary infections. After administration of Chemotherapy or Radiotherapy or combined modality, it was found that there is an acute inflammatory / vascular changes in the oral mucosa which will lead to the development of oral mucositis. A Five Phase Model has been described in the development and resolution of Oral Mucositis.[39]

Table 3: Phases of Oral Mucositis[39]

Phase 1	Initiation	Exposure of Cell to chemo- and radiotherapy will cause DNA damage and generates reactive oxygen species (ROS), which will injure cells, tissues and blood vessels
Phase 2	Signaling	ROS cause further DNA damage and stimulate expression of transcription factors that lead to tissue injury and apoptosis
Phase 3	Amplification	Release of pro-inflammatory cytokines result in further tissue damage, which amplifies the signaling cascade
Phase 4	Ulceration	Painful ulcers form that provide an entry point for bacteria, viruses and fungi. Bacterial cell wall components can further induce inflammation
Phase 5	Healing	A signal from submucosal tissue allows renewed cellular proliferation and differentiation restoring the lining of the oral cavity.

1. **Initiation:**

Here the reactive oxygen species (ROS) generated by exposure to chemotherapy or radiation therapy can cause DNA strand breaks and damage to cells, tissues, and blood vessels, which can ultimately cause apoptosis.

2. **Activation of transcription factors**

Damage to the mucosa triggers activation of transcription factors such as nuclear factor kappa B (NF-kB), which in turn causes increased production of pro-inflammatory cytokines like interleukin (IL)-1β and IL-6. These increased levels of cytokines triggers the initiation of various pathways which damage epithelial cells and surrounding fibroblasts leading to tissue injury and apoptosis.

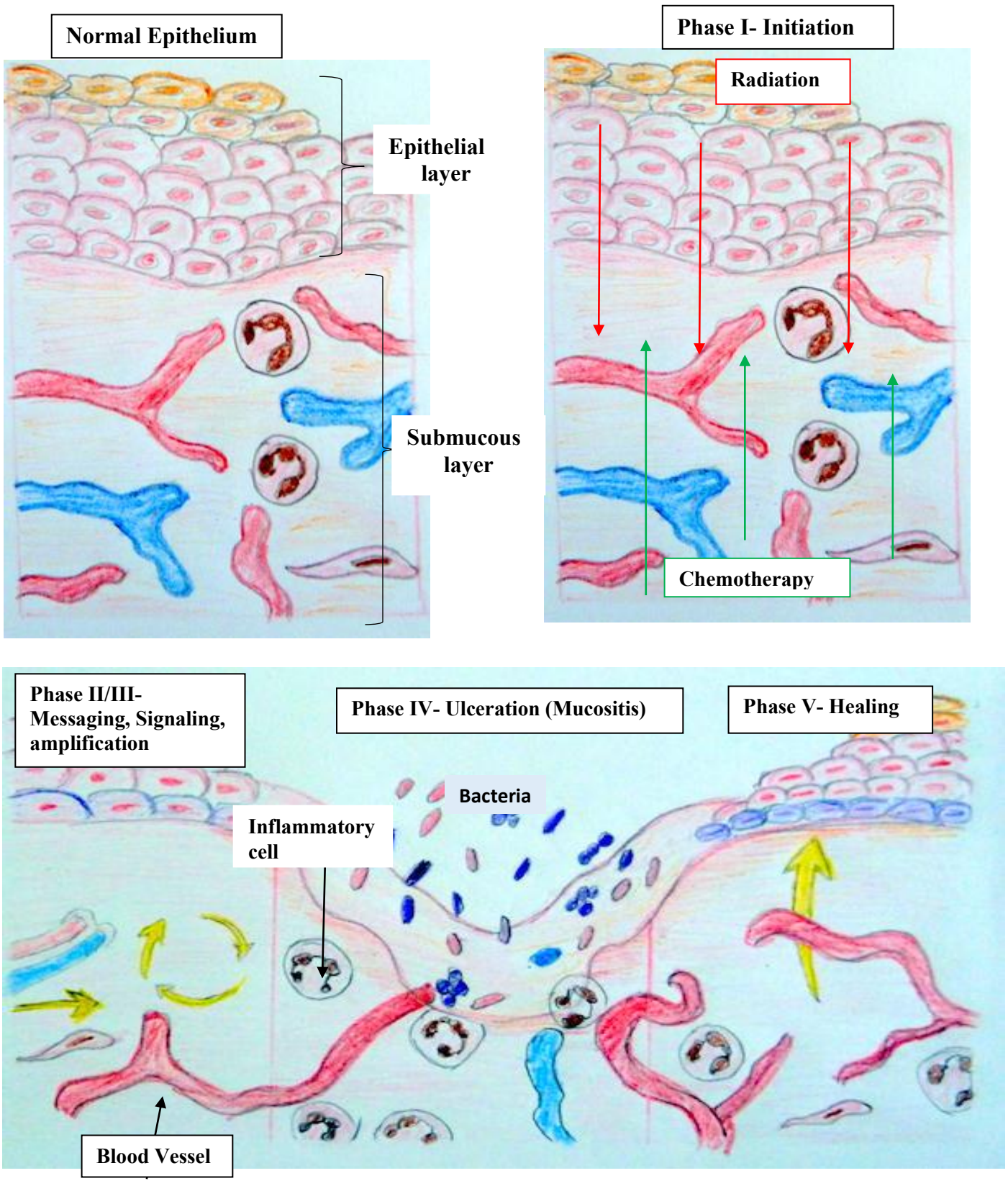


Fig 6 Phases of Oral mucositis

3. Signaling and amplification

Pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) activates ceramide and caspase pathways and these signals further increase production of TNF- α, IL1β and IL-6 and thus causing an amplification effect.

4. Ulceration and inflammation

Inflammatory infiltrate composed of polymorphonuclear and round inflammatory cells are found in the mucosa. As there is a breach in the mucosal barrier, penetration of the epithelium into the submucosa can occur and mucosa gets prone for bacterial infection which further lead to increase in the production of TNF- α, IL1β and IL-6. This further enhances the mucosal injury thus causing more severe mucositis in the form of ulceration, allowing colonization by oral bacteria and increasing the risk of sepsis. It is likely that each of these stages of mucositis pathogenesis occurs in a continuous, overlapping manner.

5. Healing:

Healing of oral lesions starts with a signal from the extracellular matrix in the Non-Myelosuppressed patient within 2 to 3 weeks following cancer treatment. Mechanisms of healing include renewal of epithelial proliferation and differentiation in parallel with white blood cell recovery, and re-establishment of normal local microbial flora.

Chemotherapy-induced stomatitis

It has been found out that approximately 40% of CT patients develop oral mucositis, and approximately half of these patients develop painful lesions requiring parenteral analgesia or total parenteral nutrition that may lead to treatment modification.[40] It has been seen that, there is a four times greater relative risk of septicemia in patients with oral mucositis and oral infections as compared with patients without oral mucositis. This has been attributed to mucosal barrier

injury, which allows pathogen to enter the peripheral circulation. Edema of the rete-pegs and vascular changes are also seen. The characteristic oral sequel of chemotherapeutic agents include epithelial hyperplasia, collagen and glandular degeneration, and epithelial dysplasia, atrophy, and localized or diffuse mucosal ulceration. It is usually the nonkeratinized mucosal areas which are most affected like the labial, buccal, and soft palate mucosa.

Radiation- induced stomatitis

Oral mucositis is seen universally in patient‟s receiving radiotherapy for oropharyngeal region and their severity depends on the type of radiation, volume of irradiated tissue, daily and cumulative dose and the duration of treatment. Oral mucositis is considered as a dose- and rate-limiting toxicity of RT for head and neck cancer.

Chemoradiation-induced oral mucositis

Cancer therapy affects rapidly dividing cells, and the epithelium in oral cavity has a turnover rate of 7 to 14 days, thus they are at risk for getting targeted by chemotherapy. After administration of chemotherapy initiation occurs as a result of DNA damage and the generation of reactive oxygen species. The relatively acute inflammatory or vascular phase occurs shortly after CT or RT administration, it involves up-regulation of transcription factors, including NF-kB, activation of cytokines and stress response genes. Signaling and amplification stage involves the production of pro-inflammatory cytokines released from epithelial tissue, including TNF-α, which is related to tissue damage, and IL- 1, which causes initiation of inflammatory response and increases the submucosal vascularity which in turn leads to increased local chemotherapeutic agents level. The ulcerative or bacterial phase usually begins after 1 week post-CT administration, which coincides with maximum neutropenia. In this phase Patients often

experience acute oropharyngeal pain, leading to dysphagia, decreased oral intake, and difficulty speaking. Mechanisms of healing include, renewal of epithelial proliferation and differentiation in parallel with white blood cell recovery and re-establishment of normal local microbial flora.

Prevention and treatment of muocositis

A standardized approach for the prevention and treatment of CT- and RT-induced oral mucositis is essential. The prophylactic measures usually used for the prevention of oral mucositis include chlorhexidine gluconate, ice-cold water, saline rinses, sodium bicarbonate rinses, acyclovir, and amphotericin B. Oral and parenteral opiates are used to relieve oral mucositis-related pain.

Oral or dental stabilization prior to CT and RT is very important to avoid serious sequel. Patients planned for Chemotherapy or head and neck RT should receive dental screening at least 2 weeks before therapy starts to allow for proper healing of extraction sites, recovery of soft tissue manipulations, and restoration of teeth. This will provide and promote optimal mucosal health before, during, and following cancer treatment.

Methods to prevent mucositis includes:

- Prior dental prophylaxis before treatment
- Brushing in a non-traumatic fashion, 2-3 times in a day with a soft-bristle toothbrush.
- Replacing the toothbrush on a regular basis.
- Using oral rinses with regular frequency.
- Avoiding irritation like hot, spicy, and coarse foods, fruits and beverages with a high acid content, and alcohol (including alcohol-containing elixirs).
- Abstaining from smoking

Management of Mucositis:

Oral rinses

To maintain oral moistness, patients should do frequent rinsing with bland solutions such as 0.9% normal saline and/or sodium bicarbonate solutions. The patient should rinse several times as often as necessary to maintain oral comfort. Sodium bicarbonate (baking soda) ½ tablespoons can be added, if viscous saliva is present. Saline solution can increase oral lubrication by acting directly as well as by stimulating salivary glands to increase salivary flow. The use of bland rinses has long been considered basis of sound oral hygiene in patients receiving cancer treatment, but there is no evidence to prove that they play a role in preventing or treating oral mucositis.[40]

Vitamins and other antioxidants

Various vitamins and anti-oxidants has been tested for efficacy with oral mucositis like Vitamin E, Vitamin C and glutathione. A study conducted by Osaki et al [41], in which 63 patients with head and neck cancer treated with chemoradiation were given vitamin C,E and glutathione in one arm and azelastine in other arm. It was found that patients in Azelastine arm, had less severe mucositis. Another antioxidant which had radio protective effect was Polaprezinc (Zinc-L-carnosine) which showed marked decrease in the incidence of mucositis, pain, xerostomia, taste disturbance and analgesic effects [42]

Amifostine

Amifostine is a thiol compound and belongs to a well-known class of free radical scavengers. It is a FDA approved drug for protection of chemotherapy as well radiotherapy induced toxicities. Amifostine at molecular level affects the redox sensitive transcription factors,

gene expression, chromatin stability, and enzymatic activity and thereby protects DNA from damaging effects of ionising radiation and chemotherapy drugs. In a study conducted on 177 patients who underwent radiotherapy for diversity of tumors were given intravenous infusion of amifostine before radiotherapy.[43] It was found that amifostine significantly reduced the severity of oral mucositis and a significant reduction in mean toxicity score was found. In a Meta-analysis that included patients who received amifostine before RT, there was a significant reduction in oral mucositis at doses above 300mg/m^2.[44] The limiting factor of amifostine which prevents its wide use is that it is expensive.

Glutamine

Glutamine is a neutral amino acid which acts as a substrate for nucleotide synthesis in most dividing cells and has immunomodulating and mucosal protective action. In one randomized trial of 17 patients who were undergoing radiotherapy for head and neck cancer, were randomized to oral glutamine (2 g swished for 3 min, four times daily during radiation) had a significantly shorter duration of objective mucositis, less severe maximum grade of mucositis, and less subjective grade three compared to the placebo group. [45]

Anti-inflammatory agents

Prostaglandins are a family of naturally occurring eicosanoids, some of which have shown cytoprotective activity. In that study conducted, topical dinoprostone was given four times a day in a non-blinded study to ten patient with oral carcinoma who were receiving 5-FU and mitomycin.[46] Benzydamine is a nonsteroidal anti-inflammatory drug with reported analgesic, anesthetic, and antimicrobial properties without activity on arachidonic acid metabolism. In a placebo-controlled clinical trial, done in 69 patients who were undergoing conventional RT, it

was found that benzydamine significantly delayed the use of systemic analgesics compared with placebo and reduced the erythema, ulceration by approximately 30% compared to placebo. [47] Current evidence does not support the use of systemic steroids to reduce the frequency or severity of oral mucositis. [48]

Epidermal Growth Factors

In vitro studies have demonstrated that epidermal growth factor is present in saliva and has the ability to affect growth, cell and migration, and repair mechanisms. Studies on epidermal growth factor (EGF) as a potential treatment option for chemotherapy and radiotherapy induced oral mucositis have reported conflicting data. EGF may function as a marker of mucosal damage and could potentially facilitate the healing process.[49] EGF mouthwashes and EGF oral sprays have been used in treatment of mucositis. In the study conducted by Girdler et al, there was not statistical significant reduction in resolution of established ulcers but a delay in onset and reduction in severity of recurrent ulcerations was found out.[50] In the study conducted by Wu et al, 50 mcg/ml dose of oral EGF was effective in treating oral mucositis, but further randomized controlled trials are needed to confirm these results.[51]

Hematopoietic Growth Factors

The results from a Radiation Therapy Oncology Group sponsored a double-blind, placebo controlled, randomized study (n= 121) to analyze the efficacy and safety of GM-CSF in reducing severity and duration of oral mucositis and related pain in head and neck cancer patients receiving RT.[52] It was found out that GM-CSF had no significant effect on the severity or duration of oral mucositis. The use of CSFs in the treatment of oral mucositis remains investigational.

Keratinocyte Growth Factors

A new drug palifermin, which is a recombinant human keratinocyte growth factor and member of FGF family, has shown efficacy in the reduction of oral mucosal injury related to cytotoxic therapy. In a study conducted by Spielberger et al.[53], it was noted that the patients receiving palifermin experienced significant reductions in grade 4 oral mucositis, soreness of the mouth and throat, decreased use of opiate analgesics, and the need of total parenteral nutrition. In a study conducted on locally advanced HNSCC treated with CTRT, palifermin decreased the incidence of oral mucositis, dysphagia, xerostomia in patients treated with hyperfractionated RT not among conventional RT.[54]

Antimicrobials

Various antimicrobial approaches have been tried including systemic antibiotics, antivirals (Acyclovir, Valacyclovir, Ganciclovir) and antifungal agent fluconazole.

Oral candidiasis is a common acute and chronic oral sequel of head and neck RT, with lesions presenting as removable (whitish) chronic or hyperplastic (non-removable) and chronic erythematous (diffused as patchy erythema), which frequently appear as angular cheilitis (first signs or symptoms). Treatment options for oral candidiasis include Mycostatin, nystatin (liquid or ointment), or clotrimazole. Pseudomembranous candidiasis is treated using topical antifungals. Chronic candidiasis usually requires much longer treatment, and it may be necessary to use oral ketoconazole, fluconazole, or intravenous amphotericin B.

For HSV and cytomegalovirus seropositive Head and neck squamous cell tumor patients, Acyclovir prophylaxis is the currently accepted modality of treatment. The reports are inconclusive regarding the use of chlorhexidine mouthwashes for relieving oral mucositis and

reducing oral complication by bacterial, candida species in patients receiving Chemoradiation.[55] In a study conducted by Sutherland and Browman for assessing prophylaxis of RT-induced oral mucositis in head and neck cancer patients, it was found that interventions chosen based on the biological etiology of oral mucositis were effective. [56] A study conducted by Spijkervet et al. evaluated the efficacy of lozenges containing polymyxin E2 2mg, tobramycin 1.8mg, amphotericin B 10 mg (PTA) taken four times daily for the oropharyngeal flora related to oral mucositis.[57] It was compared 15 patients receiving RT using PTA and two other groups of 15 patients each, one of which was using 0.1 % chlorhexidine and the other was using placebo. Results showed that the selectively decontaminated group had significant reduction in severity and oral mucositis when compared with the chlorhexidine and placebo groups.

Cryotherapy

Cryotherapy is administered as ice chips and frozen flavored ice products, and has been used to prevent oral mucositis. The Efficacy of cryotherapy for reduction of 5-FU-induced oral mucositis severity was demonstrated through a North Central Cancer Treatment Group (NCCTG) and Mayo Clinic sponsored controlled randomized trial.[58] A randomised controlled trial conducted by Svanberg et al,[59] demonstrated that this technique may reduce the development of oral mucositis and oral pain, and thereby reduces the number of days and total dose of intravenous opiates in patients treated with autologous bone marrow transplant.

Laser

Several studies have confirmed the effectiveness of low energy laser for preventing and treatment of CT-or RT induced oral mucositis.[60,61] A recent phase 3 double-blind, placebo-

controlled randomized study compared two different low level GaAlAs diode lasers (650 nm and 780 nm) to prevent oral mucositis in Hematopoietic stem cell transplantation patients treated with either CT or chemoradiotherapy.[61] It was found out that Low-level laser therapy showed to be more effective for decreasing oral mucositis and related oral pain, and it was safe without side effects. Another study using low energy Helium/Neon laser was done and it demonstrated a reduction in the severity and duration of oral mucositis. [62]

Sucralfate

Sucralfate is an aluminum salt of a sulfated disaccharide which has shown efficacy in the treatment of Gastrointestinal ulcerations and has been tested as a mouthwash for the prevention and treatment of oral mucositis. It acts by creating a protective barrier at the ulcer site via the formation of an ionic bond to proteins. Study results with sucralfate are conflicting. A double-blind, placebo controlled study with Sucralfate in 33 patients who received RT to the head and neck demonstrated no statistically significant differences in oral mucositis.[63] However, the sucralfate group did experience less oral pain and required a later start of topical and systemic analgesics throughout RT.

Opiates

Severe oral mucositis-related oropharyngeal pain may interfere with hydration and nutritional intake and may affect quality of life. For the management of this oropharyngeal pain, patient may require the use of opiates. This can be administered in various forms namely oral, transmucosal, transdermal, and parenteral and also as patient controlled analgesia pumps. Transdermal fentanyl has been shown to be an effective, convenient, and well-tolerated treatment in patients with oral mucositis pain in the RT and the Hematopoietic stem cell transplantation

setting.[64] Topical morphine for mucositis-related pain was evaluated in a sample of 26 patients following chemoradiation for head and neck cancer, it was found that patients in the morphine group demonstrated shorter duration and lower intensity of oral pain than the magic mouthwash group (equal parts of lidocaine, diphenhydramine, and magnesium, aluminum hydroxide).[65] Other agents that are currently under investigation or have shown some potential in the management of oral mucositis-related oral pain are sublingual methadone, transdermal buprenorphine, and ketamine mouthwash.

1.5 Curcumin

Curcumin (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione), the yellow pigment, is the major phenolic antioxidant in Indian saffron (Curcuma longa; also called turmeric, haldi, or haridara). It has been widely used in Indian food and also as a medicine for the treatment of inflammatory diseases. Curcumin has been found to inhibit growth of cancer cells both in in vitro and in vivo studies.[66,67] Curcumin was recently shown to induce apoptosis in several human cancer cell lines.[68]

Photo 1: Turmeric (Curcuma longa)

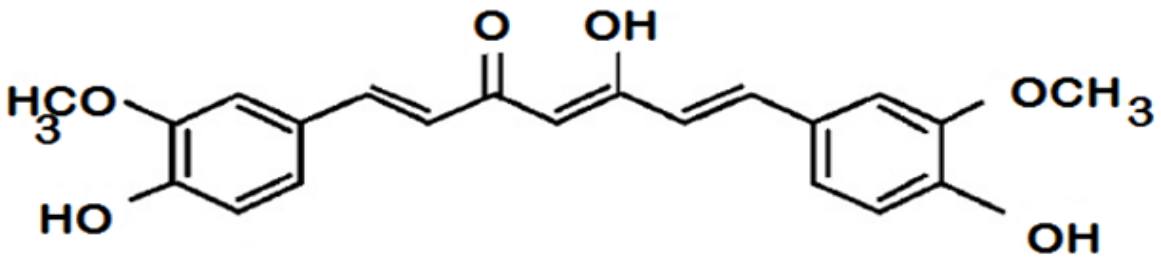


Fig 7 Curcumin (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione)

Pharmacology of curcumin[69]

Curcumin comes under the group of nutraceutics and has been approved by FSSAI (Food Safety and Standard Authority of India).

Curcumin is usually administered orally in form of capsules. It is lipophilic and relatively insoluble in aqueous solution. When administered orally, it gets rapidly metabolized into glucuronide and sulfate conjugates like curcumin glucuronide, curcumin sulfate, hexahydrocurcumin, tetrahydrocurcumin, and dihydrocurcumin, which are excreted primarily in bile and to a lesser extent in urine. Low or undetectable blood levels of unchanged curcumin were observed after oral administration. This poor oral bioavailability resulted in the research of development of curcumin derivatives, curcumin analogs and curcumin-drug vehicle combinations, so as to enhance the oral bioavailability. One such formulation is Biocurcumax curcumin. A study done to compare the bioavailability between curcumin and biocurcumax showed that curcumin when administered orally, the mean t1/2 of curcumin is 2.63 hours, time of peak plasma concentration was 2 hours and the peak plasma concentration was 149.8 ng/g. While the administration of Biocurcumax curcumin showed an increased bioavailability with mean t1/2 of 4.96 hours, the time of peak plasma concentration was 3.44 hours and the peak plasma concentration was 456.88 ng/g. [69]

Studies have demonstrated that curcumin even at high doses (upto 8g/day) was non-toxic to patients and can be given for prolonged periods of time with minimal side effects.[70] It should be used with caution in patients with gastrointestinal disease (peptic ulcer disease, ulcerative colitis, Crohn's). It should be avoided in patients with history of allergy/hyper sensitivity to turmeric, any of its constituents or other members of the Zingiberaceae (ginger) family.

Anticarcinogenic mechanism of Curcumin

Anticarcinogenic effects of curcumin and its underlying mechanisms have been investigated in various animal tumor cell lines, including skin, colon, lung, duodenal, stomach, esophageal, and oral cancers. The various proposed mechanisms by which curcumin acts as an anti-carcinogen are:[71]

1. Causing cell cycle arrest
2. Induction of apoptosis
3. Increased autophagy
4. Inhibition of angiogenesis and metastasis

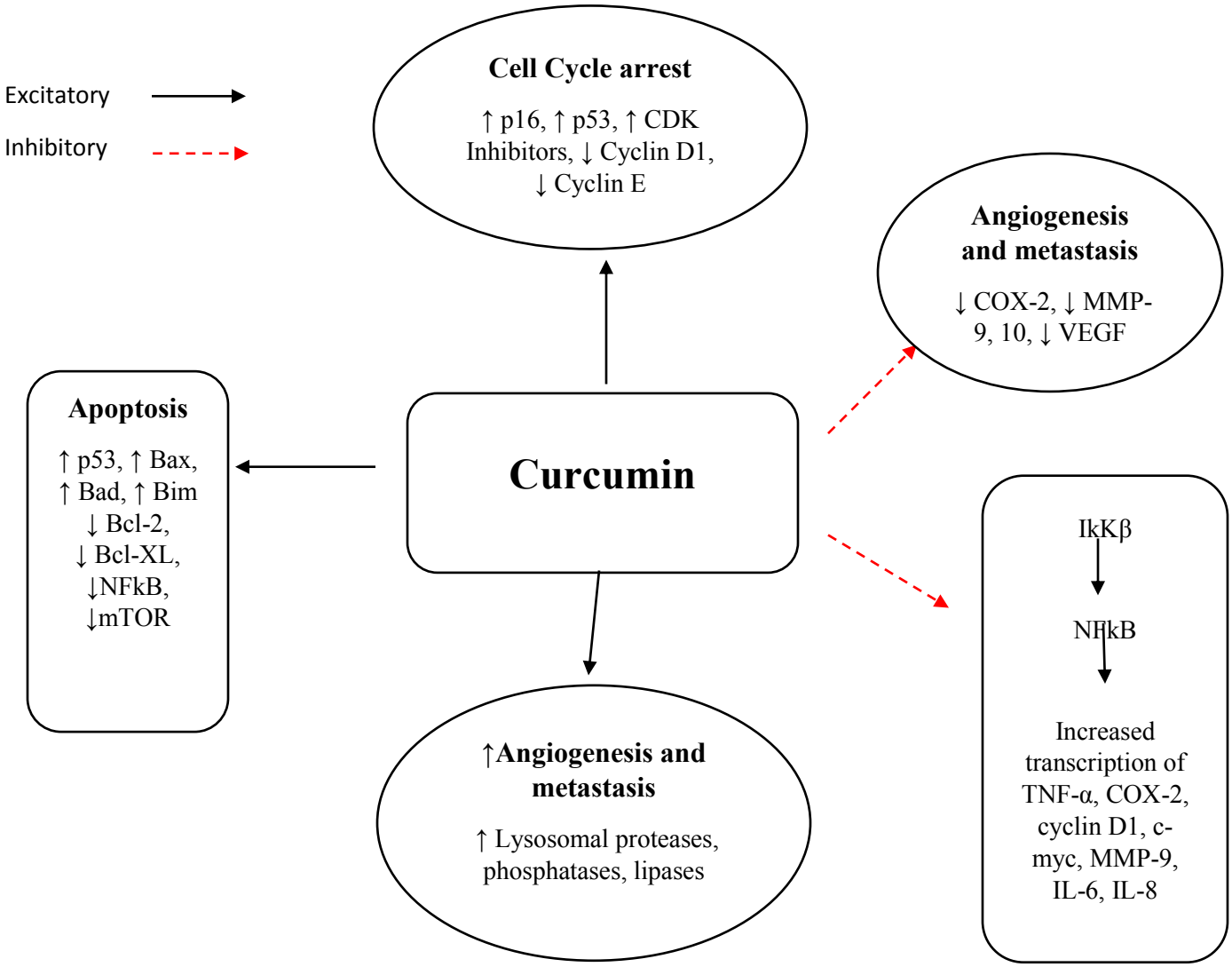


Fig 8 Various mechanisms through which curcumin exerts its anti- cancerogenic action

1. Cell cycle arrest

Cellular growth and proliferation is a highly regulated event in normal cells, and derangements of the cell cycle will result in uncontrolled proliferation and malignant transformation of cells. The cell cycle is controlled by the coordinated interaction of cyclins with their respective cyclin-dependant kinases (CDKs). There are 2 distinct family of CDK inhibitors: the INK-4 family (p15, p16, p18, p19) and the Cip/Kip family (p21, p27, p57). At the G1/S

transition, the Cyclin D/CDK4, 6 complexes promote progression of cell division by phosphorylating the pRB protein, thereby releasing the transcription factor E2F and it results in transcription of genes required for cell division. Curcumin has been shown to upregulate the expression of the Cip/Kip family of CDK inhibitors, thereby inhibiting the association of cyclin D1 with CDK4, 6 and causes arrest of cell cycle.[70]

Curcumin also causes decreased phosporylation of Rb gene and suppresses transcription of E2F-regulated genes, thereby arresting cell division. Cyclin D1 over expression has been associated with many types of cancers including various solid tumors and hematologic malignancies. Curcumin has been shown to suppress the expression of cyclin D1 by inhibiting the NFkB activation and subsequent suppression of resulting gene products.[70]

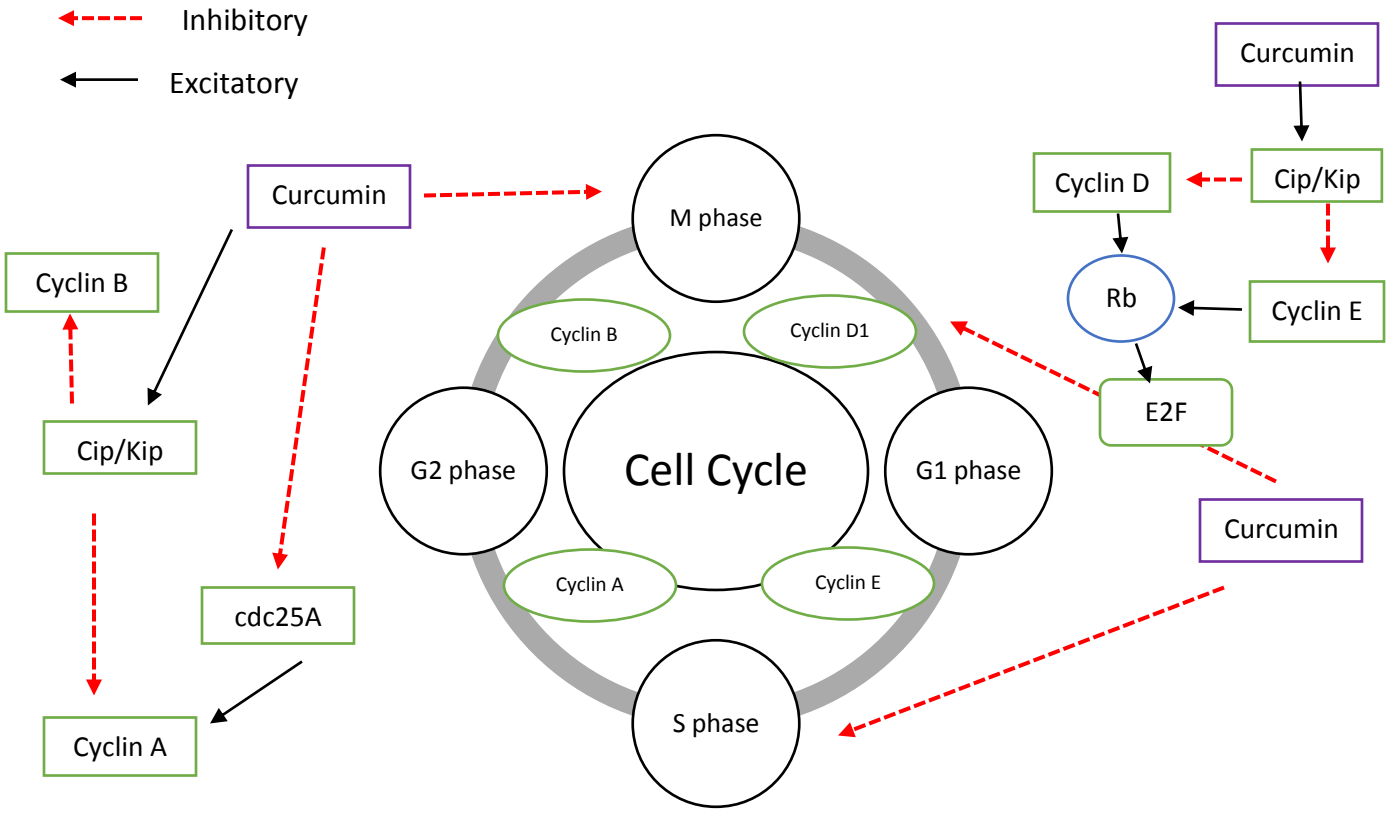


Fig 9 Various mechanisms by which curcumin causes cell cycle arrest

2. **Induction of cancer cell apoptosis**

The anticancer potential of curcumin is attributed to the ability of curcumin to induce apoptosis selectively in cancerous and transformed cells. Curcumin has been reported to induce apoptosis in various cell lines including HL-60, K562, MCF-7, and HeLa.[70] One of the major signaling pathways involved in apoptotic cell death includes the intracellular caspase cascade.[70]

There are two main apoptotic pathways: the intrinsic (mitochondrial) pathway which involves p53 functioning as a transcription factor, which in turn up regulates the expression of pro-apoptotic protein Bax and the extrinsic (death receptor) pathway which is by activation of TNF-α and Fas Ligand. Bax is a pro-apoptotic protein that antagonizes Bcl-2, an anti-apoptotic protein which is present in the mitochondrial membrane. When Bax/Bcl-2 ratio is increased, the protective effect of Bcl-2 on mitochondrial membrane is interrupted and the membrane permeability increases, leading to leakage of cytochrome c to cytosol. This Cytochrome c binds to Apaf-1 (apoptotic protease activating factor-1) to form an apoptosome complex, which in turn initiates the caspase pathway via caspase-9 and causes cell death. Curcumin has been shown to induce apoptosis in tumor cells by acting selectively at the G2 phase by up regulating p53 expression and initiation of the mitochondrial apoptotic pathway via increased Bax expression and cytochrome c release.[70]

Curcumin also has a stimulatory effect on the extrinsic apoptotic pathway. It causes activation of TNF-α and Fas Ligand, which in turn activates caspase-8 through receptor-attached FADD adapter molecule and initiation of the caspase cascade. Curcumin has been shown to induce apoptosis in mouse-rat retinal ganglion cells by increasing the levels of Fas and FADD.[72]

3. Increased autophagy

Autophagy is a catabloic process which involves the breaking down of cell components by engulfment in vacuoles and its degradation through lysosomal system. There is formation of "autophagosomes" which is a double layered vacoules containing cytoplasmic proteins and organelles targeted for degradation on fusing with lysosome. In addition to promoting cell survival and function, autophagy is also a method by which cells may undergo programmed cell death. Curcumin has been shown to be an inducer of autophagic cell death in chronic myelogenous leukemia, esophageal cancer and malignant glioma cells through the inhibition of Akt /mTOR/p70S6 kinase pathway and inhibition of the ERK1/2 pathway, which are both involved in regulation of autophagy caused by nutrient stress.[70]

4. Inhibition of angiogenesis and metastasis

Angiogenesis is regulated by various signaling molecules such as VEGF, EGF, platelet derived growth factors, angiopoetin-1 and 2 and metalloproteinases. Curcumin has been shown to inhibit angiogenesis by regulating these signaling molecules in in vivo studies done in xenograft models of various carcinomas like glioblastoma, hepatocellular carcinoma and ovarian carcinomas. Curcumin has also shown to inhibit angiogenic response to FGF-2 stimulation in mouse endothelial cells and decrease the expression of enzyme MMP-9, which is involved in growth of new blood vessels. [70]

Curcumin as a Radiosensitiser

Curcumin has been found to have dual mode of action after irradiation depending on its dose. It has been found to protect the cells from harmful effects of radiation which is induced by radiation and also enhances the effect of radiation.

The mechanisms through which curcumin exerts its effects have been put forward by many studies, these are: (1) Causing cell cycle arrest at S/G2M phases of cell cycle (2)Down regulation of COX-2 and (3) Inhibiting EGFR phosphorylation. Curcumin has shown to arrest S/G2 phase of cell cycle which is the most sensitive phase of cell cycle to radiation. When curcumin was combined with radiation, there was a decrease in tumor weight and tumor size in mice models.[68]

COX-2 is commonly up regulated in HNSCC, recent studies have shown that, down regulation of COX-2 may enhance the chemoradiotherapy response while sparing the normal tissue. Curcumin has shown to decrease COX-2 expression in SCC cell lines. Activation of EGFR signaling pathway leads to elevated COX-2 transcription and enhanced PGE_2 production. Curcumin is known to inhibit EGFR pathway which forms another mechanism of radiosensensitising action of curcumin. Similar radioenhancing effect has been seen, when curcumin was given along with gamma radiation on prostate cancer human cell lines and on hamster ovary cells.[68]

Role of Curcumin in chemoprevention

Regression of premalignant lesions of bladder, soft palate, gastrointestinal tract, cervix and skin have been noted in various studies done in India, Taiwan, USA and UK. Ikezaki et al. investigated the modifying effects of curcumin on glandular stomach carcinogenesis in male Wistar rats treated with N-methyl-N-nitro-N nitroso guanisine and sodium chloride.[73] The total incidence of combined atypical hyperplasias and adenocarcinomas produced in the glandular stomach was 10% lower in the curcumin-fed group (0.05% curcumin) than that observed in the basal diet fed group. In a study conducted by Singletary et al, intraperitoneal administration of curcumin at the dose of 100 mg/kg or 200 mg/kg significantly decreased the number of palpable

mammary tumors and suppressed the production of mammary adenocarcinomas in Sprague–Dawley rats.[74] It has been found that doses up to 8-10g could easily be administered daily to patients with pre-malignant lesions for 3 months without any toxicity.[68]

Antioxidant and Anti- Inflammatory properties of Curcumin

The antioxidant activity of the curcuminoids is attributed to their chemical structure. The curcuminoids consist of two methoxylated phenols connected by two α, B unsaturated carbonyl groups that exist in a stable enol form. It has been found that curcumin acts as an antioxidant by breaking chain at the 3' position, there by resulting in an intramolecular Diels-Alder reaction and causes neutralization of the lipid radicals. Curcumin has free radical-scavenging activity and has been shown to scavenge various reactive oxygen species produced by macrophages (including superoxide anions, hydrogen peroxide and nitrite radicals) both in vitro as well as in vivo studies.[75]

The Inducible nitric oxide synthase (iNOS) is an enzyme found in macrophages which generates large amounts of Nitric oxide to provide the 'oxidative burst' which is needed for the defense against pathogens. iNOS is induced in response to an oxidative environment, and the Nitric oxide which is generated reacts with superoxide radicals to form peroxynitrite, which is highly toxic to cells. It has been shown that curcumin decreases the iNOS activity in macrophages, thus reducing the amount of ROS generated in response to oxidative stress. [75]

The NF-kB, which is a transcription factor that regulates a host of pro-survival /anti-apoptotic genes. Activation of these genes results in the regulation of the expression of COX-2, cyclin D1, and VEGF, all of which have a role in regulating proliferation of cells and inflammation.

Curcumin inhibits NF-kB pathway by blocking the IκK-mediated phosphorylation and degradation of IκBα, thus NF-κB remains bound to IκBα in the cytoplasm and will not be able to enter the nucleus to activate transcription. Therefore by inhibiting NF-kB pathway, curcumin causes down-regulation of COX-2 and iNOS and decreased production of inflammatory markers. Apart from NF-kB pathway curcumin also has suppressive effects on other inflammatory pathways.

The arachidonic acid pathway for eicosanoid biosynthesis is an important participant pathway in the producing inflammatory response. It acts by generating a host of reactive lipid products including leukotrienes, prostaglandins, prostacyclins and thromboxanes. Curcumin has been shown to decrease the metabolism of arachidonic acid by down regulating the activity of LOX and COX-2, both at the transcriptional level as well as via post-translational enzyme inhibition.[70]

Other Clinical uses of Curcumin

A number of clinical studies, most of which were single-arm phase II design, have shown that curcumin might be beneficial in diseases such as chronic inflammation, malignancies, and premalignant lesions. Unfortunately, most of these studies has only small numbers of patients, and none of these observations has been verified by other groups of investigators.

In a study conducted by Deodhar et al[76], to find out improvement in 18 patients with rheumatoid arthritis, it was found out that patients who received curcumin (1200 mg/day) or phenylbutazone. The administration of curcumin caused no discernible side effects. However, the long-term effect of curcumin in rheumatoid arthritis has not been reported.

Kuttan and colleagues had done a study on the use of turmeric as a topical treatment for oral cancers and leukoplakia. It was found that of 62 patients enrolled in the study, 10% showed

a reduction in lesion sizes.[77] Even though there is no widely done phase II trial studies for curcumin in treatment of human cancers.

It has been considered as an antiviral agent for human immunodeficiency virus (HIV). However, in a 40-patient cohort, there was no evidence that curcumin reduced viral load or increased CD4 counts.[78] In a study conducted in 10 healthy volunteers, who received 500 mg of curcumin per day for 7 days, a significant decrease in the level of serum lipid peroxides (33%), an increase in high-density lipoprotein cholesterol (29%), and a decrease in total serum cholesterol (11.63%) was found.[79]

MATERIALS AND METHODS

Our study is a randomized, single blinded clinical study coordinated by a multidisciplinary team including Head & Neck surgeon, medical oncologist and radiation oncologist to evaluate all eligible patients during December 2012 to June 2014 in the Department of Otorhinolaryngology and Head and Neck Surgery of R.L.Jalappa Hospital and Research Centre.

The study was approved by the Institutional ethical committee.

Detailed clinical examination was carried out and patients were staged according to AJCC 2012 TNM classification. All the patients underwent biopsy for histopathological diagnosis. Other required tests like complete blood investigations, including liver and renal function tests, x-ray mandible, chest x-ray, electrocardiogram, were done.

Written informed consent was taken from all the patients included in the study.

Inclusion criteria:

All adult patients undergoing radiotherapy or concurrent chemo-radiotherapy for Head and Neck Squamous Cell Carcinoma.

Exclusion criteria:

1. Patients with Non Squamous Head and Neck cancers.
2. Patients not giving consent for the treatment.
3. Patients with severe acid-peptic disease.
4. Patients with distant metastasis.
5. Patients with recurrent tumors

All Patients enrolled in the study were randomized using 4x4 block randomization. In this method four groups were made and allotted into 1,2,3,4 groups, then random numbers were generated from 1 to 4 using Research Randomizer. The patients were randomised into 2 groups - Study group (Group A) and Control group (Group B). Patients in group A received daily dose of 500mg of curcumin capsules thrice a day (total dose 1.5gm/day) and were asked to take after food, while patients in control group received placebo capsules thrice a day. The patients started consuming the capsules on the first day of radiation till the completion of radiotherapy.

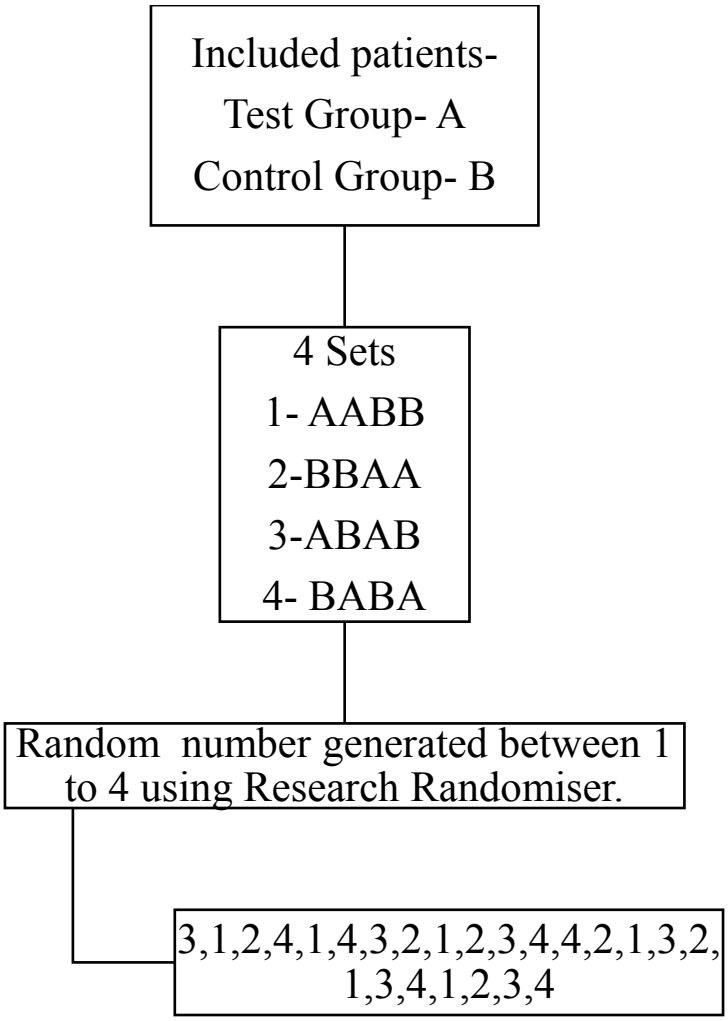


Fig 10 Randomisation pattern done in the study

The curcumin (Biocurcumax) used in our study was obtained from Arjuna naturals, Aluva, Kerala. Each capsule of curcumin contained 500mg of curcumin powder and it comes under the group of nutraceutics and has been approved by FSSAI (Food Safety and Standards Authority of India). The placebo capsule contained starch powder. Both the capsules are identical in colour and shape.

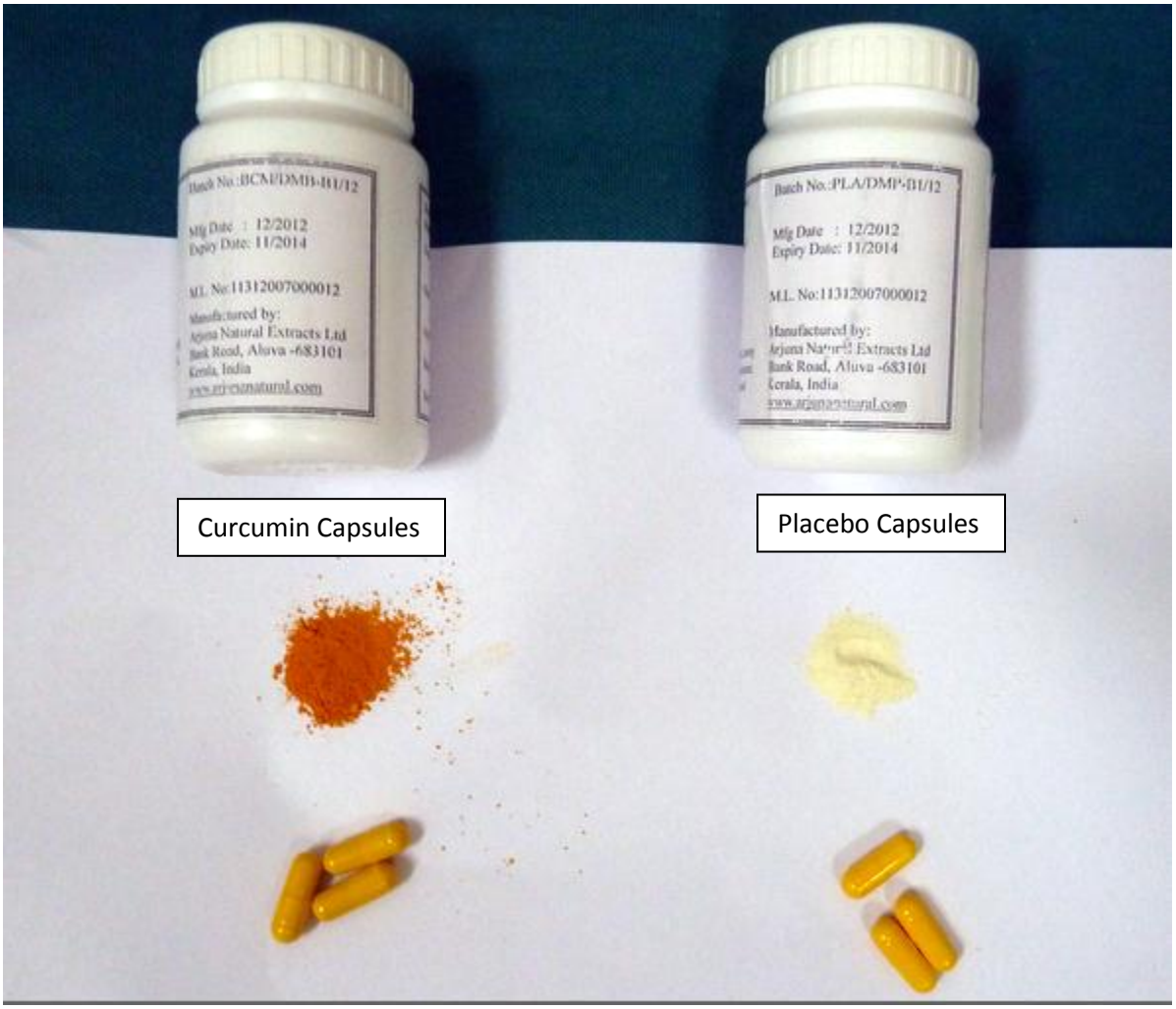


Photo 2: Curcumin capsules and the placebo capsules used in the study

A total of 64 patients were included in the study, and all of them received External Beam Radiotherapy using Cobalt 60, in that 3 patients were excluded from the study as they defaulted the treatment. At the end of the study there were 61 patients who completed the study. All the patients received 1 fraction (2Gy) of radiotherapy per day, five times a week, for a total dose of 66 Gy, spinal cord was excluded after 46Gy. Patients planned for Chemoradiation received Cisplatin infusion (50mg/m^2) weekly along with radiotherapy. They remained as inpatients during their entire course of treatment and a constant check was kept on their dental, medical parameters and supportive care was given to subjects of both the arms. Patients were asked to maintain a good oral hygiene.

Among 61 patients included in the study, 21 patients were qualified to study the radiosensitisation potential of curcumin. They were randomized to group A with 12 patients and group B with 9 patients. They underwent Base-line Contrast enhanced computerized tomography (CECT) scan and pre-treatment antero-posterior diameter, transverse diameter and volume of tumour were documented. Three months post treatment, patient underwent a repeat CECT scan to know the response. RECIST criteria (Response Evaluation Criteria in Solid Tumors) was used to assess the response[80] and the response were documented as Complete Response (CR), Partial Response (PR), Progressive disease (PD) or Stable disease (SD).

Table 4 RECIST Criteria used for assessing the tumor response[80]

Response assessment	**RECIST guideline, version 1.0**
Target lesions	
Complete Response (CR)	Disappearance of all target lesions
Partial Response (PR)	≥30 percent decrease in the sum of the longest diameter (SLD) of the target lesions compared with baseline
Progressive disease (PD)	≥20 percent increase in the sum of the longest diameter (SLD) of the target lesions compared to the smallest sum of the longest diameter recorded since treatment started OR The appearance of one of more new lesions
Stable disease (SD)	Neither Partial Response (PR) nor Progressive disease (PD)

All 61 patients included were assessed for mucositis weekly during treatment and 2 months after treatment using a subjective and an objective scale. The subjective scale used for assessment was the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.0 and the objective scale was the WHO scale for oral mucositis.

Table 5 NCI CTCAE version 4.0 Subjective Assessment of mucositis[39]

Grade 1	Asymptomatic or mild symptoms; intervention not indicated.
Grade 2	Moderate pain; not interfering with oral intake; modified diet indicated
Grade 3	Severe pain; interfering with oral intake
Grade 4	Life-threatening consequences; urgent intervention indicated
Grade 5	Death

Table 6 WHO scale for oral mucositis objective Assessment scale[39]

Grade 0	No oral mucositis
Grade 1	Erythema and soreness
Grade 2	Ulcers, able to eat solids
Grade 3	Ulcers, requires liquid diet (due to mucositis)
Grade 4	Ulcers, alimentation not possible (due to mucositis)

Statistical Analyisis:

We used the IBM SPSS software (v.22) to perform the statistical analysis. Mean, SD and standard error, chi-square test were used for categorical variables. Independent t-test for quantitative data and 2 tailed p value. P value less than or equal to 0.05 was considered as statistically significant.

Observation and Results

Table - 7

Age Distribution of patients included in the study (n=61)

Age group	Group A (n-30)		Group B (n-31)	
	Number of Cases	%	Number of Cases	%
30-40	7	23.3	8	25.8
41-50	5	16.6	10	32.2
51-60	7	23.3	4	12.9
61-70	10	33.3	6	18.7
71-80	1	3.3	2	6.4
81-90	0	0	1	3.2

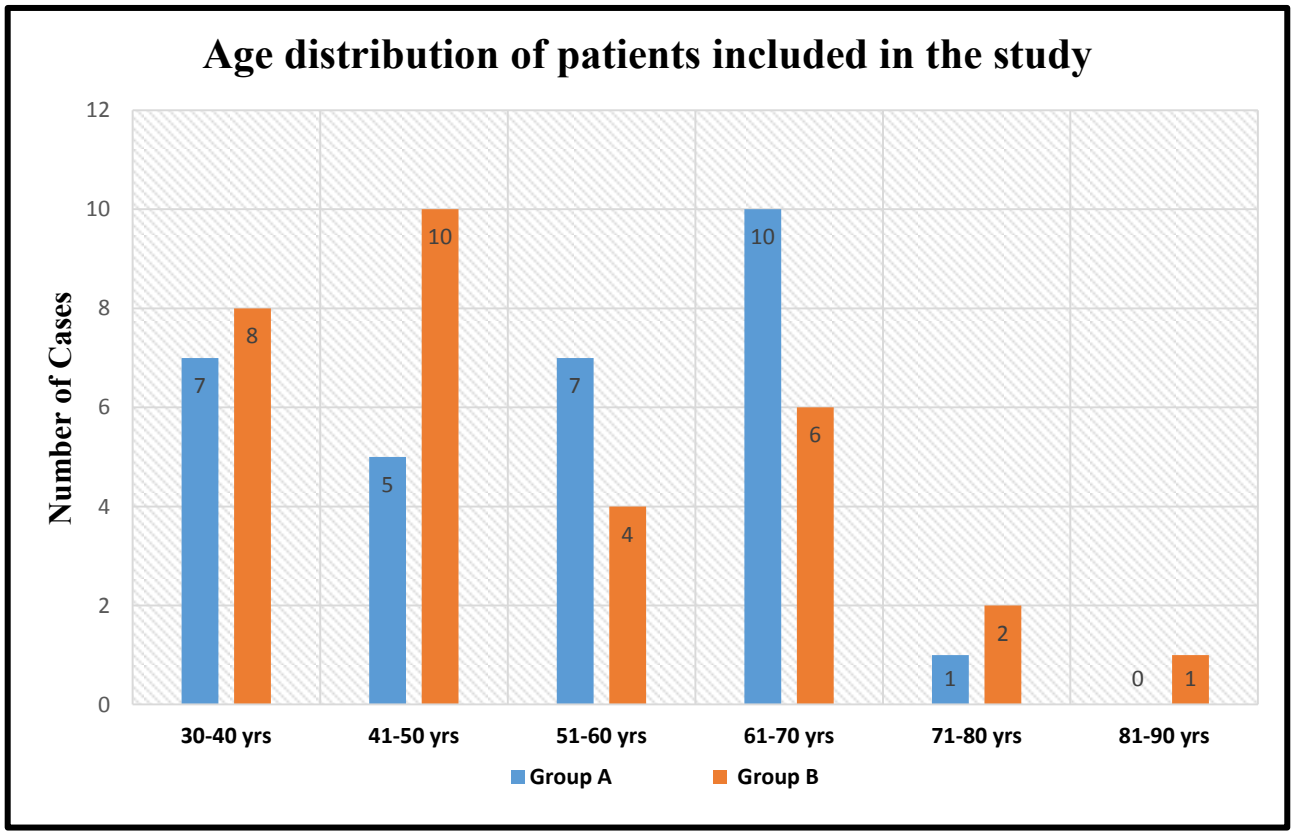


Fig 11 Distribution of patients in the study. The study included patients in the age group of 30 to 80 years in group A and 35 to 85 years in group B.

Table – 8

Sex Distribution of Patients included in the study

Sex	Group A (n-30)		Group B (n-31)	
	Number of Cases	%	Number of Cases	%
Female	15	50	18	58.1
Male	15	50	13	41.9

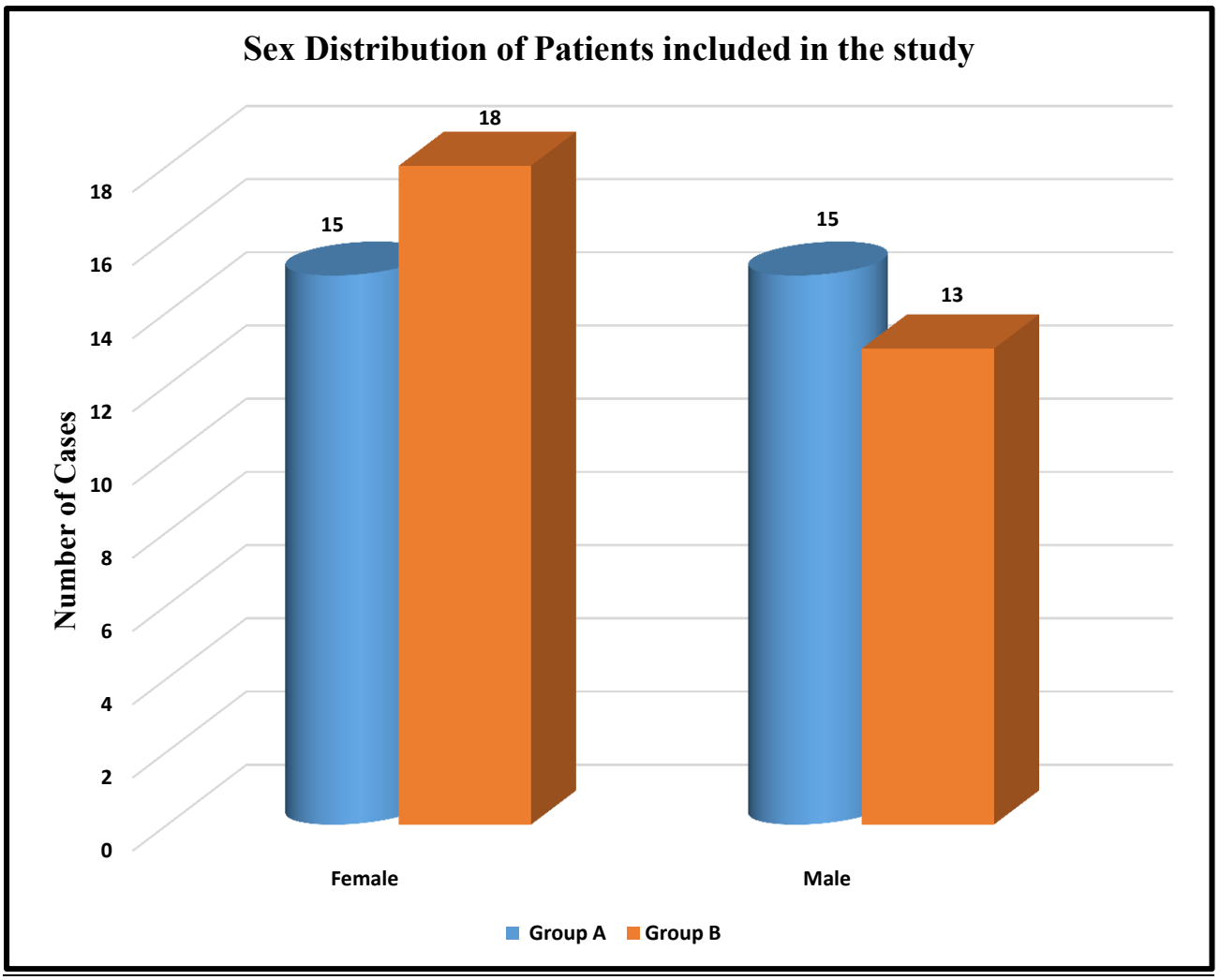


Fig 12 Sex distribution of patients. In group A, the Female is to Male ratio was 1:1 while in group B, Female is to Male ratio was 1:1.4

Table – 9

Habit Profile

Habits	Group A (n-30)		Group B (n-31)	
	Number of Cases	%	Number of Cases	%
Pan Chewers	30	100	31	100
Smokers	10	31.25	7	22.6
Alcohol consumption	2	6.25	4	12.5

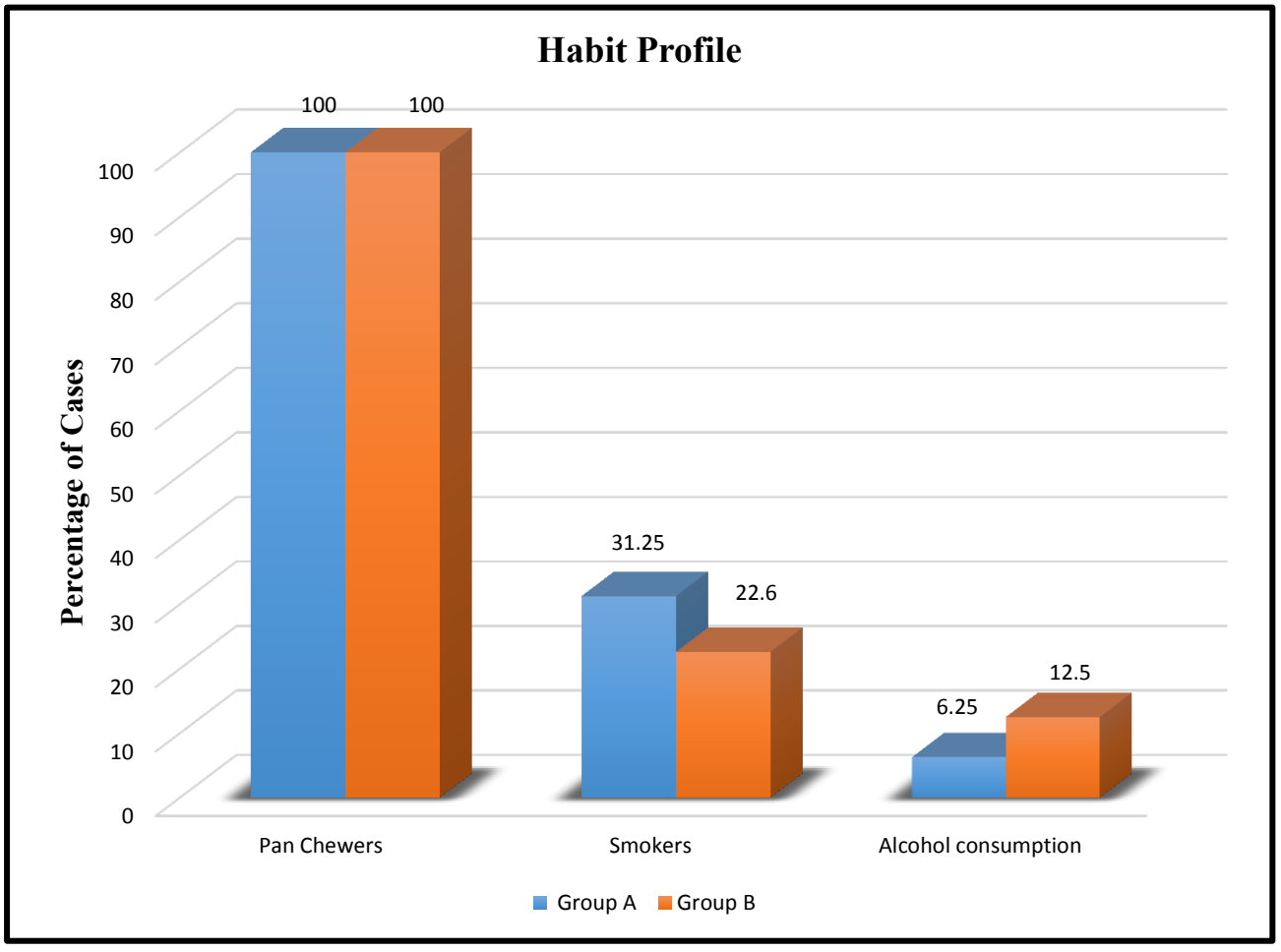


Fig 13 Habit profile of patients included in the study. All the patients in both the groups had the habit of pan chewing. Habit of smoking was seen in 10 patients in Group A and 7 patients in Group B. Only 2 patients in Group A and 4 patients in Group B had the habit of alcohol consumption.

Table – 10

Site of Primary Tumor

Site of Primary Tumor	Group A (n-30)		Group B (n-31)	
	Number of Cases	%	Number of Cases	%
Oral Cavity	22	73.3	26	83.9
Oropharynx	5	16.7	3	9.7
Glottis	2	6.7	1	3.2
Supraglottis	1	3.3	1	3.2

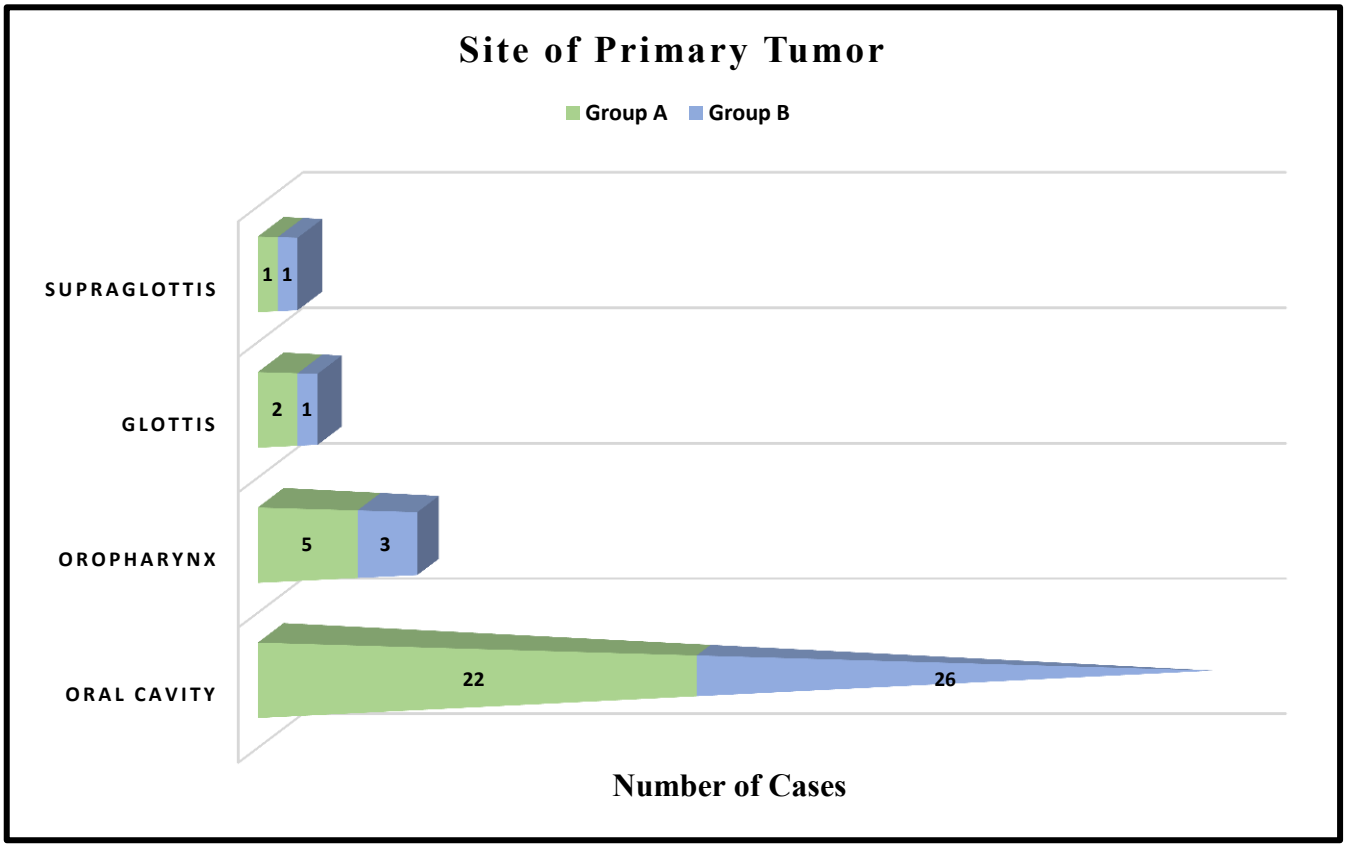


Fig 14 Site of Primary Tumour. Most of the patients in both the groups had suffered from malignancy of oral cavity malignancy with 22 patients (73.3%) in Group A and 6 patients (83.9%) in Group B. Rest of the patient suffered from malignancies of oropharynx and larynx.

Table – 11

Distribution of patients according to TNM staging (AJCC 2012 Classification)

Stage of Primary Tumor	Group A (n-30)		Group B (n-31)	
	Number of Cases	%	Number of Cases	%
Stage IVa	24	73.3	19	61.3
Stage III	6	20	10	32.2
Stage IVb	0	0	1	3.2
Stage II	0	0	1	3.2

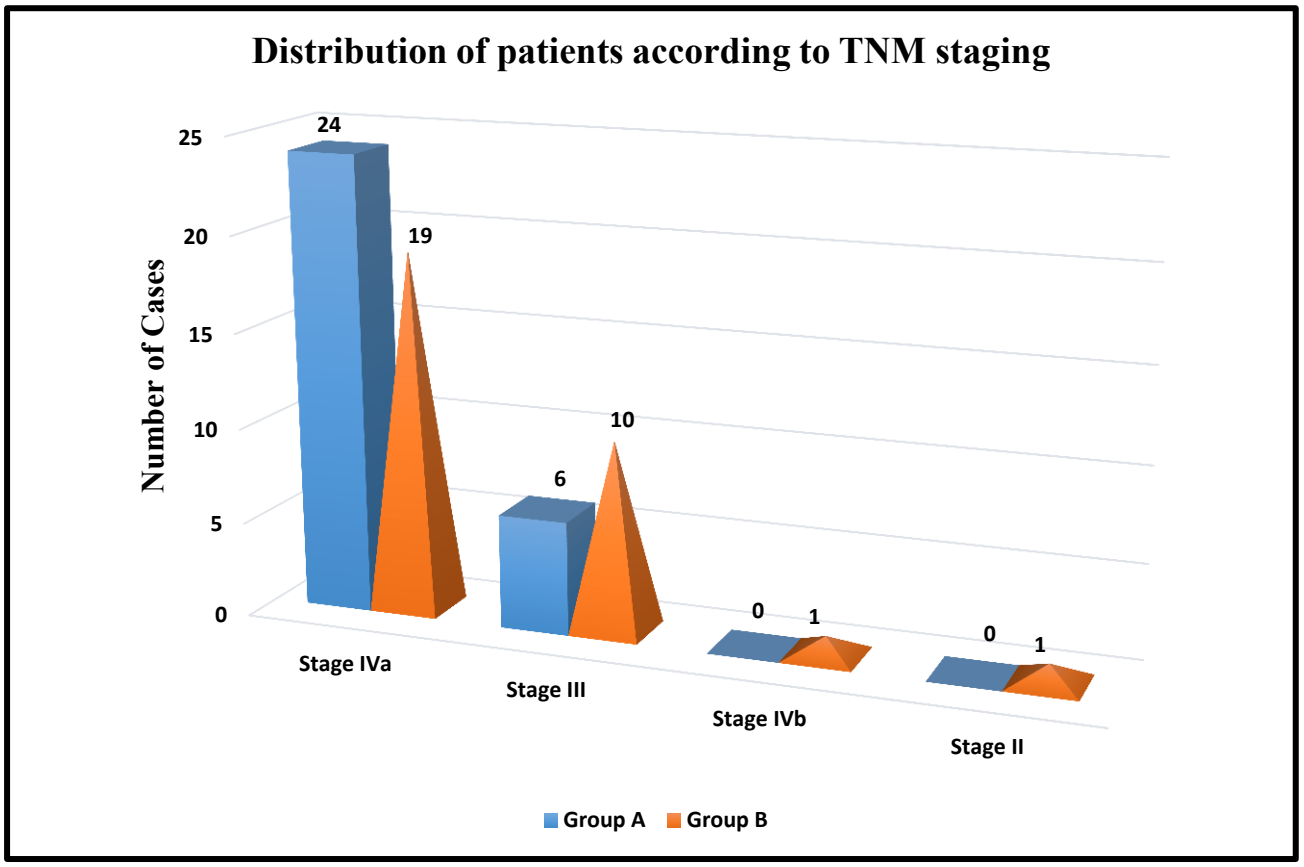


Fig 15 Distribution of patients according to TNM staging. There was a predominance of Stage IV cancers in our study in both the groups with 73.3% (n=24) in group A and 61.3% (n=19) in group B, followed by stage III cancers. One patient each with Stage II and IVB cancer were included in group B.

Table – 12

Overall distribution of patient based on Treatment Modality

Primary Modality of Treatment	Total Number of Cases (n=61)	Percentage
Surgery + Radiotherapy	36	59.1
Radiotherapy+ Chemotherapy	21	34.4
Surgery + Radiotherapy+ Chemotherapy	4	6.5

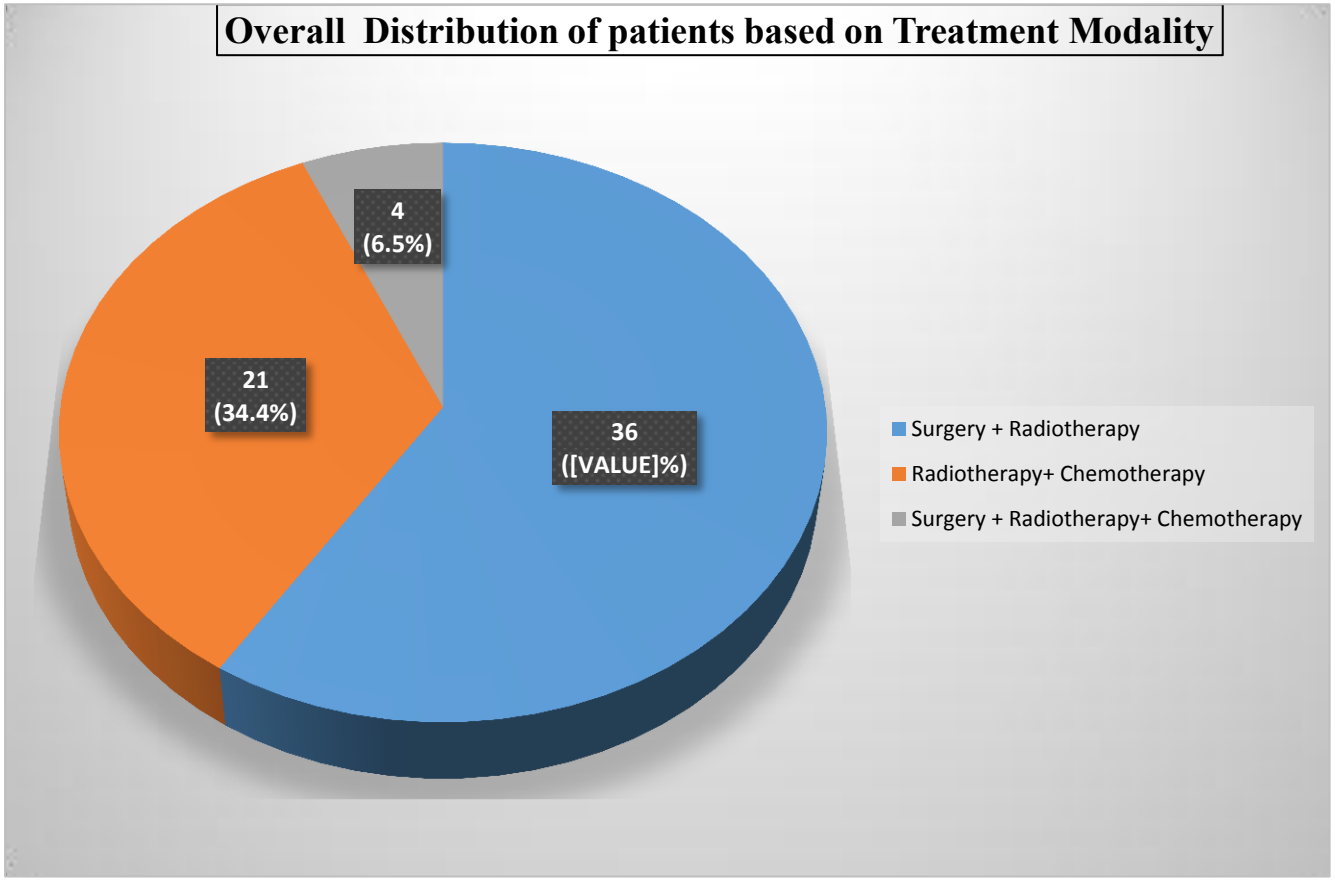


Fig 16 Overall distribution of patient based on the treatment modality. Most of the patients included in the study underwent surgery and received post-operative adjuvant radiotherapy (36 patients). Twenty one patients (34.4%) received chemoradiation and 4 patients received chemoradiation as an adjuvant treatment after surgery.

Among the 61 patients included in the study, 21 patients qualified to study the radiosensitisation effect of curcumin. They were randomized into group A with 12 patients and group B with 9 patients.

Table – 13

Stage matched distribution of Patients included in the study to assess the radiosensitivity of curcumin

Stage of disease	Group A (n-12)	Group B (n-9)
	Number of Cases	Number of Cases
Stage III	1	1
Stage IV	11	8

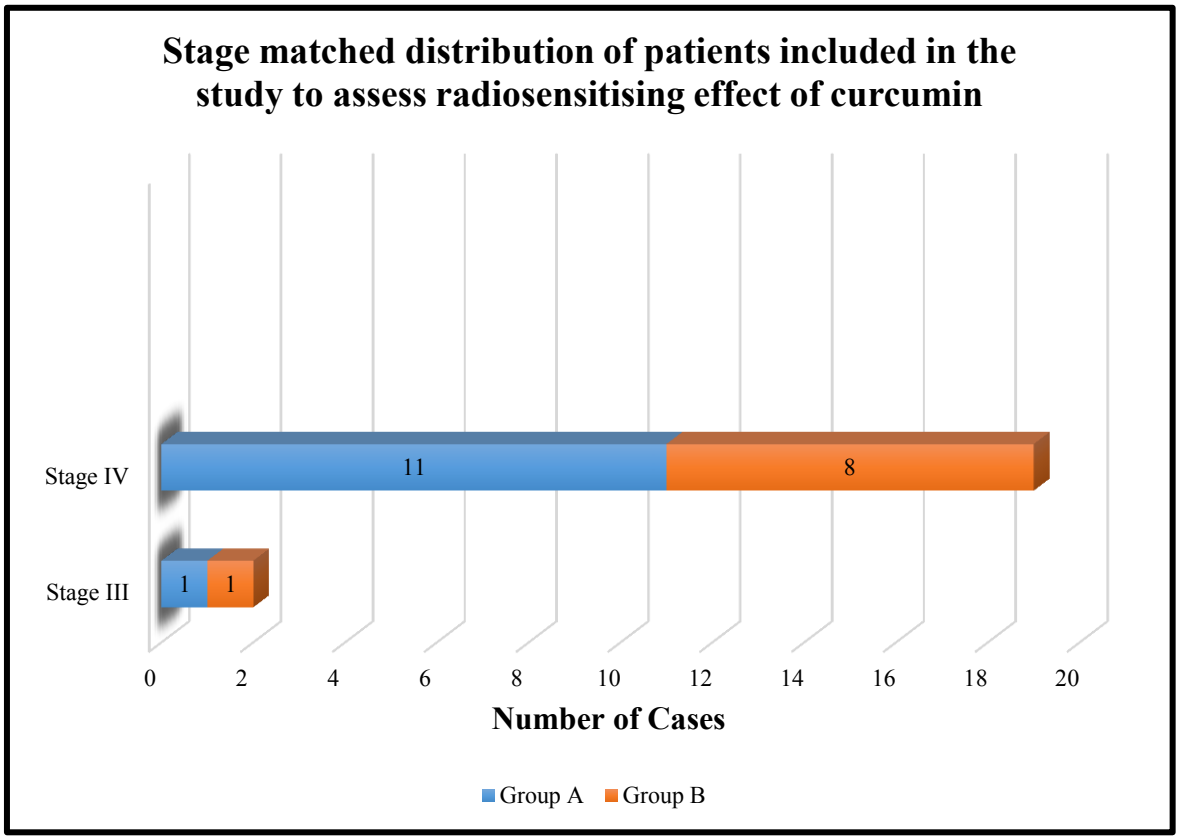


Fig 17 Stage match distribution of patients included in the study to assess the radiosensitising effect of curcumin. After randomization, group A included 11 patients of stage IV cancers and one patient had stage III cancer. In group B, 8 patients had stage IV cancers and one patient had stage III cancer.

Table – 14

Overall Reduction in tumor size 3 months Post Chemoradiation

Overall reduction in tumor size 3 months Post Chemoradiation	Mean AP diameter before Treatment	Mean AP diameter after Treatment	Mean Transverse diameter before Treatment	Mean Transverse diameter after Treatment
Group A (in cm)	3.69	2.43	2.89	1.87
Group B (in cm)	3.6	2.46	2.93	2.11

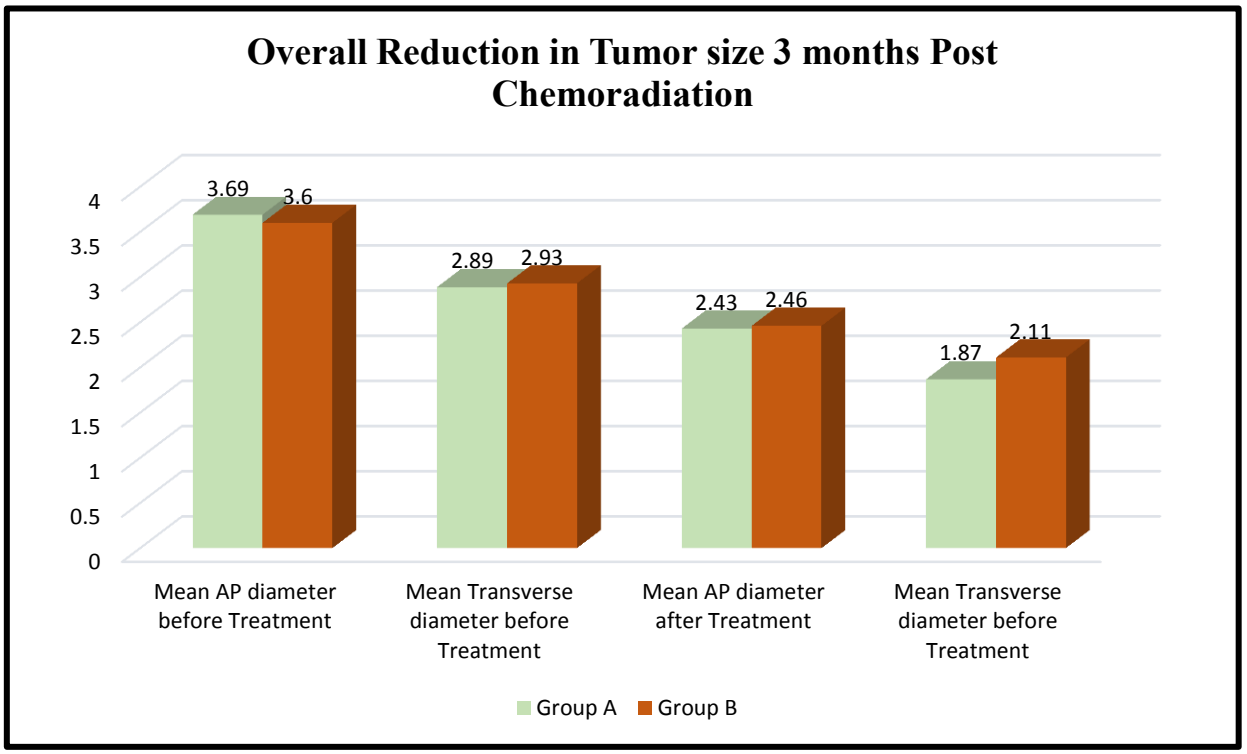


Fig 20 Overall reduction in tumor size 3 months Post chemoradiation. A difference in reduction in tumor size is seen in both the groups. But a statistical significance could not be attained due to less number of cases.

Table – 15

Overall Reduction in tumor Volume 3 months Post Chemoradiation

Overall reduction in Tumor Volume 3 months Post Chemoradiation	Mean Tumor Volume before Treatment	Mean Tumor Volume after Treatment
Group A (in cm^3)	41.47	12.74
Group B (in cm^3)	18.73	8.40

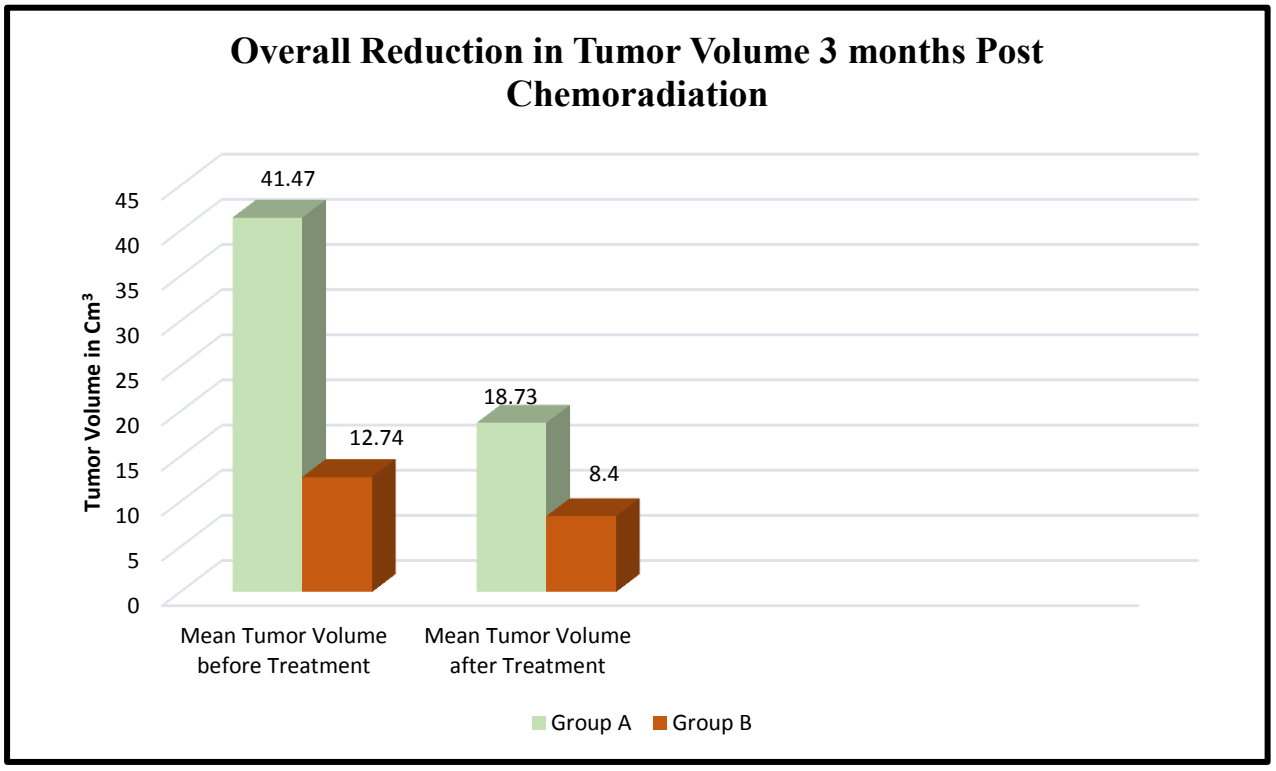


Fig 21 Overall reduction in tumor volume 3 months Post chemoradiation. A difference in reduction in tumor volume is seen in both the groups. But a statistical significance could not be attained due to less number of cases.

Table – 16

Tumor Response at 3 months Post Treatment in Patients with Stage IV cancers

Tumor Response at 3 months Post Treatment in patients with Stage IV	Group A (n-11)		Group B (n-8)	
	Number of Cases	%	Number of Cases	%
Partial Response	6	54.5	3	37.5
Stable Disease	5	45.5	5	62.5

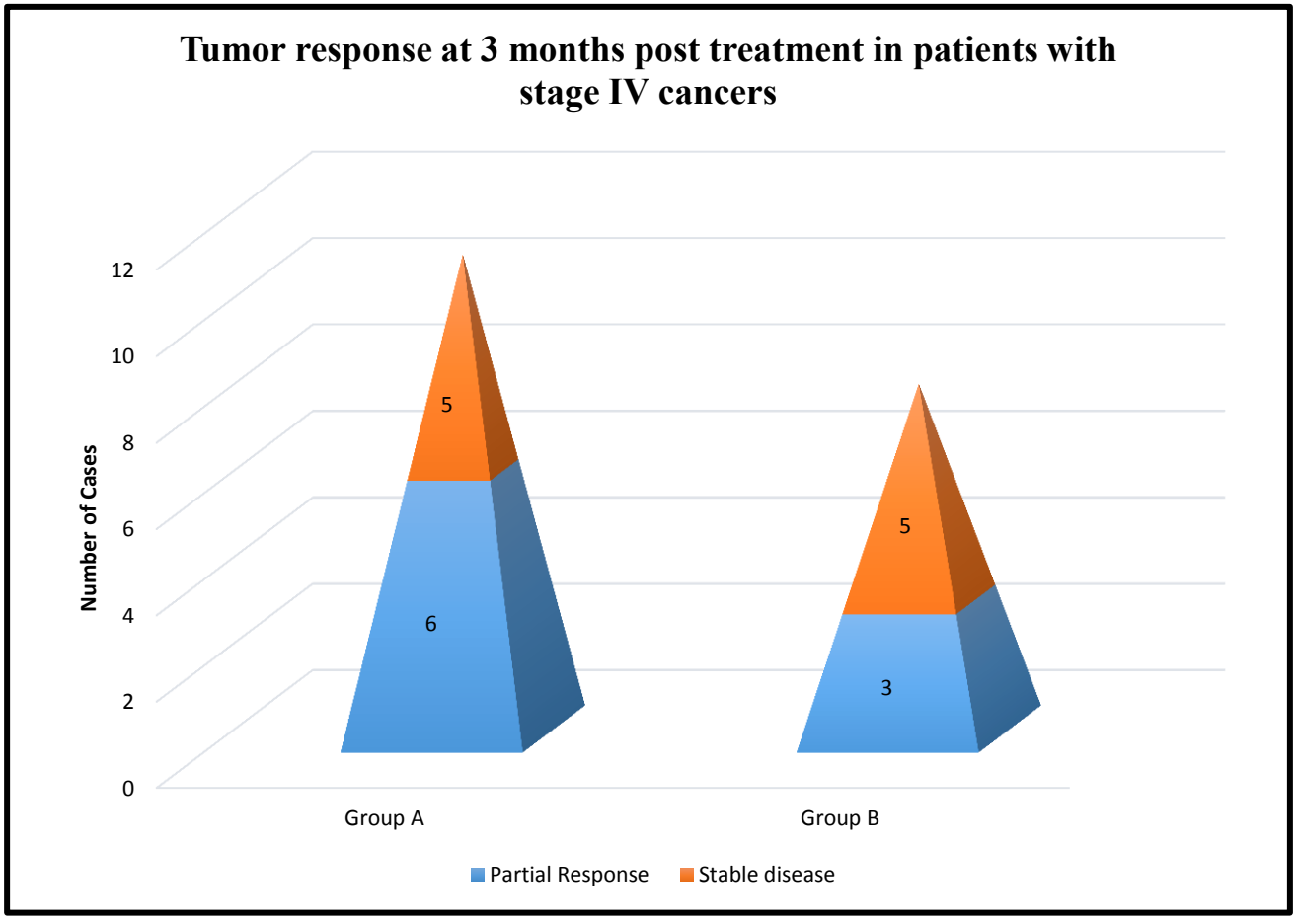


Fig 18 Tumor response at 3 months post treatment in patients with stage IV cancers. We could get stage match only for stage IV cancers and at 3 months post treatment in Group A, the PR was seen in 54.5% and SD was seen in 45.5%. In group B, 37.5% had PR and 62.5% had SD. None of the patients in both the groups had progressive disease.

Table - 17

Overall Tumor Response 3 months Post Chemoradiation

Tumor Response 3 months Post Chemoradiation	Group A (n-12)		Group B (n-9)	
	Number of Cases	%	Number of Cases	%
Partial Response	7	58.3	3	33.3
Stable Disease	5	41.7	6	66.6

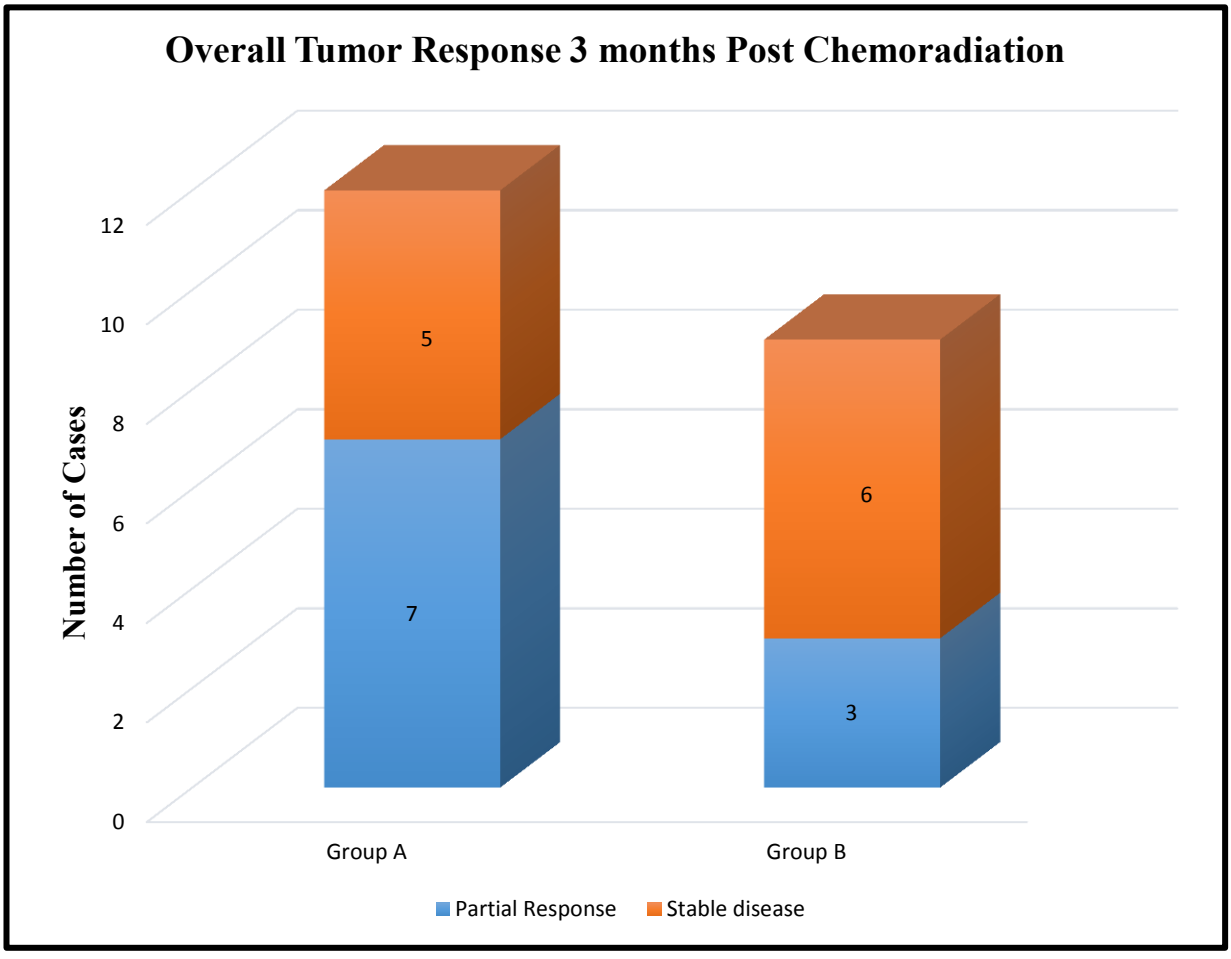


Fig 19 Overall tumour response 3 months post chemoradiation. In group A, the overall PR was seen in 58.3% and SD was seen in 41.7%. In group B, 33.3% had PR and 66.6% had SD. None of the patients in both the groups had progressive disease.

A total of 61 patients were included to study the role of curcumin in treatment of mucositis in HNSCC patients treated with RT/CTRT. The patients were randomized and 30 patients were included in group A and 31 in group B
Table – 18

Distribution of patient based on Treatment Modality

Primary Modality of Treatment	Study group (n-30) A		Control Group (n-31) B	
	Number of Cases	%	Number of Cases	%
Surgery + Radiotherapy	16	53.3	20	64.5
Radiotherapy+ Chemotherapy	12	40	9	29.1
Surgery + Radiotherapy+ Chemotherapy	2	6.7	2	6.4

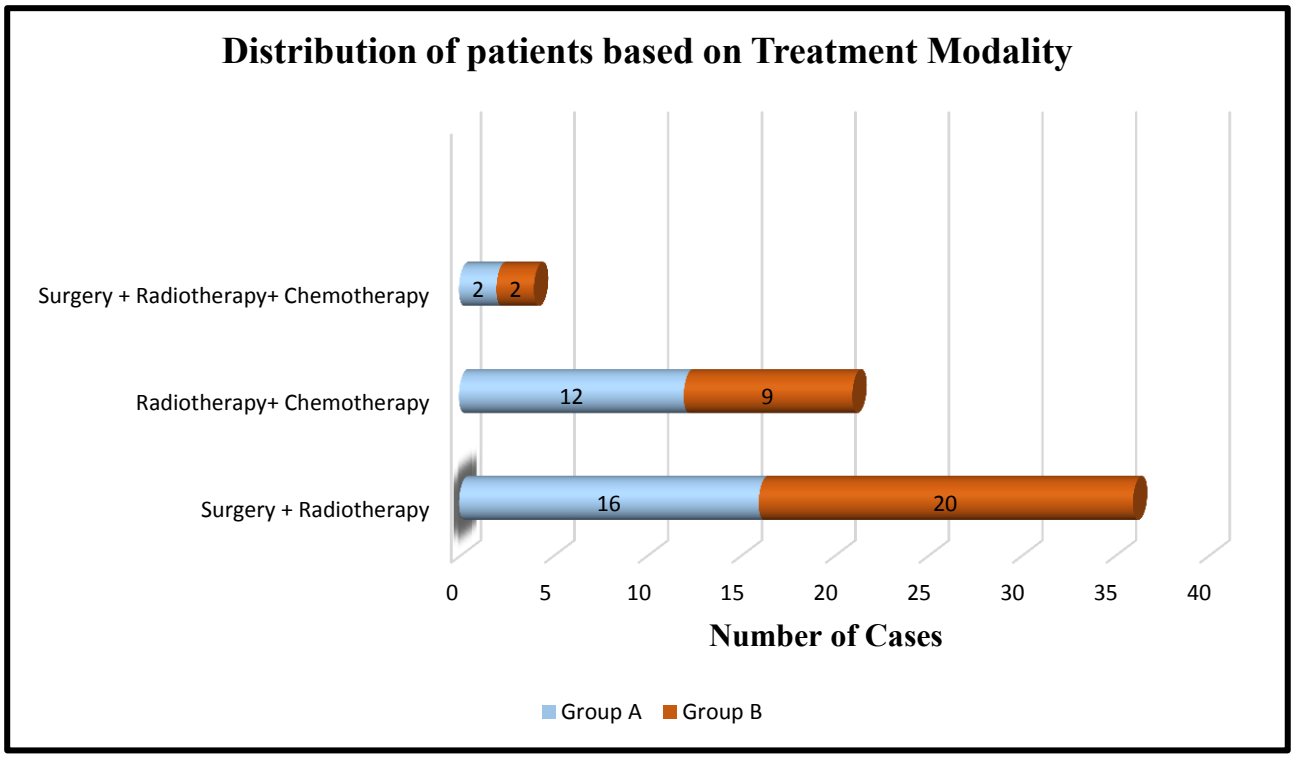


Fig 22 Distribution of patient based on the treatment modality. Nearly 53% (16 patients) in group A and 64.5% (20 patients) in group B underwent surgery and post-operative radiotherapy. Twenty one patients underwent chemoradiation and 4 patients underwent Surgery with post-operative chemoradiotherapy.

Table - 19

Objective grading of Mucositis at end of Week 1

Objective grading of Mucositis at end of Week 1	Group A (n-30)		Group B (n-31)	
	Number of Cases	%	Number of Cases	%
Grade 0	29	96.7	29	93.6
Grade 1	1	3.3	2	6.4
Grade 2	0	0	0	0
Grade 3	0	0	0	0
Grade 4	0	0	0	0

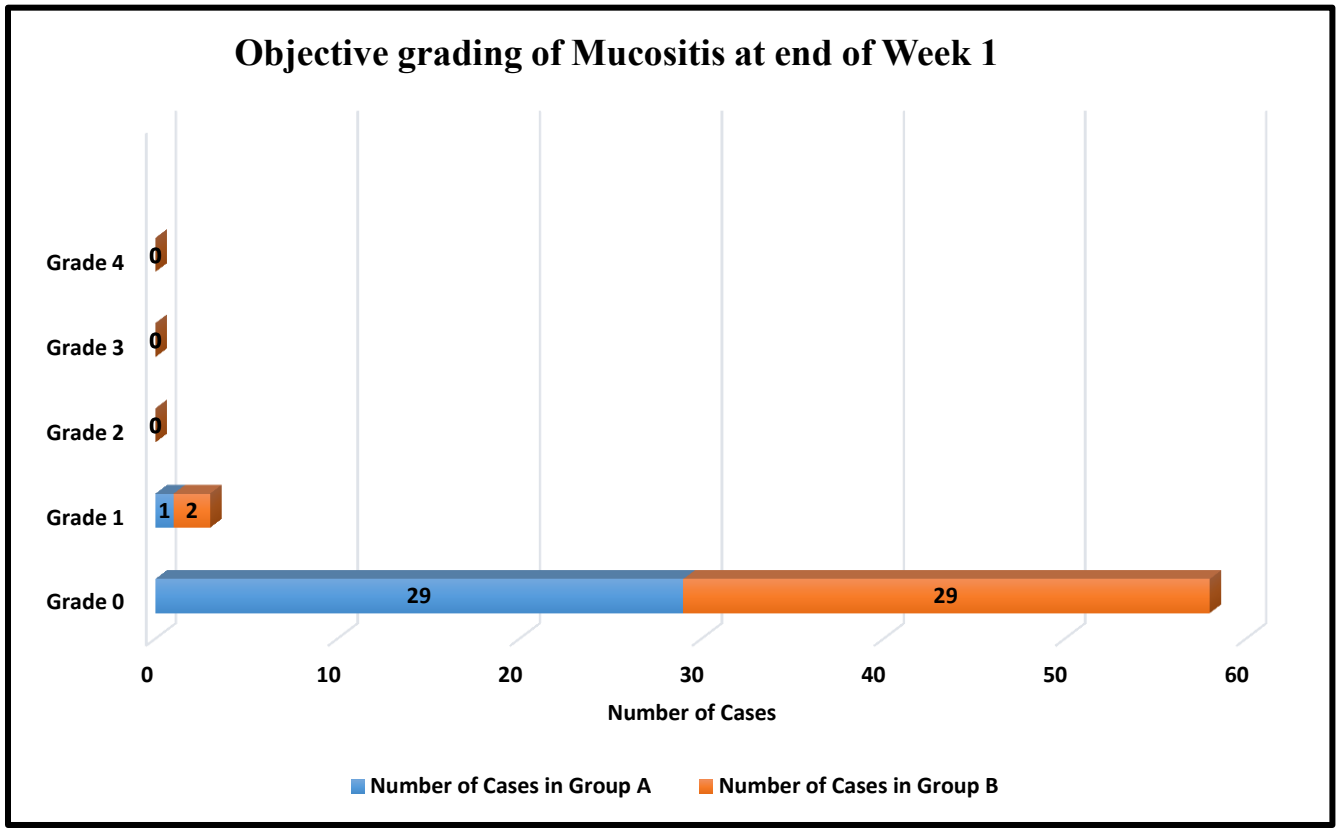


Fig 23 Objective grading of mucositis at the end of week 1. At the end of one week, one patient in group A and 2 patient in group B had developed grade I mucositis. Rest all of the patients were free of mucositis. The difference was not found to be statistically significant. (p=0.488)

Table - 20

Subjective grading of Mucositis at end of Week 1

Subjective grading of Mucositis at end of Week 1	Group A (n-30) A		Group B (n-31)	
	Number of Cases	%	Number of Cases	%
Grade 1	29	96.7	29	93.6
Grade 2	1	3.3	2	6.4
Grade 3	0	0	0	0
Grade 4	0	0	0	0
Grade 5	0	0	0	0

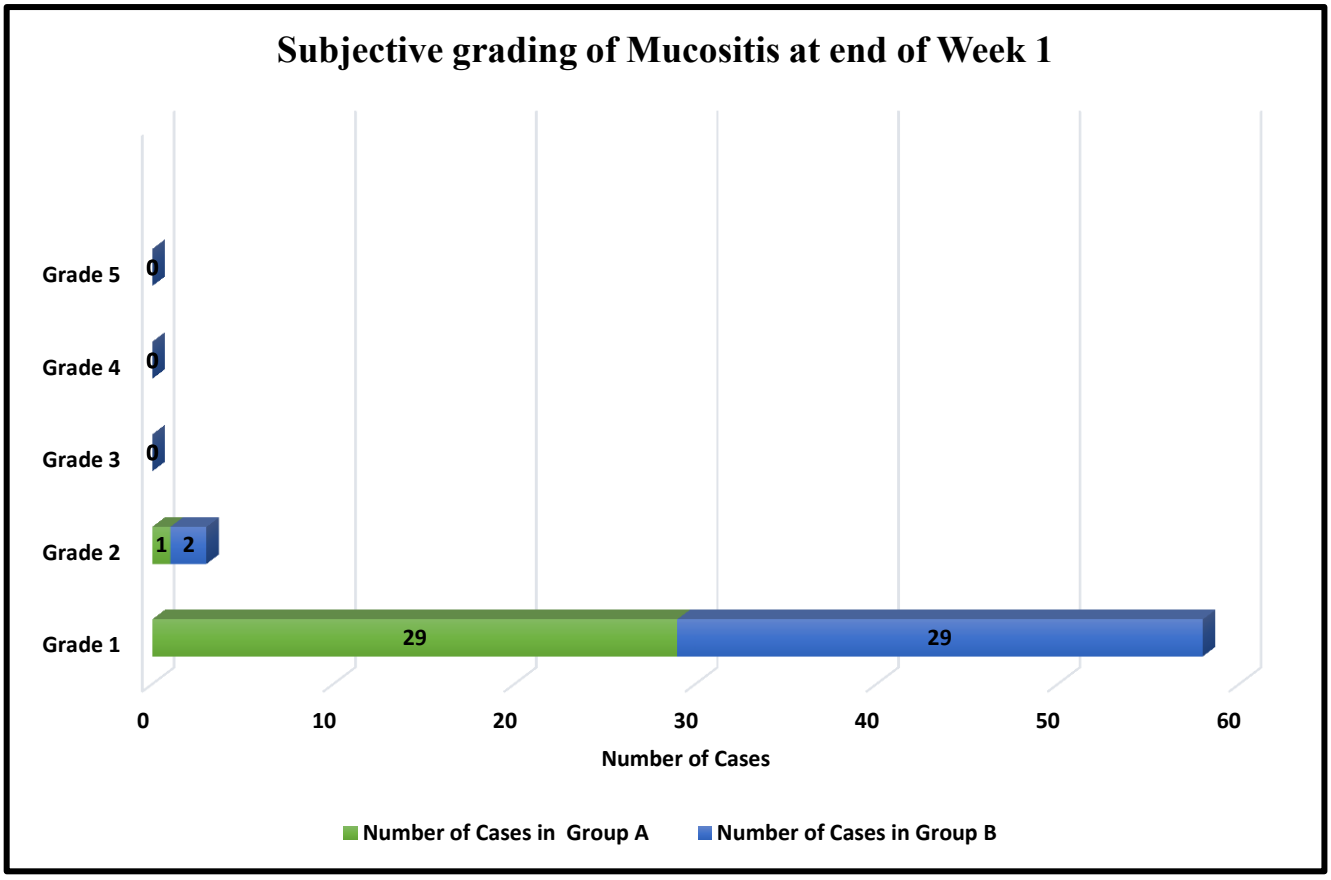


Fig 24 Subjective Assessment of mucositis at the end of week 1. At the end of one week, most of the patients were asymptomatic. One patient in group A and 2 patients in group B had moderate symptoms (grade II) of mucositis. The difference found was not found statistically significant. (p=0.488)

Table - 21

Objective grading of Mucositis at end of 2nd Week

Subjective grading of Mucositis at end of 2nd Week	Group A (n-30)		Group B (n-31)	
	Number of Cases	%	Number of Cases	%
Grade 0	13	43.3	6	19.4
Grade 1	16	53.3	23	74.2
Grade 2	1	3.3	2	6.4
Grade 3	0	0	0	0
Grade 4	0	0	0	0

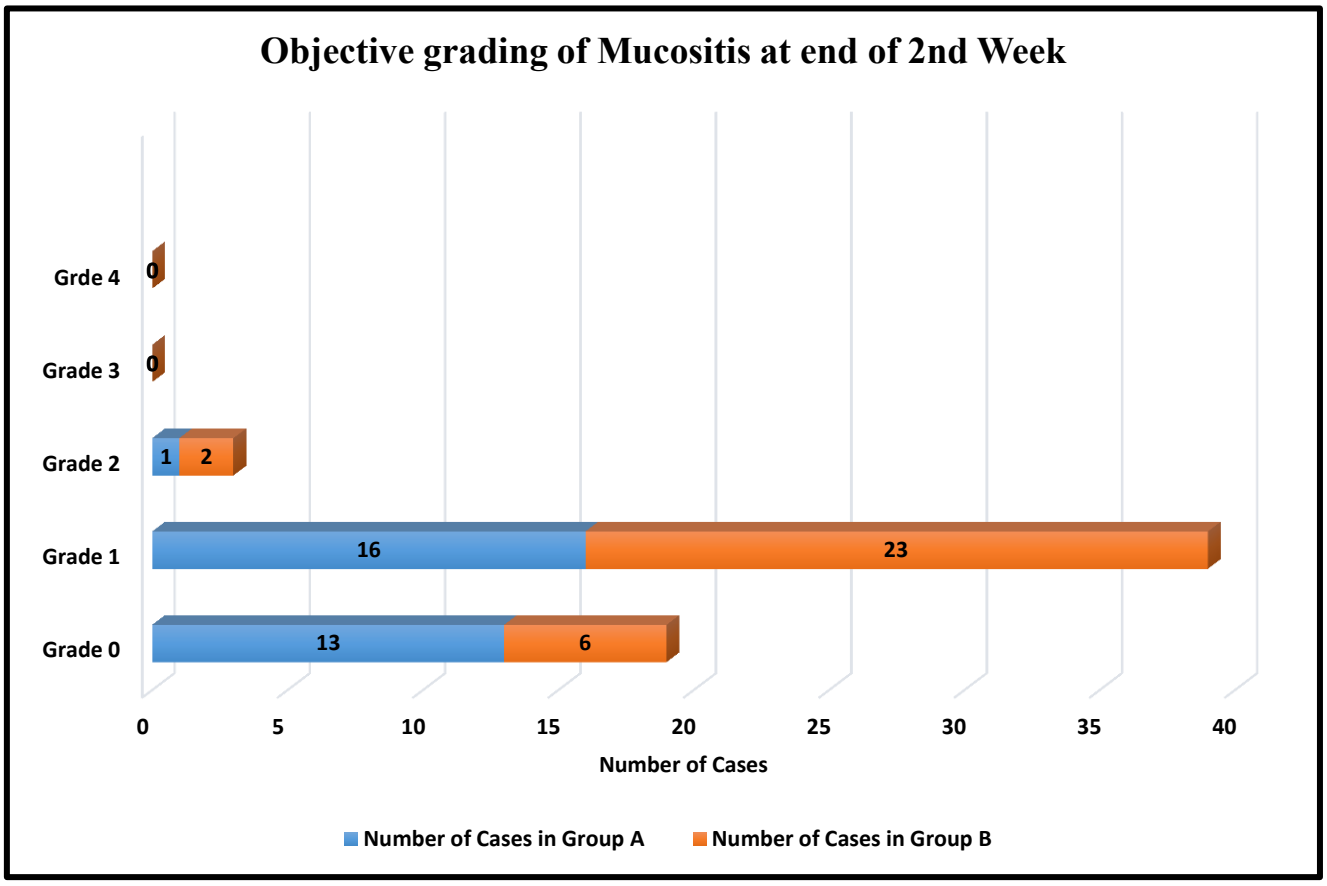


Fig 25 Objective Assessment of mucositis at the end of week 2. At the end of 2 weeks of treatment, 53.3% (n=16) in group A and 74.2% (n=23) in group B developed grade I mucositis. One patient in group A and 2 patient in group B had developed grade II mucositis. Rest all of the patients were free of mucositis. The difference was not found statistically significant. (p=0.171)

Table - 22

Subjective grading of Mucositis at end of 2nd Week

Subjective grading of Mucositis at end of 2nd Week	Group A(n-30)	Group B(n-31)		
	Number of Cases	%	Number of Cases	%
Grade 1	13	43.3	6	19.4
Grade 2	16	53.3	23	74.2
Grade 3	1	3.3	2	6.4
Grade 4	0	0	0	0
Grade 5	0	0	0	0

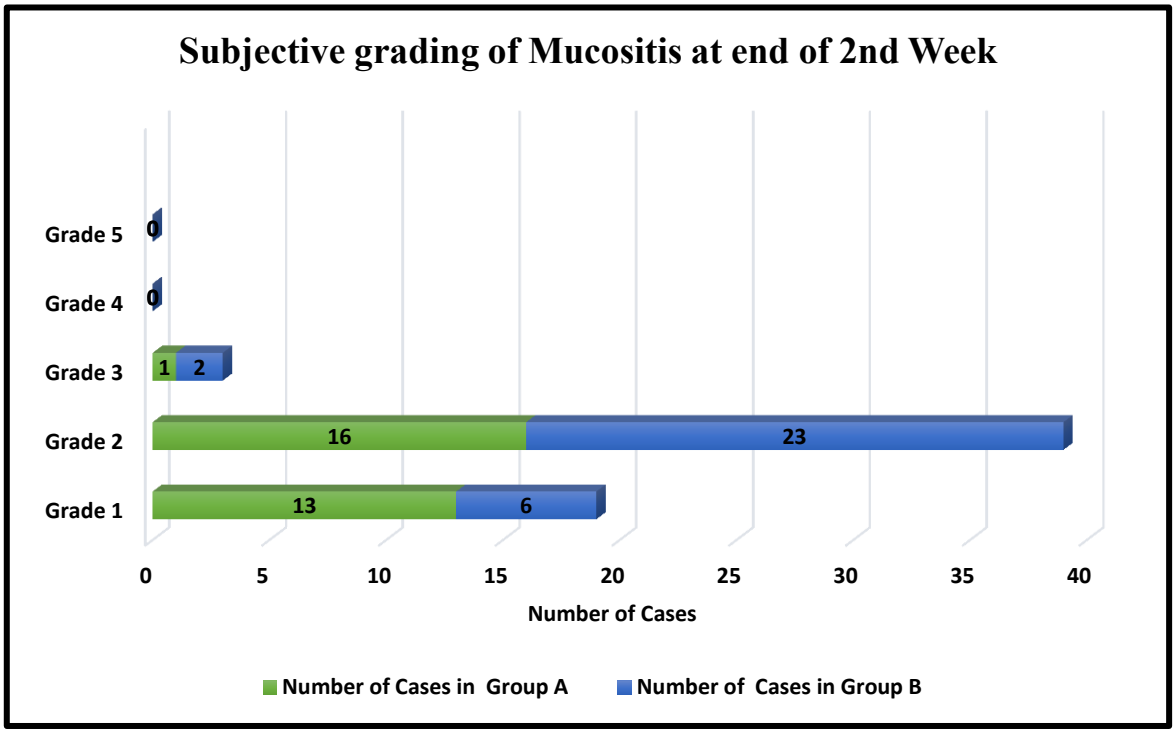


Fig 26 Subjective Assessment of mucositis at the end of 2nd week. At the end of 2 weeks of treatment, 53.3% (n=16) in group A and 74.2% (n=23) in group B developed moderate mucositis (grade II) One patient in group A and 2 patient in group B had developed grade II mucositis. Rest all of the patients were free of mucositis. The difference was not found statistically significant. (p=0.171)

Table - 23

Objective grading of Mucositis at end of 3rd Week

Subjective grading of Mucositis at end of 3rd Week	Group A(n-30)		Group B(n-31)	
	Number of Cases	%	Number of Cases	%
Grade 0	1	3.3	0	0
Grade 1	26	86.7	7	22.6
Grade 2	3	10	22	71
Grade 3	0	0	2	6.4
Grade 4	0	0	0	0

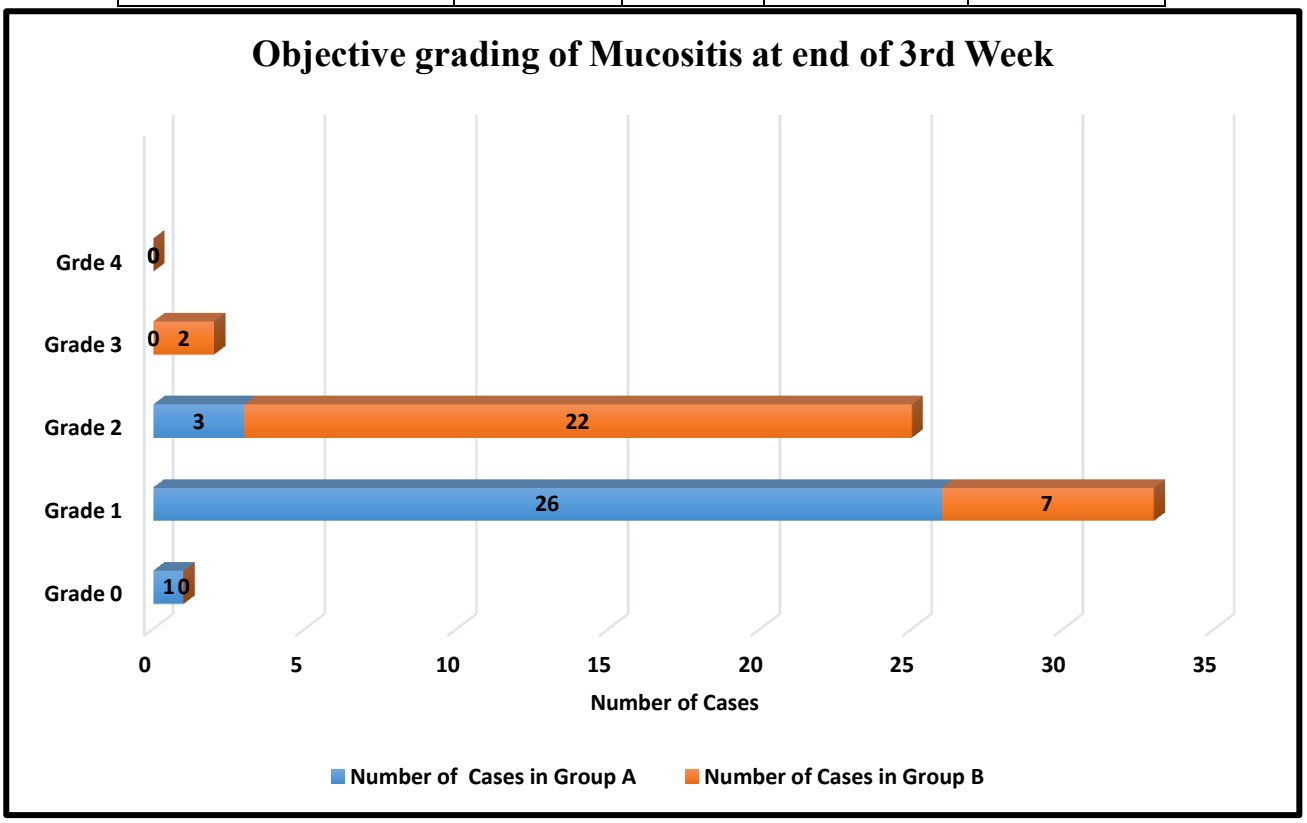


Fig 27 Objective Assessment of mucositis at the end of 3rd week. At the end of 3 weeks of treatment, 71% of the patients (n=22) progressed to grade II mucositis in group B, while only 10% (n=3) had grade II mucositis in group A. 86.7% (n=26) of patients in group A had grade I mucositis. Two patients in group B had progressed to grade III mucositis. The difference found between the groups was statistically significant ($p<0.001$)

Table - 24

Subjective grading of Mucositis at end of 3rd Week

Subjective grading of Mucositis at end of 3rd Week	Group A (n-30)		Group B (n-31)	
	Number of Cases	%	Number of Cases	%
Grade 1	1	3.3	0	0
Grade 2	25	83.3	7	22.6
Grade 3	4	13.3	22	71
Grade 4	0	0	2	6.4
Grade 5	0	0	0	0

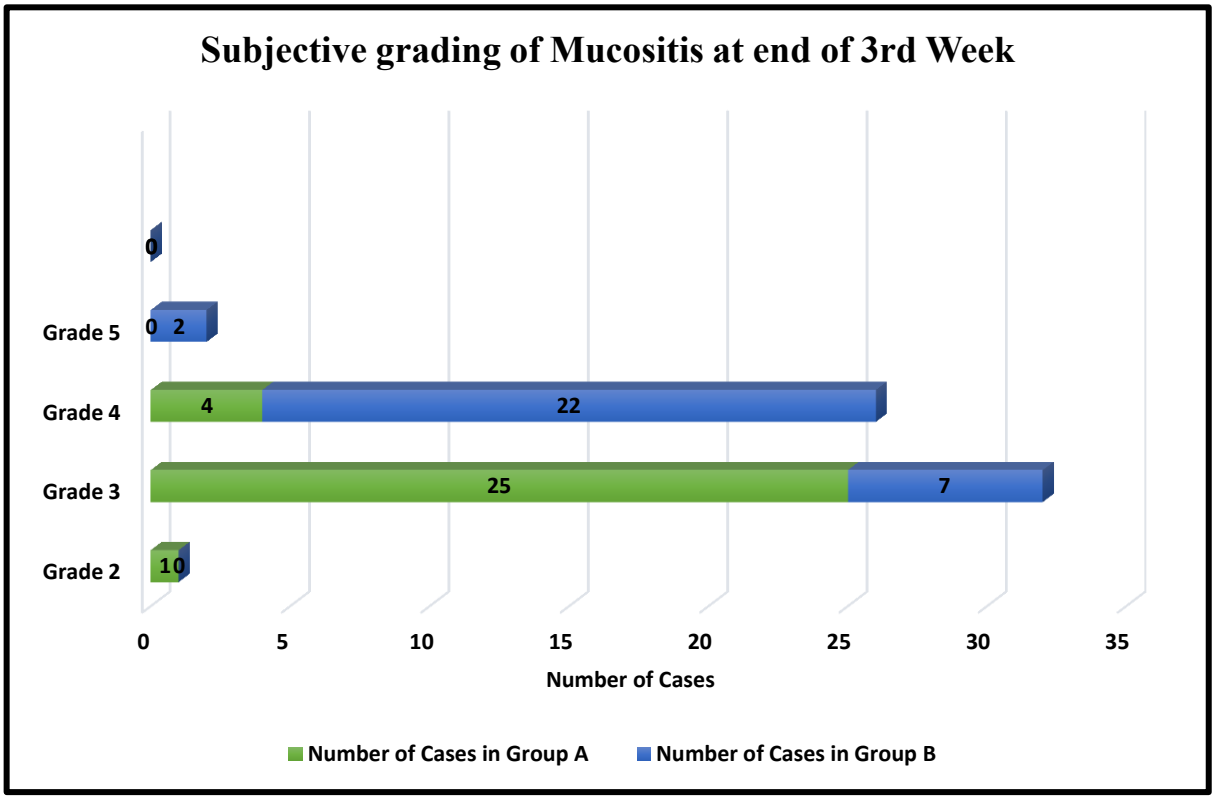


Fig 28 Subjective grading of mucositis at the end of 3rd week. At the end of 3rd week of treatment, in group B, 71% (n=22) patients developed severe pain interfering with oral intake (grade III). Two patients in group B progressed to grade 4 mucositis, requiring intervention, where as 83% of the patients (n=22) were having moderate symptoms (grade II). The difference was found to be statistically significant ($p<0.001$).

Table - 25

Objective grading of Mucositis at end of 4th Week

Objective grading of Mucositis at end of 4th Week	Group A (n-30)		Group B (n-31)	
	Number of Cases	%	Number of Cases	%
Grade 0	2	6.7	0	0
Grade 1	22	73.3	6	19.3
Grade 2	6	20	21	67.7
Grade 3	0	0	4	12.9
Grade 4	0	0	0	0

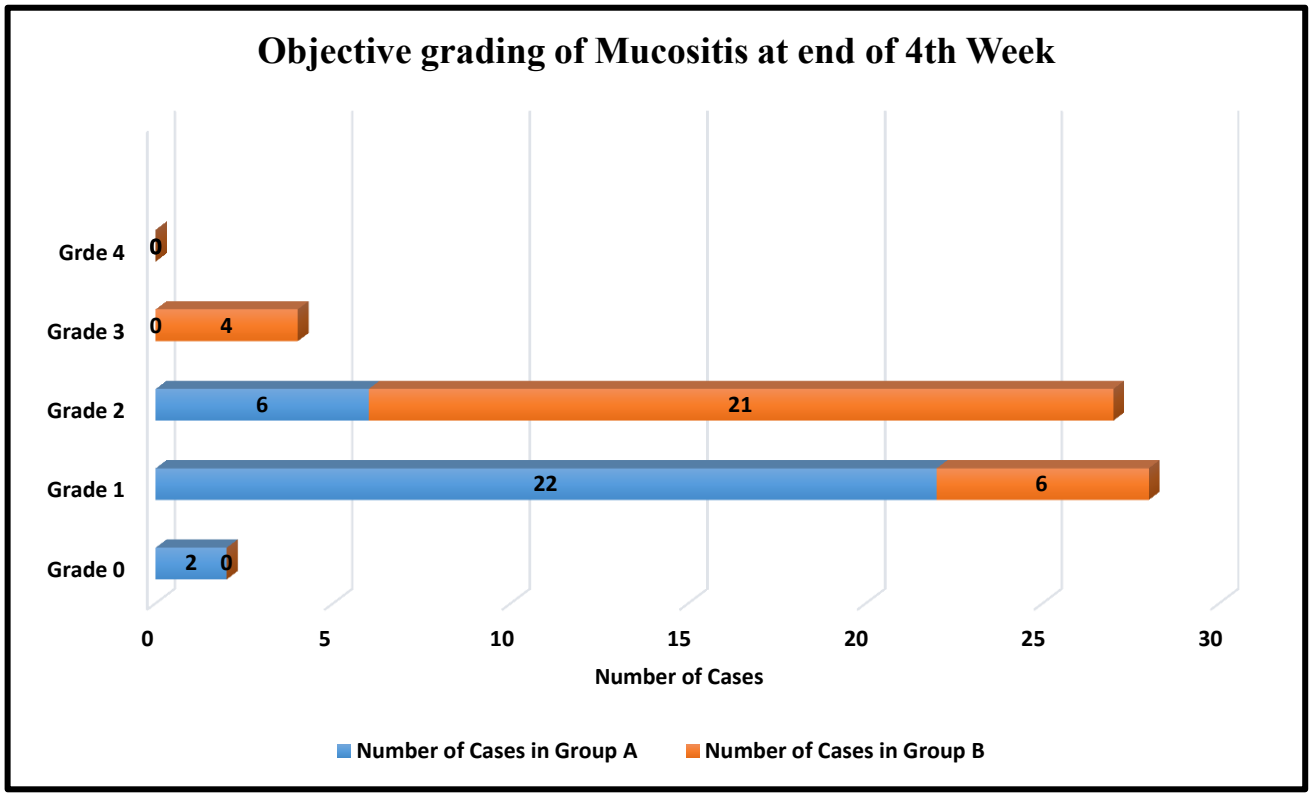


Fig 29 Objective grading of mucositis at the end of 4th week. At the end of 4th week of treatment, 4 patients in group B progressed to grade III mucositis and nearly 68% (21) patients had grade II mucositis. In comparison, none of the patients had grade III mucositis in group A and 73.3% (n=22) had grade II mucositis. The difference was found to be statistically significant ($p<0.001$).

Table - 26

Subjective grading of Mucositis at end of 4th Week

Subjective grading of Mucositis at end of 4th Week	Group A(n-30)		Group B(n-31)	
	Number of Cases	%	Number of Cases	%
Grade 1	1	3.3	0	0
Grade 2	23	76.7	6	19.3
Grade 3	6	20	21	67.7
Grade 4	0	0	4	12.9
Grade 5	0	0	0	0

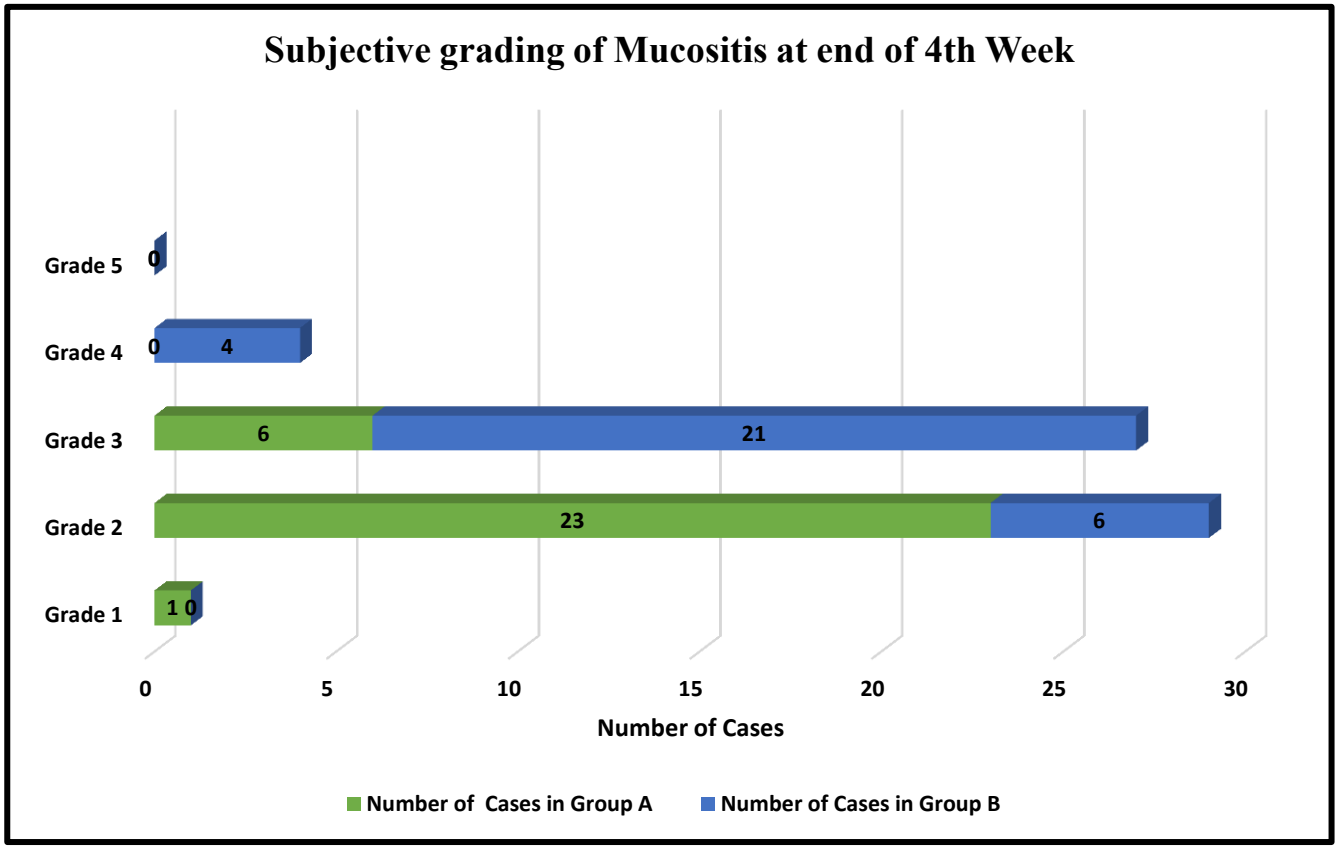


Fig 30 Subjective grading of mucositis at the end of 4th week. At the end of 4th week of treatment, in group B, 67.7% (n=22) patients developed severe pain interfering with oral intake (grade III mucositis). Two patients in group B progressed to grade 4 mucositis, requiring intervention, where as 83% of the patients (n=22) were having moderate symptoms (grade II mucositis). The difference was found to be statistically significant ($p<0.001$).

Table - 27

Objective grading of Mucositis at 2 months follow up

Objective grading of Mucositis after 2 months of treatment	Group A (n-30)		Group B(n-31)	
	Number of Cases	%	Number of Cases	%
Grade 0	2	6.7	0	0
Grade 1	26	86.6	6	19.3
Grade 2	2	6.7	24	77.5
Grade 3	0	0	1	3.2
Grade 4	0	0	0	0

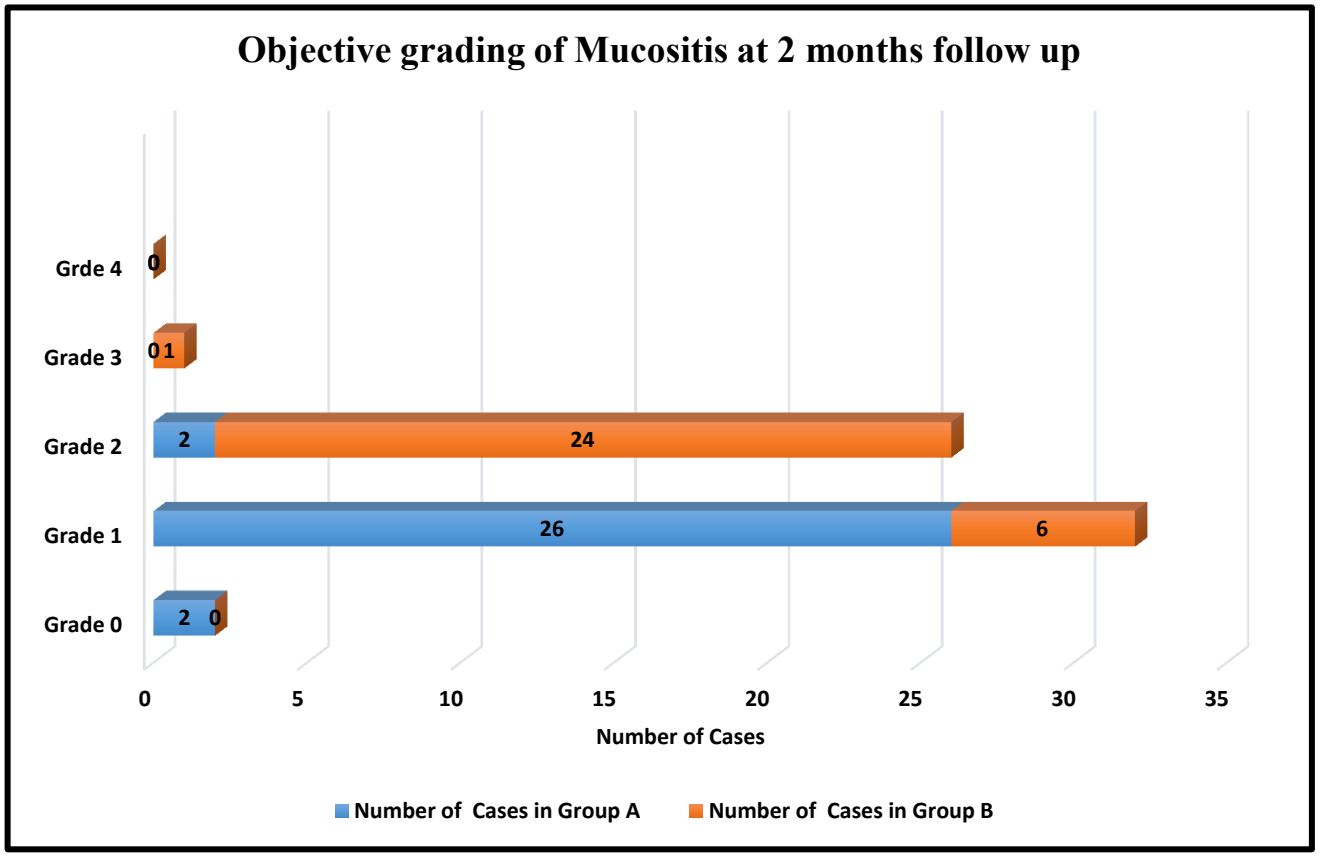


Fig 31 Objective grading of mucositis at 2 months follow up. At follow up after 2 months of treatment, nearly 78% (n=24) patients had persistent mucositis in group B, whereas only 2 patients had grade II mucositis in group A. In group A most of the patients 86.6% (n=26) were having only grade I mucositis. The difference was found to be statistically significant ($p<0.001$)

Table - 28

Subjective grading of Mucositis at 2 months follow up

Subjective grading of Mucositis at 2 months follow up	Group A(n-30)		Group B (n-31)	
	Number of Cases	%	Number of Cases	%
Grade 1	1	3.3	0	0
Grade 2	27	90	6	19.3
Grade 3	2	6.7	24	77.5
Grade 4	0	0	1	3.2
Grade 5	0	0	0	0

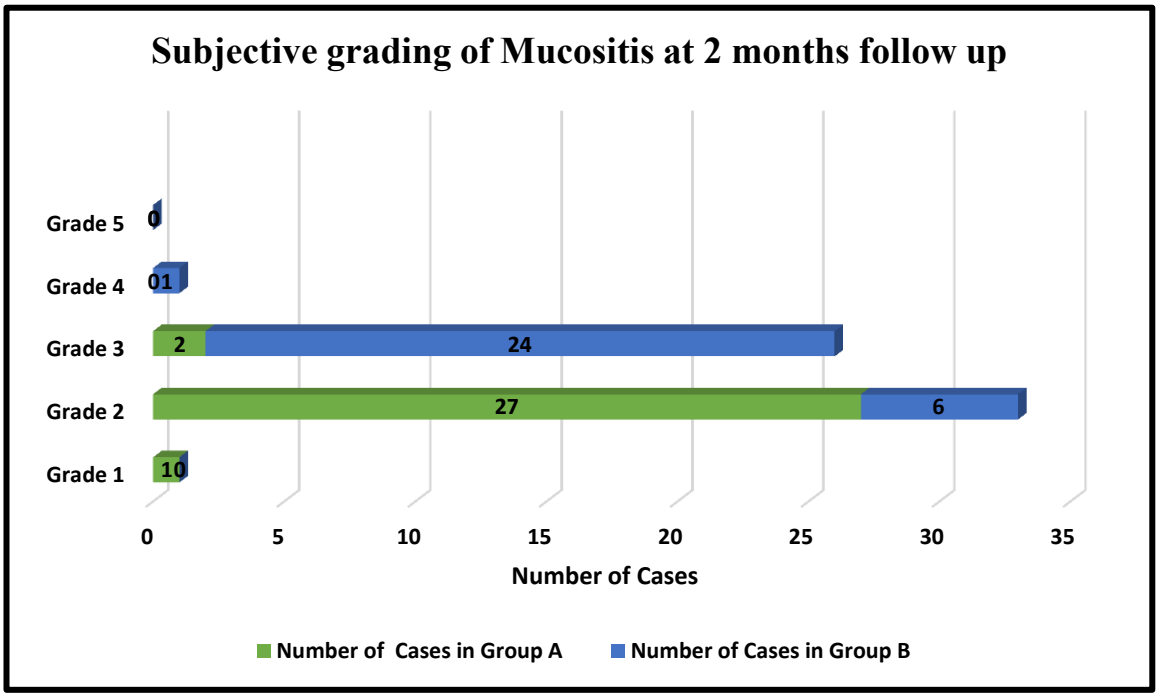


Fig 32 Subjective grading of mucositis at 2 months follow up. At follow up after 2 months of treatment, in group B, 77.5% (n=24) patients were having severe pain interfering with oral intake (grade III mucositis). One patient in group B was still having grade 4 mucositis, whereas 90% of the patients (n=27) were having moderate symptoms (grade II mucositis) and 2 patients were having grade III mucositis. The difference was found to be statistically significant ($p<0.001$).

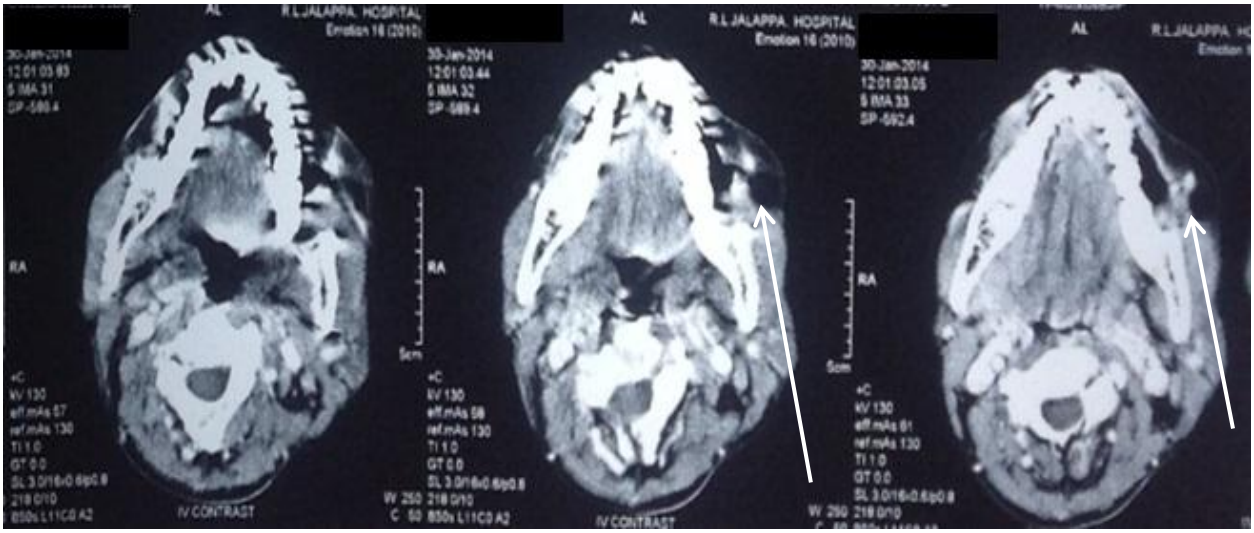


Photo 3 Baseline CT scan of patient with carcinoma buccal mucosa before initiation of treatment, arrow pointing towards tumor site.

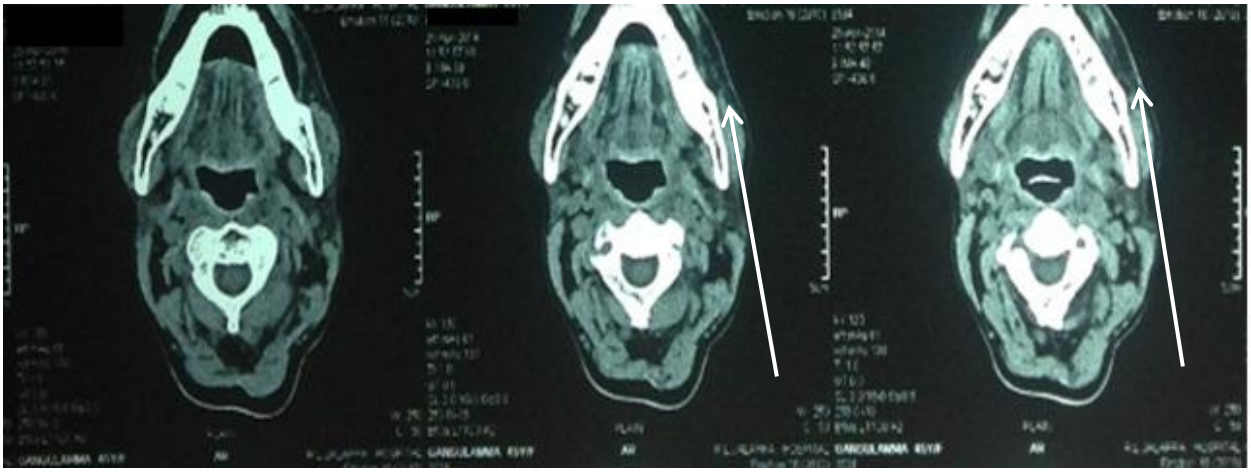


Photo 4 Post treatment CT scan showing reduction in tumor size, arrow pointing towards tumor site.

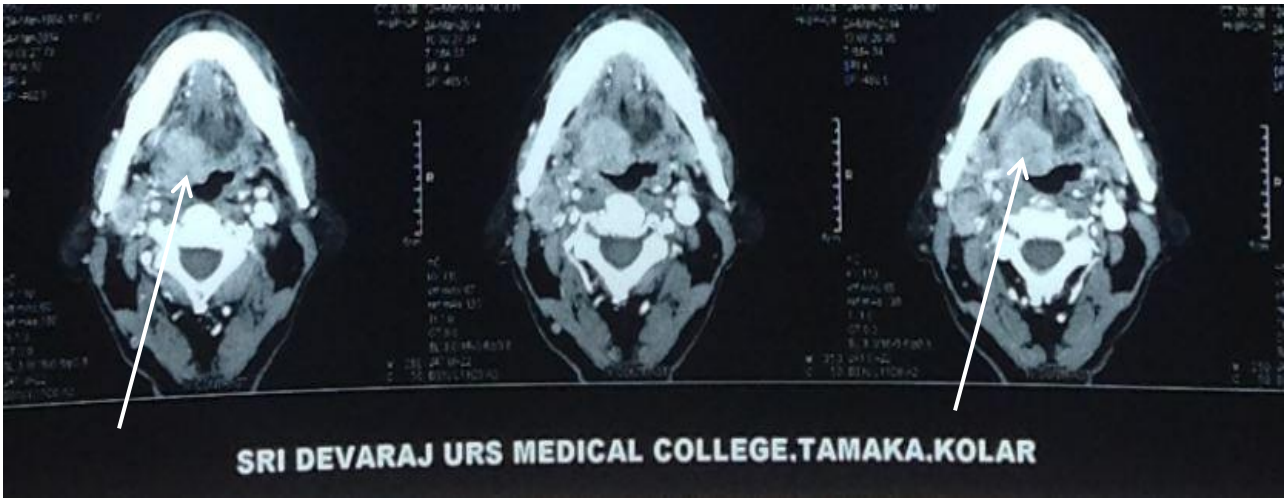


Photo 5 Baseline CT scan of patient with carcinoma oropharynx before initiation of treatment, arrow pointing towards tumor site.

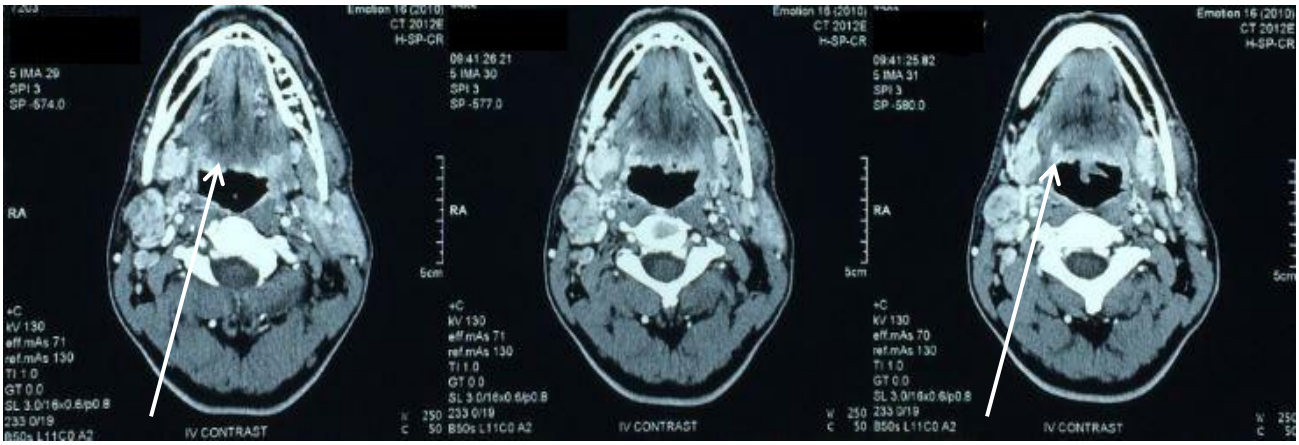


Photo 6 Post treatment CT scan showing reduction in tumor size, arrow pointing towards tumor site.

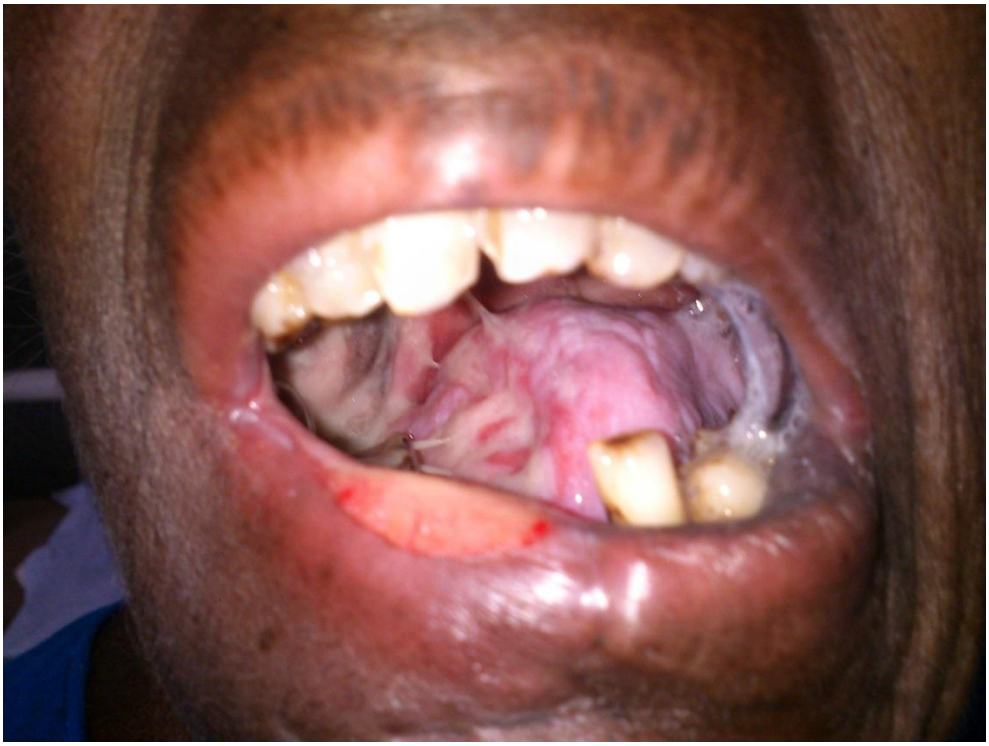


Photo 7 Post op patient of Carcinoma Buccal Mucosa with grade 3 mucositis in control group at the end of 3rd week

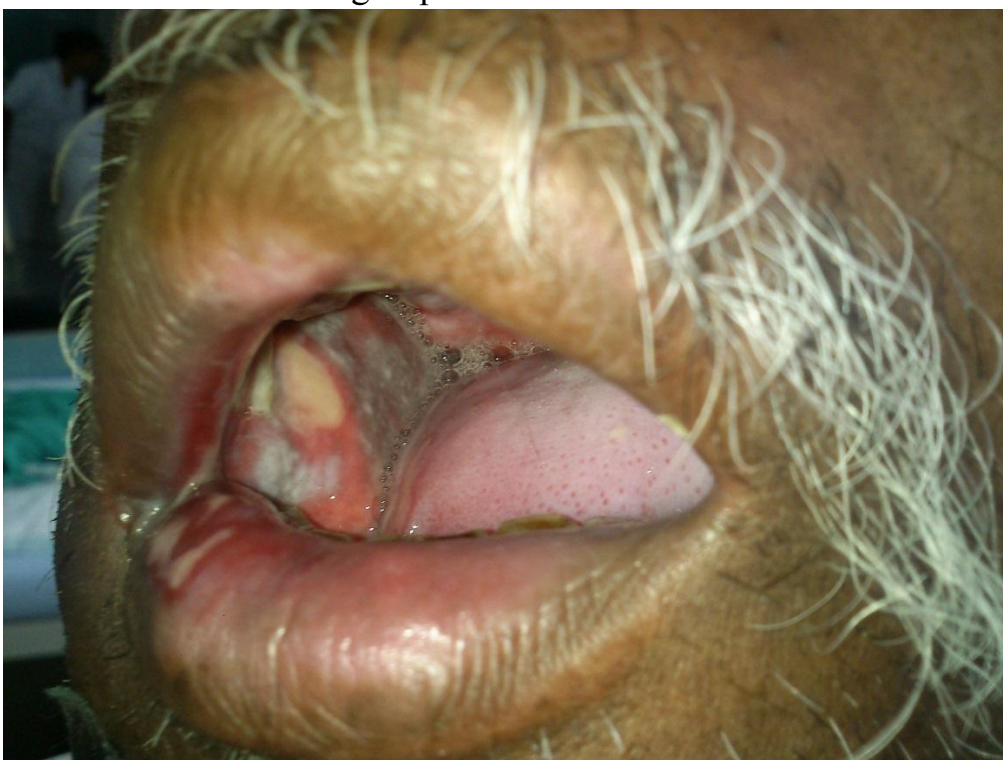


Photo 8 Post op patient of Carcinoma Buccal Mucosa with grade 3 mucositis in control group at the end of 3rd week

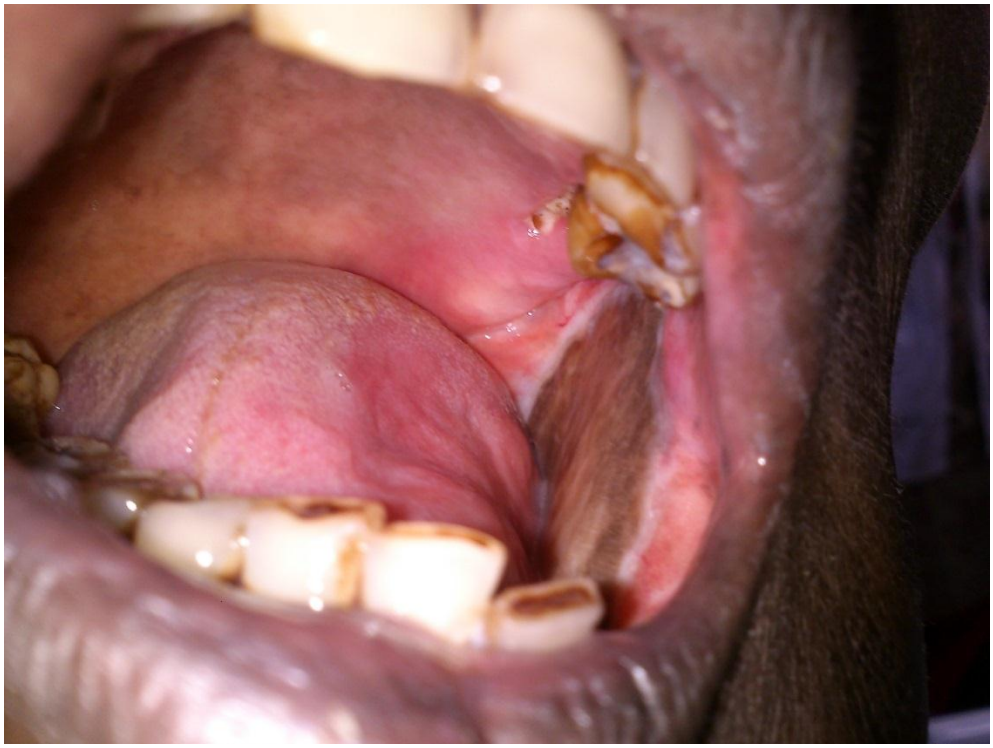


Photo 9 Post op patient of Carcinoma Buccal mucosa, with grade 1 mucositis in test group at the end of 3rd week

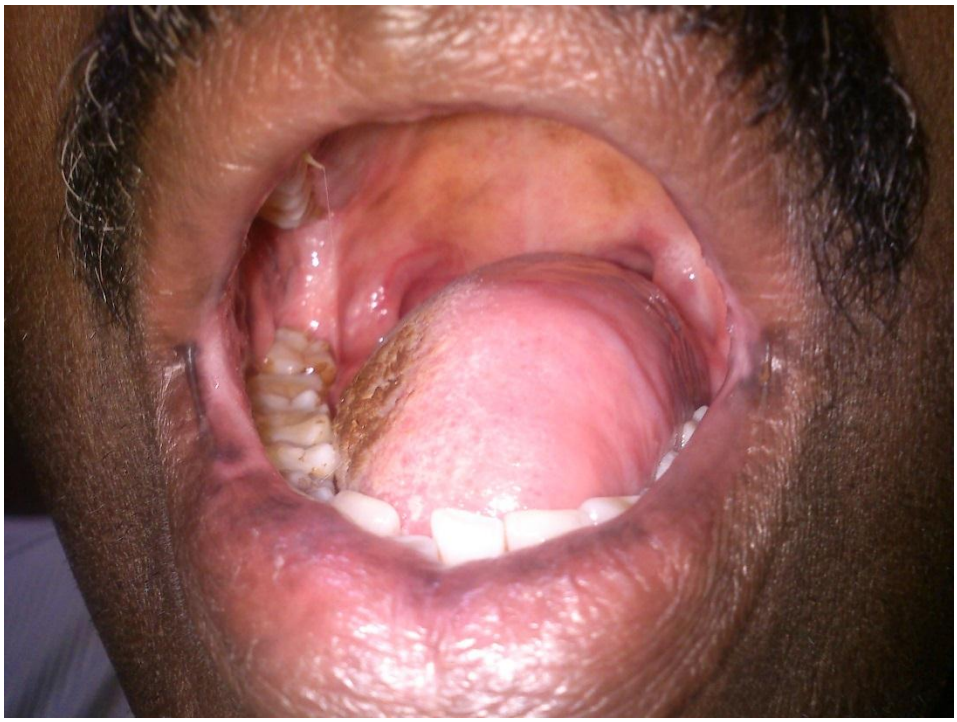


Photo 10 Post op patient of Carcinoma tongue with grade 1 mucositis in test group at the end of 3rd week

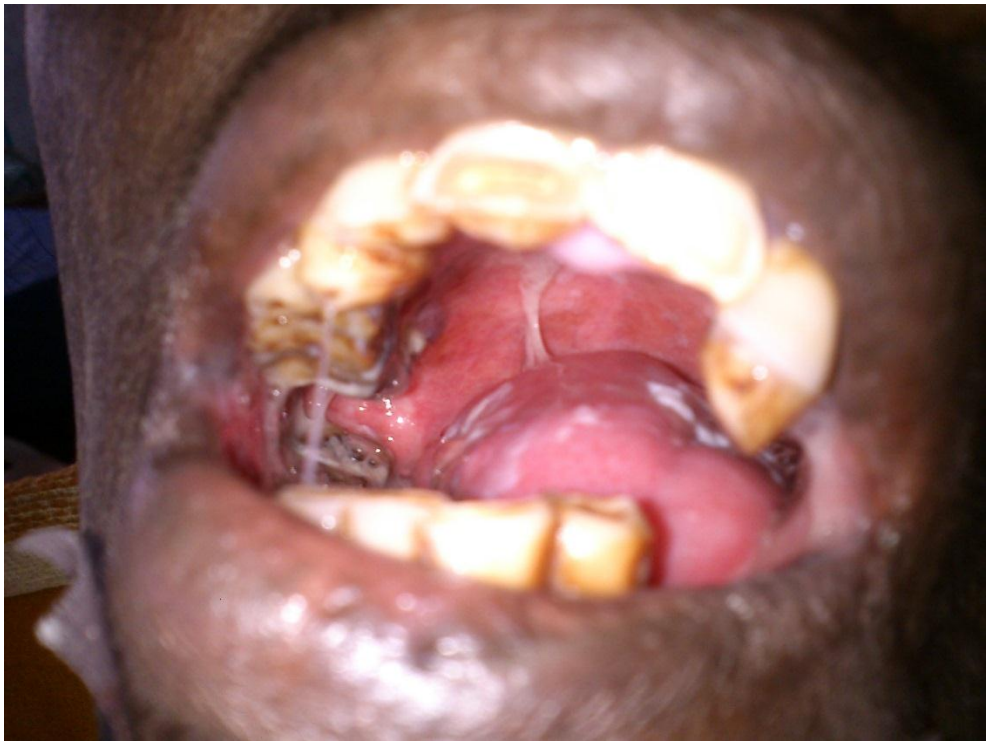


Photo 11 Patient with Carcinoma Retromolar trigone having grade 1 mucositis in test group at the end of 4th week of chemoradiation.

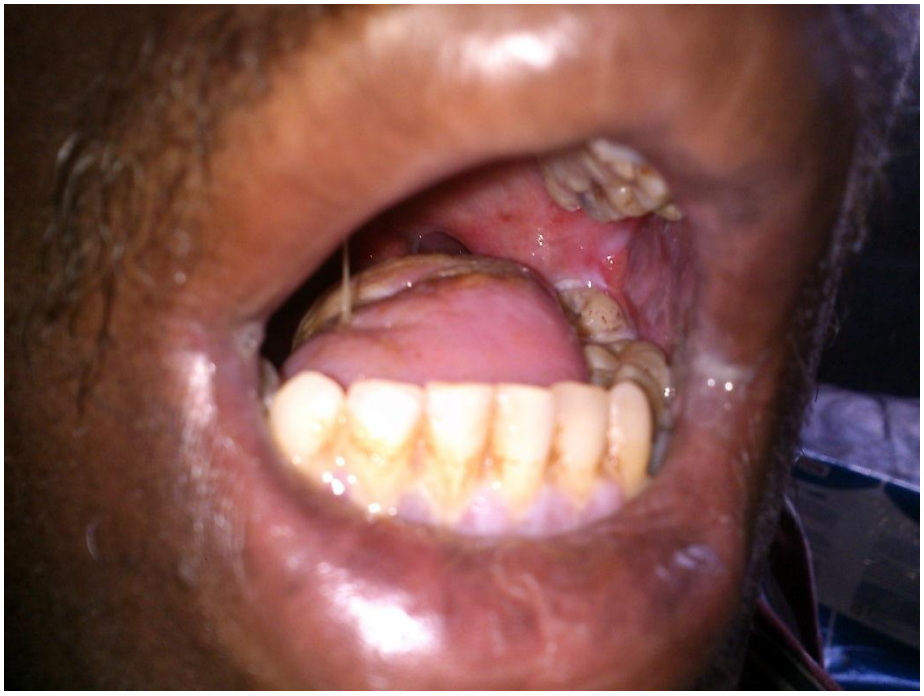


Photo 12 Patient with carcinoma tongue having grade 1 mucositis in test group at the end of 4th week of chemoradiation.

DISCUSSION

Multimodality of treatment using combination of surgery, radiation, chemotherapy has become the preferred treatment for HNSCC, more so in the advanced tumors. Chemotherapeutic agents are commonly used as an adjuvant along with radiation. They play a key role of radiosensitiser. The most commonly used radiosensitisers are Cisplatin, 5 –fluorouracil, Paclitaxel and Gemcitabine. Cytotoxic action of the radiosensitiser are usually associated with damage to normal cells with varying consequences, which can be acute or delayed. Randomised trials RTOG 9501 and EORTC 22931 have shown satisfactory evidence of improvement in terms of LRC and DFS in patients undergoing chemotherapy along with EBRT, but grade 3 toxicity was reported in 77% and 44% of patients.[81] Hence there is a continued search for potential alternatives with less toxicity profile, one such agent of interest is curcumin.

Studies have shown that multiple molecular pathways such as NF-kB activation, STAT 3 expression, MAP kinase cascade and VEGF mediated angiogenesis are dysregulated in HNSCC and are potential targets of therapeutic intervention. In vitro and in vivo studies have shown curcumin to have a diversified inhibitory effect on the various molecular pathways of tumorogenesis.[70-75] In vitro studies on various head and neck cancer cell lines such as CCL23 (laryngeal), CAL27, UM-SCC14A and UMSCC1 (oral), treated with curcumin have demonstrated inhibitory effect on molecular pathways involved in cell proliferation. The inhibitory action of curcumin was shown to be mediated via inhibition of NF-kB and STAT3 signaling protein.[70] In SAS oral cancer cell lines, curcumin has shown to up-regulate insulin like growth factor and C/EBPα protein, which are potent suppressors of head and neck cancers. This inhibitory effect of curcumin was mediated via activation of p38.[70]

Several phase I and phase II trials are underway in several countries, studying the role of curcumin as an adjuvant in treatment of premalignant conditions of GIT and oral cavity and also in advanced malignancies of pancreas and colon.[82]

Curcumin as a radiosensitiser has been studied only in human cancer cell lines and in animal models. Curcumin significantly enhanced the effect of gamma radiation in xenograft nude mice models with colon cancer by suppressing NF-kB activity. In prostate cancer cell line PC-3, curcumin showed anti-cancer and radiosensitising effect by down regulating MDM2 levels, and also by inhibition of TNF-α mediated NF-kB activity.[68]

Curcumin has had promising result as a radiosensitiser in both in vitro and in vivo studies on head and neck cancer cells. In vitro studies on HNSCC cell lines such as SCC1, SCC-9, A431 and KB, which were treated with curcumin, radiation and combination of both have shown that curcumin along with radiation had an independent and addictive effect and inhibited cell viability in all cell lines. In the same study, orthotopic mouse models implanted with SCC-1 cells, treated with curcumin and RT, also showed significant reduction in tumor weight and size.[68] The mechanism of radiosensitisation by curcumin in this study was attributed to the inhibitory action on COX-2 pathway and also on phosphorylation of EGFR. Studies have shown COX-2 and EGFR upregulation in most head and neck cancers. Curcumin as a combined inhibitor of COX-2 and EGFR has a potential role in the treatment of these cancers. The down regulation of COX-2 expression has also shown to enhance chemoradiotherapy response while sparing the normal tissues.[70]

Curcumin has been shown to cause alteration in the mitotic spindle structures and arrest cells in G2/M and S phase of cell cycle, which is the most radiosensitive phase of cell cycle. This mechanism is very similar to the action of taxols, which are potent radiosensitisers. In a phase I

trial on 14 patients of advanced/metastatic breast cancer, combination of docetaxel and curcumin have shown to arrest progression of cancer. Out of the 14 patients enrolled in the study, 5 patients had PR, and 3 patients had SD and none of the patients had progressive disease.[83]

Nearly 6-7 phase I clinical trials have tested the safety profile of curcumin in treatment of various cancers and found no dose-limiting toxicity. Our study was a pilot study, to study the radiosensitisation potential of curcumin. At the end of treatment, none of the patients in both the groups had progressive disease. Stage match could be achieved only with patients having stage IV head and neck squamous cell cancers. The partial response (PR) in study group was 54.5% compared to 37.5% in control group. The overall tumor response (Stage III +Stage IV), the study group had PR of 58.3% and SD of 41.7%, while the control group had 33.3% PR, and 66.6% SD. The difference in the groups was not statistically significant due to lack of adequate number of cases. The diverse inhibitory effect on various pathways of carcinogenesis, lack of systemic toxicity and synergestic effect with radiation makes curcumin an ideal adjuvant in the treatment of head and neck squamous cell cancers. Further studies are required with larger sample size to understand the radiosensitising effect of curcumin.

Studies have shown curcumin to be highly pleotropic. It is known to interact on various molecular levels of inflammation. Curcumin modulates its anti-inflammatory action by down-regulating the activity of COX-2, lipoxygenase, inducible nitric oxide synthase (iNOS) enzymes by inhibition of NF-kB transcription factor and production of inflammatory cytokines like TNF-α, IL-1,-2,-6,-8 and -12, MCP, MIP by activating transcription factors like AP-1(activating protein-1).[70]

Anti-inflammatory action of curcumin has been studied in various animal model studies. Curcumin had anti-inflammatory action similar to cortisone in reducing carrageenan induced

paw edema in mice and rats. Curcumin has been reported to reduce the inflammation and symptomatic improvement in mice with experimentally induced colitis. Intraperitoneal injection of curcumin extract have shown to significantly inhibit joint inflammation in animal models.[82]

Several clinical studies have shown curcumin to have beneficial anti-inflammatory properties. Curcumin in doses of 1200mg/day in patients with rheumatoid arthritis, has shown to benefit patients in terms of decreasing joint swelling and morning stiffness.[76] In a comparative study of 45 patients, post-surgical spermatic cord edema was significantly reduced by 84.2% in patients using curcumin, its effect was found to be similar to that of phenylbutazone.[82] In a cross over Randomised controlled Trial in osteoarthritis patients, curcumin demonstrated a significant improvement in pain severity and disability scores.[84] Inflammatory orbital conditions like anterior uveitis and idiopathic orbital inflammatory pseudotumors have shown to improve with the use of curcumin.

In clinical trials on patients suffering from lichen planus, high doses of curcumin used as oral rinses was effective in reducing the severity of mucositis. [85] Curcumin has both anti-inflammatory and anti-bacterial activity. In vitro oral mucositis model using human pharyngeal cell line Detroit 562 exposed to bacterial stimuli and treated with curcumin demonstrated reduction in bacterial adherence and reduction of pro-inflammatory cytokine release in the cell lines. Bacterial adherence and cytokine adhesion are the key initial steps in pathogenesis of mucositis. This inhibitory action may have therapeutic benefit in treatment of oral mucositis.[86]

Animal studies have also shown curcumin to reduce radiation induced mucositis. Topical application of curcumin reduced the severity of mucositis in rats exposed to local radiation to tongue.[70]

Curcumin mouth rinses have shown to benefit patients with radiation induced mucositis. In a single blind, randomized comparative clinical study conducted on HNSCC patients treated by radiation or chemoradiation. Curcumin was compared with povidone-iodine mouth washes in 80 patients. Group using curcumin had delayed onset and less severe mucositis compared to povidone-iodine groups, which was statistically significant with $p<0.001$. Fourteen out of 39 patients developed high grade mucositis in curcumin group whereas 34 of 40 patients in povidone iodine group developed high grade mucositis.[85]

In our study there was no statistical difference in the severity of oral mucositis in both groups till the end of 2nd week. But from 3rd week the severity of mucositis in group A was significantly lower compared to group B. A the end of 3 weeks, nearly 86% of patients in group A had grade I mucositis in comparison to 71% developing grade II mucositis in group B, which was statistically significant with p value $p<0.001$. At the end of 4 weeks, majority of patients in group A had only grade I mucositis 73.3% in comparison to 67.7% patients of group B developing grade II mucositis and 12.9% progressing to grade III mucositis. The difference was found to be statistically significant ($p<0.001$). During follow up at 2 months after completion of treatment, nearly 87% patients in group A had only mild mucositis, while nearly 78% patients had grade II mucositis in group B and one patient had grade III mucositis.

Mucositis is a complex process. At the cellular level, radiation causes damage to DNA, generation of free radicals, release of cytokines and activation of NFkB. Various invitro and in vivo studies have shown that curcumin inhibits activity of NFkB, decreases the release of inflammatory cytokines and scavenges free radicals. This could reflect the protective action of curcumin in reducing the oral mucositis.

The lack of systemic toxicity and diverse inhibitory effect of curcumin in various pathways of inflammation makes it an ideal agent in treatment of radiation induced mucositis. As highlighted in our study the curcumin has a significant benefit in reducing the severity of mucositis which can benefit patients undergoing radiation/ chemoradiation.

The limitation of our study was lack of number of stage matched patients for assessing the radiosensitisation property of curcumin.

CONCLUSION

- There was a marginal decrease in tumor dimensions and volume in patients receiving curcumin along with chemoradiation a statistical significance could not be achieved due to inadequate number of cases and lack of stage-match controls.
- In patients who received curcumin along with chemoradiation, partial response was seen in 58.3% patients and stable disease in 41.7% patients. A statistical significance could not be attained due to inadequate stage-match controls.
- Oral administration of curcumin (1.5g/day) reduced the severity of mucositis compared to patients in control group who underwent same modality of treatment. Its role in reducing the severity of mucositis can benefit patients undergoing radiation/ chemoradiation.
- Apart from gastritis, there were no major side effects noticed in patients taking curcumin.
- Further studies are required to validate the role of curcumin as an adjuvant (radiosensitiser) in the treatment of head and neck squamous cell carcinomas.

SUMMARY

Our study was a single blinded, randomized, clinical study done from December 2012 to June 2014 in Department of Otorhinolaryngology in R. L. Jalappa Hospital and Research Centre, Kolar to find out the radiosensitisation potential of Curcumin in patients receiving radiotherapy or chemoradiotherapy for squamous cell carcinoma of head and neck and to assess the efficacy of curcumin in reducing radiation induced mucositis in patients undergoing radiotherapy or chemoradiotherapy.

Patients were randomized and divided into Group A (Test group) and Control group (Group B). A total of 64 patients was included in the study, 3 patients were dropped from the study as they defaulted the treatment. Thirty patients were included in Group A and they received curcumin (500mg thrice a day) along with radiation. Thirty one patient were included in group B and they received placebo along with radiation.

Among the 61 patients only 21 patients were assessed for the radiosensitisation potential of curcumin. There were a total of 12 patients in group A and 9 patients in group B. Stage-wise matching was done, 11 patients in group A and 8 patients in group B were having Stage IV cancer and remaining one patient in each group were having stage III cancer. The tumor size was documented clinically, a baseline CECT scan was taken and follow up CECT scan was taken at 3 months post treatment. The tumor response was assessed using RECIST criteria. It was found that overall 58.3% (n=7) patients had partial response and 41.7% (n=5) patients had stable disease in group A. In group B 33.3% (n=3) patients had a partial response and 66.6% (n=6) patient had a stable disease. None of the patients in either group had progressive disease. A statistical significance could not be attained due to paucity of cases.

All 61 patients included in the study were assessed to find out the effect of curcumin in treatment of mucositis. Thirty patients were present in group A and 31 patients in group B. It was found that in first 2 weeks of radiotherapy, there were no significant difference in mucositis between group A and B. The objective and subjective assessment showed overall similar results. From 3rd week onwards, it was found that, the patients in group A had a less severe mucositis compared to group B, with majority of patients (73.3%) in group A having grade I mucositis at end of 4th week, while 67.7% of group B patients were having grade II mucositis and 12.9% having grade III mucositis. The difference noted between the groups were statistically significant with p value <0.001.

None of the patients included in the study developed any serious adverse effects, few of the patients had mild gastritis which subsided on conservative management.

BIBLIOGRAPHY

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr, accessed on 14/01/2014.

2. Kalyani R, Das S, Bindra Singh MS, Kumar H. Cancer profile in Kolar: A ten years study. Indian J Cancer 2010; 47:160-5.

3. Head and Neck Cancers in Developing Countries; Joshi P, Dutta S, Chaturvedi P, Nair S Rambam Maimonides Med J. 2014; 5(2):1-5.

4. Hecht SS. Tobacco. *In*: DeVita VT, Lawrence TS, Rosenberg SA. Cancer principles and practice of oncology. 9th Ed. Philadelphia: Wolters Klawer Health; 2011. p150-57.

5. Mashberg A, Boffetta P, Winkelman R, Garfinkel L. Tobacco smoking, alcohol drinking, and cancer of the oral cavity and oropharynx among U.S. veterans. Cancer. 1993; 72(4):1369-75.

6. Davidson BJ, Epidemiology and Etiology. *In*: Shah JP. Cancer of the Head and Neck.1st Ed. London: BC Decker; 2001.p5-8.

7. Bhisey R A. Chemistry and toxicology of smokeless tobacco. Indian J Cancer. 2012; 49(4):364-72.

8. Flanders WD, Rothman KJ. Interaction of alcohol and tobacco in laryngeal cancer. Am J Epidemiol.1982; 115(3):371-9.

9. Cruz IB, Snijders PJ, Steenbergen RD, Meijer CJ, Snow GB, Walboomers JM et al. Age-dependence of human papillomavirus DNA presence in oral squamous cell carcinomas. Eur J Cancer B Oral Oncol. 1996; 32:55–62.

10. Sethi S, Ali-Fehmi R, Franceschi S, Struijk L, Van Doorn LJ, Quint W et al. Characteristics and survival of head and neck cancer by HPV status: a cancer registry-based study. Int J Cancer. 2012; 131(5):1179-86.

11. Paterson IC, Eveson JW, Prime SS. Molecular changes in oral cancer may reflect aetiology and ethnic origin. Eur J Cancer B Oral Oncol. 1996; 32:150–3.

12. Bockmuhl U, Schluns K, Schmidt S, Matthias S, Petersen I. Chromosomal alterations during metastasis formation of head and neck squamous cell carcinoma. Genes Chromosomes Cancer. 2002; 33:29–35.

13. Gasco M, Crook T. The p53 network in head and neck cancer. Oral Oncol. 2003; 39: 222–31.

14. Bussink J, van der Kogel AJ, Kaanders JH. Activation of the PI3-K/AKT pathway and implications for radioresistance mechanisms in head and neck cancer. Lancet Oncol. 2008; 9: 288–96.

15. Kalyankrishna S, Grandis JR. Epidermal growth factor receptor biology in head and neck cancer. J Clin Oncol. 2006; 24:2666–72.

16. Minhas KM, Singh B, Jiang WW, Sidransky D, Califano JA. Spindle assembly checkpoint defects and chromosomal instability in head and neck squamous cell carcinoma. Int J Cancer. 2003; 107:46–52.
17. Sturgis EM, Wei Q. Genetic susceptibility – molecular epidemiology of head and neck cancer. Curr Opin Oncol. 2002; 14:310–17.
18. So S, Davis AJ, Chen DJ. Autophosphorylation at serine 1981 stabilizes ATM at DNA damage sites. J Cell Biol. 2009; 187(7):977-90.
19. Elkind MM, Sutton-Gilbert H, Moses WB, Alescio T, Swain RW. Radiation response of mammalian cells grown in culture. V. Temperature dependence of the repair of x-ray damage in surviving cells (aerobic and hypoxic). Radiat Res. 1965; 25:359.
20. Fu KK, Phillips TL. Biologic rationale of combined radiotherapy and chemotherapy. Hematol Oncol Clin N Am. 1991; 5:737-751.
21. Blanchard P, Baujat B, Holostenco V, Bourredjem A, Baey C, Bourhis J, Pignon JP; MACH-CH Collaborative group. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): a comprehensive analysis by tumour site. Radiother Oncol. 2011; 100(1):33-40.
22. Steel GG, Peckham MJ. Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. Int J Radiat Oncol Biol Phys. 1979; 5(1):85-91.
23. Hennequin C, Favaudon V. Biological basis for chemo-radiotherapy interactions. Eur J Cancer. 2002; 38:223-230.
24. Gupta T, Agarwal JP, Ghosh-Laskar S, Parikh PM, D'Cruz AK, Dinshaw KA. Radical radiotherapy with concurrent weekly cisplatin in loco-regionally advanced squamous

cell carcinoma of the head and neck: a single-institution experience. Head Neck Oncol. 2009; 1:17.

25. Rampino M, Ricardi U, Munoz F, Reali A, Barone C, Musu AR et al. Concomitant adjuvant chemoradiotherapy with weekly low-dose cisplatin for high-risk squamous cell carcinoma of the head and neck: a phase II prospective trial. Clin Oncol. 2011; 23(2):134-40.
26. Forastiere AA, Metch B, Schuller DE, Ensley JF, Hutchins LF, Triozzi P et al. Randomized comparison of cisplatin plus fluorouracil and carboplatin plus fluorouracil versus methotrexate in advanced squamous-cell carcinoma of the head and neck: a Southwest Oncology Group study. J Clin Oncol. 1992; 10:1245–1251.
27. Jacobs C, Lyman G, Velez-Garcia E, Sridhar KS, Knight W, Hochster H et al. A phase III randomized study comparing cisplatin and fluorouracil as single agents and in combination for advanced squamous cell carcinoma of the head and neck. J Clin Oncol 1992; 10:257–263.
28. Argiris A, Karamouzis MV, Johnson JT, Heron DE, Myers E, Eibling D et al. Long-term results of a phase III randomized trial of postoperative radiotherapy with or without carboplatin in patients with high-risk head and neck cancer. Laryngoscope. 2008; 118(3):444-9.
29. Browman GP, Cripps C, Hodson DI, Eapen L, Sathya J, Levine MN. Placebo-controlled randomized trial of infusional fluorouracil during standard radiotherapy in locally advanced head and neck cancer. J Clin Oncol. 1994; 12(12):2648-53.
30. Schiff PB, Fant J, Horwitz SB. Promotion of microtubule assembly in vitro by Taxol. Nature. 1979; 277(5698):665-7.

31. Lee JJ, Swain SM. Peripheral neuropathy induced by microtubule-stabilizing agents. J Clin Oncol. 2006; 24(10):1633-42.
32. Garden AS, Harris J, Vokes EE, Forastiere AA, Ridge JA, Jones C et al. Preliminary results of radiation therapy oncology group 97- 03: a randomized phase II trial of concurrent radiation and chemotherapy for advanced squamous cell carcinomas of the head and neck. J Clin Oncol 2004; 22(14):2856–64.
33. Popovtzer A, Normolle D, Worden FP, Prince ME, Chepeha DB, Wolf GT et al. A Phase I trial of radiotherapy concurrent with twice-weekly gemcitabine for head and neck cancer: translation from preclinical investigations aiming to improve the therapeutic ratio. Transl Oncol. 2014; 7(4):479-83.
34. Eisbruch A, Shewach DS, Bradford CR, Littles JF, Teknos TN, Chepeha DB et al. Radiation concurrent with gemcitabine for locally advanced head and neck cancer: a phase I trial and intracellular drug incorporation study. J Clin Oncol. 2001;19(3):792-9.
35. Gerber DE, Chan TA. Recent Advances in Radiation Therapy. Am Fam Physician. 2008; 78(11): 1254-1262.
36. Lichter SA. Radiation therapy. *In*: Abeloff MD, Armtage JO, Lichter AS, Niederherber JE. Clinical oncology, 2nd ed. New York: Churchill Livingstone;2000. p423-70.
37. Trotti A, Bellm LA, Epstein JB, Frame D, Fuchs HJ, Gwede CK et al. Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: a systematic literature review. Radiother Oncol. 2003; 66(3):253-62.

38. Dodd MJ, Miaskowski C, Shiba GH, Dibble SL, Greenspan D, MacPhail L, Paul SM et al. Risk factors for CT-induced oral: dental appliances, oral hygiene, previous oral lesion, and a history of smoking. Cancer Invest. 1999; 17(4):278-84.

39. Sonis ST, Elting LS, Keefe D, Peterson DE, Schubert M, Hauer-Jensen M.Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients.Cancer.2004; 100:1995-2025.

40. McGuire DB, Fulton JS, Park J, Brown CG, Correa ME, Eilers J et al; Systematic review of basic oral care for the management of oral mucositis in cancer patients. Mucositis Study Group of the Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO). Support Care Cancer. 2013; 21(11):3165-77.

41. Osaki T, Ueta E, Yoneda K, Hirota J, Yamamoto T. Prophylaxis of oral mucositis associated with chemoradiotherapy for oral carcinoma by Azelastine hydrochloride (azelastine) with other antioxidants. Head Neck 1994; 16(4):331-9.

42. Watanabe T, Ishihara M, Matsuura K. Polaprezinc prevents oral mucositis associated with radiochemotherapy in patients with head and neck cancer. Int J Cancer 2010; 127(8):1984.

43. Kouloulias V, Kouvaris JR, Kokakis JD, Kostakopoulos A, Mallas E, Metafa A et al. Impact on cytoprotective efficacy of intermediate interval between amifostine administration and radiotherapy: a retrospective analysis. Int J Radiat Oncol Biol Phys 2004; 59:1148.

44. Sasse AD, Clark LG, Sasse EC. Amifostine reduces side effects and improves complete response rate during radiotherapy: results of a meta-analysis. Int J Radiat Oncol Biol Phys. 2006; 64(3):784-91.

45. Huang EY, Leung SW, Wang CJ, Chen HC, Sun LM, Fang FM, Yeh SA, Hsu HC, Hsiung CY. Oral glutamine to alleviate radiation-induced oral mucositis: a pilot randomized trial. Int J Radiat Oncol Biol Phys. 2001; 46(3):535-9.

46. Porteder H, Rausch E, Kment G, Watzek G, Matejka M, Sinzinger H. Local prostaglandin E2 in patients with oral malignancies undergoing chemo and radiotherapy. J Craniomaxillofac Surg. 1988; 16(8):371-4.

47. Epstein JB, Stevenson-Moore P, Jackson S, et al. Prevention of oral mucositis in RT: a controlled study with benzydamine hydrochloride rinse. Int J Radiat Oncol Biol Phys. 1989; 16:1571-5.

48. Leborgne J H , Leborgne F, Zubizarreta E, Ortega B, Mezzera J .Corticosteroids and radiation mucositis in head and neck cancer: a double-blind placebo-controlled randomized trial. Radiother Oncol. 1998; 47(2):145-8.

49. Hong JP, Lee SW, Song SY, Ahn SD, Shin SS, Choi EK, Kim JH. Recombinant human epidermal growth factor treatment of radiation-induced severe oral mucositis in patients with head and neck malignancies. Eur J Cancer Care. 2009; 18(6):636-41.

50. Girdler NM, Mcgurk M, Aqual S, Prince M. The effect of epidermal growth factor mouthwash on cytotoxic-induced oral ulceration: a phase I clinical trial. Am J Clin Oncol. 1995; 18(5):403-6.

51. Wu HG, Song SY, Kim YS, Oh YT, Lee CG, Keum KC, Ahn YC, Lee SW. Therapeutic effect of recombinant human epidermal growth factor (RhEGF) on

mucositis in patients undergoing radiotherapy, with or without chemotherapy, for head and neck cancer: a double-blind, placebo-controlled prospective phase 2 multi-institutional clinical trial. Cancer. 2009; 115(16):3699-708.

52. Ryu JK, Swann S, LeVeque F, Scarantino CW, Johnson D, Chen A et al. The impact of concurrent granulocyte macrophage-colony stimulating factor on radiation-induced mucositis in head and neck cancer patients: a double-blind placebo-controlled prospective phase III study by Radiation Therapy Oncology Group 9901. Int J Radiat Oncology Biol Phys. 2007; 67(3):643-50.
53. Spielberger R, Stiff P, Bensinger W, Gentile T, Weisdorf D, Kewalramani T et al. Palifermin for oral mucositis after intensive therapy for hematologic cancers. N Engl J Med. 2004; 351(25):2590-8.
54. Brizel DM,Murphy BA, Rosenthal DI, Pandya KJ, Gluck S, Brizel HE et al. Phase II study of palifermin and concurrent chemoradiation in head and neck squamous cell carcinoma.J Clin Oncol. 2008; 26(15):2489-96.
55. Wahlin BY. Effects of chlorhexidine mouth rinse on oral health in patients with acute leukemia. Oral Surg Oral Med Oral Pathol. 1989; 68(3):279-87.
56. Sutherland SE, Browman GP. Prophylaxis of oral mucositis in irradiated head-and-neck cancer patients: a proposed classification scheme of interventions and meta-analysis of randomized controlled trials. Int J Radiat Oncol Biol Phys. 2001; 49(4):917-30.
57. Spijkervet FK, Saene HK, van Saene JJ, Panders AK, Vermey A, Mehta DM et al. Effect of selective elimination of the oral flora on mucositis in irradiated head and neck cancer patients. J Surg Oncol. 1991; 46(3):167-73.

58. Mahoud DJ, Dose AM, Loprinzi CL, Veeder MH, Athmann LM, Therneau TM et al. Inhibition of fluorouracil-induced stomatitis by oral cryotherapy. J Clin Oncol. 1991; 9(3):449-52.

59. Svanberg A, Birgegård G, Öhrn K. Oral cryotherapy reduces mucositis and opioid use after myeloablative therapy: a randomized controlled trial. Support Care Cancer. 2007; 15(10):1155-61.

60. Bensadoun RJ, Ciais G, Schubert MM, Viot M, Dejou J, Tardieu C et al. Low-energy He/Ne laser in the prevention of radiation-induced mucositis. A multicenter phase III randomized study in patients with head and neck cancer. Support Care Cancer. 1999; 7(4):244-52.

61. Genot-Klastersky MT, Klastersky J, Awada F, Awada A, Crombez P, Martinez MD et al. The use of lower-energy laser (LEL) for the prevention of chemotherapy- and/or radiotherapy-induced oral mucositis in cancer patients: results from two prospective studies. Support Care Cancer. 2008; 16(12):1381-7.

62. Cowen D, Tardieu C, Schubert M, Peterson D, Resbeut M, Faucher C et al. Low energy helium-neon laser in the prevention of oral mucositis in patients undergoing bone-marrow transplant: results of a double-blind randomized trial. Int J Radiat Oncol Biol Phys.1997; 38(4):697-703.

63. Epstein JB, Wong FLW. The efficacy of sucralfate suspension in the prevention of oral mucositis due to RT. Int J Radiat Oncol Biol Phys. 1994; 28(3):693-8.

64. Kim JG, Sohn SK, Kim DH, Baek JH, Chae YS, Bae NY et al. Effectiveness of transdermal fentanyl patch for treatment of acute pain due to oral mucositis in patients receiving stem cell transplantation. Transplant Proc. 2005; 37(10):4488-91.

65. Cerchietti LC, Navigante AH, Bonomi MR, Zaderajko MA, Menéndez PR, Pogany CE et al. Effect of topical morphine for mucositis-associated pain following concomitant chemoradiotherapy for head and neck cancer. Cancer. 2005; 37(10):4488-91.

66. Li M, Zhang Z, Hill DL, Wang H, Zhang R. Curcumin, a dietary component, has anticancer, chemosensitization, and radiosensitization effects by down-regulating the MDM2 oncogene through the PI3K/mTOR/ETS2 pathway. Cancer Res. 2007; 67(5):1988-96.

67. Lin YG, Kunnumakkara AB, Nair A, Merritt WM, Han LY, Armaiz-Pena GN et al. Curcumin inhibits tumor growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-kappaB pathway. Clin Cancer Res. 2007; 13(11):3423-30.

68. Khafif A, Lev-Ari S, Vexler A, Barnea I, Starr A, Karaush V, Haif S, Ben-Yosef R. Curcumin: a potential radio-enhancer in head and neck cancer. Laryngoscope 2009; 119(10):2019-26.

69. Antony B, Merina B, Iyer V S,Judy N, Lennertz K, Joyal S. A Pilot Cross-Over Study to Evaluate Human Oral Bioavailability of BCM-95®CG (Biocurcumax™), A Novel Bioenhanced Preparation of Curcumin Indian J Pharm Sci. 2008; 70(4): 445–449.

70. Wilken R, Veena MS, Wang MB, Srivatsan ES. Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. Mol Cancer. 2011; 10:12.

71. Aggarwal BB, Surh YJ, Shishodia S. The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease. Advances in experimental medicine and biology. 2007; (595): 159-161.

72. Sa G, Das T. Anti cancer effects of curcumin: cycle of life and death. Cell Div. 2008; 3:14.

73. Ikezaki S, Nishikawa A, Furukawa F,Kudo K, Nakamura H, Tamura K, Mori H. Chemopreventive effects of curcumin on glandular stomach carcinogenesis induced by N-methyl-N-nitro-N-nitrosoguanidine and sodium chloride in rats. Anticancer Res. 2001; 21(5):3407-11.

74. Singletary K, MacDonald C, M. Iovinelli, Fisher C, Wallig M. Effect of the betadiketones diferuloylmethane (curcumin) and dibenzoylmethane on rat mammary DNA adducts and tumors induced by 7,12- dimethylbenz[a]anthracene. Carcinogenesis 1998; 19(6):1039-43.

75. Lev-Ari S, Zinger H, Kazanov D, Yona D, Ben-Yosef R, Starr A, Figer A, Arber N. Curcumin synergistically potentiates the growth inhibitory and pro-apoptotic effects of celecoxib. Biomed Pharmacother. 2005; 59 Suppl 2:S276-80.

76. Deodhar SD, Sethi R, Srimal RC. Preliminary study on antirheumatic activity of curcumin (diferuloyl methane). Indian J Med Res.1980; 71: 632-4.

77. Kuttan R, Sudheeran PC, Josph CD. Turmeric and curcumin as topical agents in cancer therapy. Tumori. 2010; 9(1):8-14.

78. James JS. Curcumin: Clinical trial finds no antiviral effect. AIDS Treat News 1996; (242):1-2.

79. Soni KB, Kuttan R. Effect of oral curcumin administration on serum peroxides and cholesterol levels in human volunteers. Indian J Physiol Pharmacol. 1992 Oct; 36(4):273-5.

80. Meerten EL, Gelderblom H, Bloem JL.RECIST revised: implications for the radiologist. A review article on the modified RECIST guideline. Eur Radiol. 2010; 20:1456–1467.

81. Geiger JL, Lazim AF, Walsh FJ, Foote RL, Moore EJ, Okuno SH. et al. Adjuvant chemoradiation therapy with high-dose versus weekly cisplatin for resected, locally-advanced HPV/p16-positive and negative head and neck squamous cell carcinoma. Oral Oncol. 2014; 50(4):311-8.

82. Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research.Altern Med Rev. 2009; 14(2):141-53.

83. Bayet-Robert M, Kwiatkowski F, Leheurteur M, Gachon F, Planchat E, Abrial C, Mouret-Reynier MA, Durando X, Barthomeuf C, Chollet P. Phase I dose escalation trial of docetaxel plus curcumin in patients with advanced and metastatic breast cancer.Cancer Biol Ther. 2010; 9(1):8-14.

84. Kulkarni RR, Patki PS, Jog VP, et al. Treatment of osteoarthritis with a herbomineral formulation: a double-blind, placebo controlled, cross-over study. J Ethnopharmacol 1991; 33:91-95.

85. Rao S, Dinkar C, Vaishnav LK, Rao P, Rai MP, Fayad R, Baliga MS. The Indian Spice Turmeric Delays and Mitigates Radiation-Induced Oral Mucositis in Patients Undergoing Treatment for Head and Neck Cancer: An Investigational Study. Integr Cancer Ther. 2013; 13(3):201-210.

86. Lüer SC, Goette J, Troller R, Aebi C. Synthetic versus natural curcumin: bioequivalence in an in vitro oral mucositis model. BMC Complement Altern Med. 2014; 14:53.

Annexure-I

PROFORMA OF CASE SHEET

Efficacy of Curcumin as a radio-sensitiser and in minimising mucosal damage in patients receiving radiotherapy for head and neck squamous cell cancers

Name of the patient: **Age:** **Sex: M/F** **Date:**

Occupation: **Hospital no:** **Phone:**

Address:

COMPLAINTS OF	YES/NO	SINCE
Ulcer/mass in oral cavity		
Mass/swelling in neck		
Restricted mouth opening		
Burning sensation in oral cavity upon taking spicy food		
Difficulty in swallowing solid food		
Difficulty in swallowing liquid food		
Voice change		

COMORBIDITIES	YES/NO	SINCE
Hypertension		
Diabetes Mellitus		
Pulmonary Tuberculosis		
Acid Peptic Disease		

Family History:

PERSONAL HISTORY	
Sleep, bowel, bladder habits	
Appetite	

HABITS	YES/NO	QUANTITY/DAY	SINCE
Tobacco chewing If yes stopped			
Bidi If yes stopped			
Cigarette If yes stopped			
Alcohol If yes stopped			
Others			

Diagnosis:

Plan of Treatment: RT / CTRT / Post op RT /

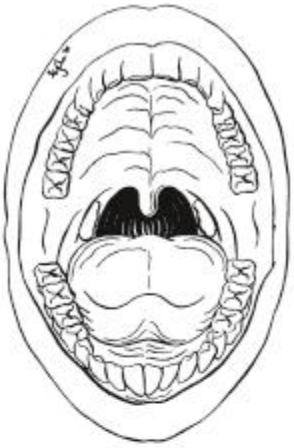
Local examination:

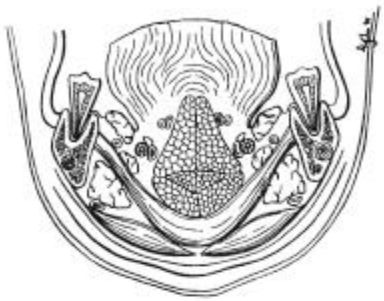
Oral cavity:

Lips:

Mouth opening: Trismus : +/-

Lesion	Site	Greatest Antero Posterior diameter	Greatest Transverse diameter	CT Scan Measurement





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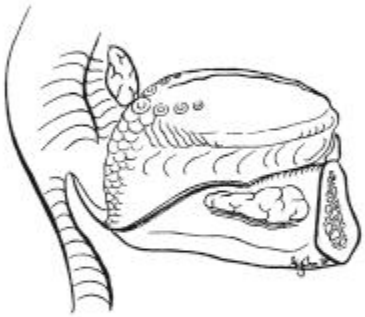

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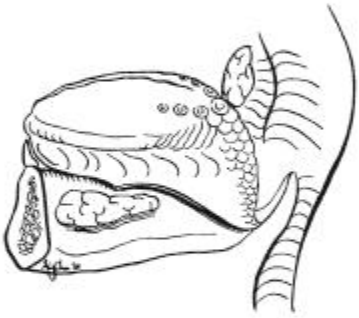

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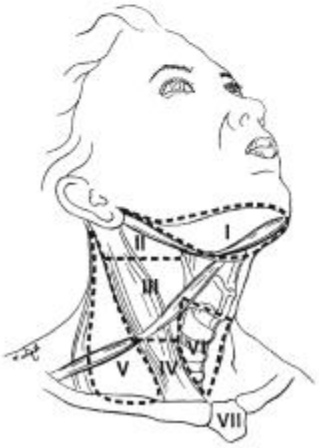

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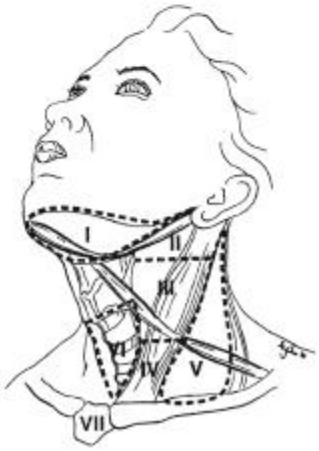

agram primary
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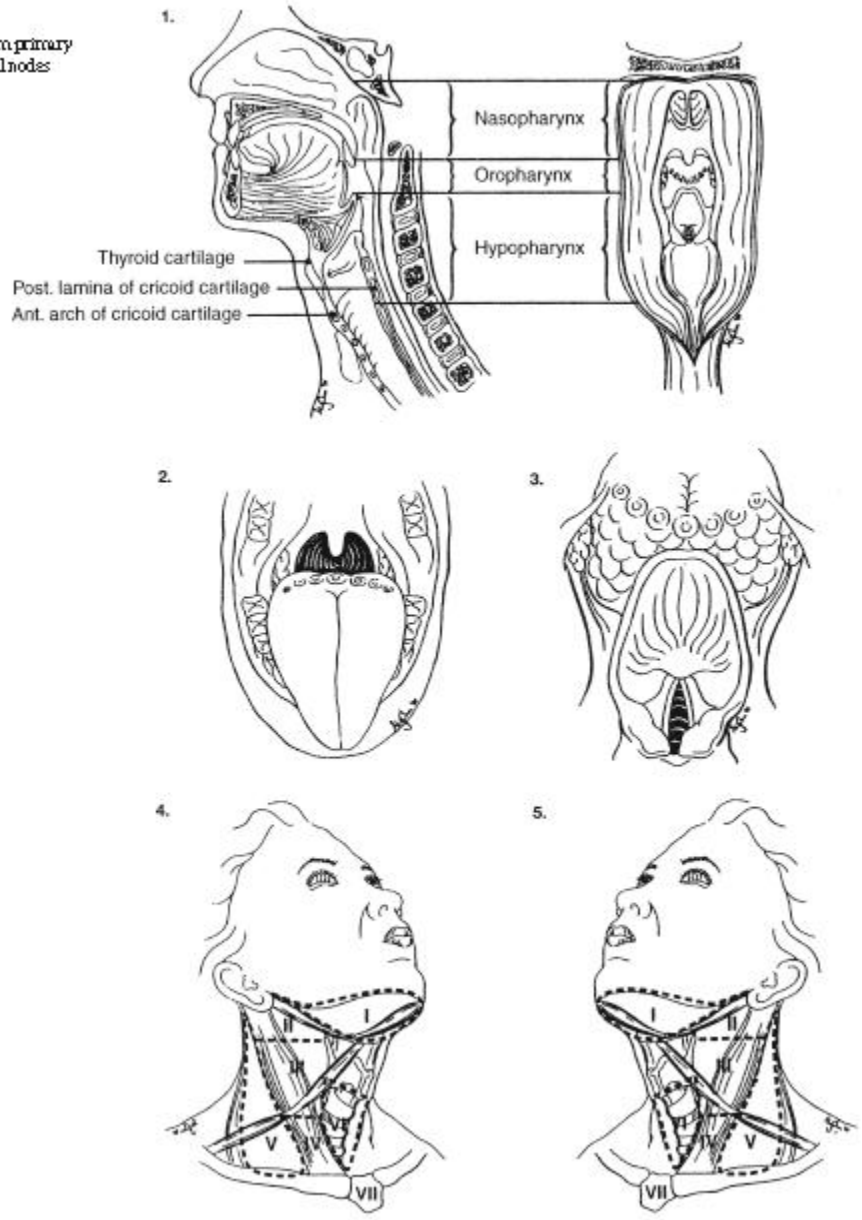

Investigations:

Hb: TC: DC: Plt Count: BT: CT: HIV: HbsAg:

Histopathological Diagnosis:

Final Diagnosis:

CT scan findings before treatment:

Dimensions of tumor:		
Volume of tumor:		

Treatment: **Surgery+ CTRT / Surgery +RT / CT+RT / RT**

If any break in treatment/defaulter, if YES Reason:

CT scan findings after treatment (3months followup):

Dimensions of tumor:		
Volume of tumor:		

Group | **A** | **B** | **A -Test Group, B - Control Group**

Size and extend of Primary Tumor

Size in cm	**Before starting of Rx**	**During 1st wk Rx**	**During 2nd wk Rx**	**During 3rd wk Rx**	**During 4th wk Rx**	**After 3 months**
Greatest AP Diameter						
Transverse Diameter						

Oral mucositis (Objective grading) WHO scale for oral mucositis

	During 1st wk Rx	During 2nd wk Rx	During 3rd wk Rx	During 4th wk Rx	After 2 months
Grade 0 **None**					
Grade 1 **Soreness with erythema**					
Grade 2 **Erythema, ulcers, can eat solids**					
Grade 3 **Ulcers, liquid diet only**					
Grade 4 **Alimentation not possible**					

Oral mucositis (Subjective grading) NCI-CTAE v.4.0 scale for oral mucositis

	During 1st wk Rx	**During 2nd wk Rx**	**During 3rd wk Rx**	**During 4th wk Rx**	**After 2 months**
Grade 1 Asymptomatic or mild symptoms; intervention not indicated.					
Grade 2 Moderate pain; not interfering with oral intake; modified diet indicated					
Grade 3 Severe pain; interfering with oral intake					
Grade 4 Life-threatening consequences; urgent intervention indicated					
Grade 5 Death					

Annexure II

Consent for the study

Study Title: Efficacy of Curcumin as a radio-sensitiser and in minimising mucosal damage in patients receiving radiotherapy for head and neck squamous cell cancers.

I have read the consent form / has been read to me and I understand the purpose of this study, the procedures that will be used, the risks and benefits associated with my involvement in the study and the confidential nature of the information that will be collected and disclosed during the study.

I understand that I remain free to withdraw from the study at any time and this will not change my future care.

I have had the opportunity to ask questions regarding various aspects of this study and my question have been answered to my satisfaction.

I, the undersigned agree to participate in this study and authorize the collection and disclosure of my personal information as outlined in this consent form.

Subject's / Guardian's name and signature / thumb impression Date:

Name and signature of witness Date:

Name and signature of principle investigator Date:

Annexure III

KEY TO MASTERCHART

F	⇨	Female
M	⇨	Male
SCC	⇨	Squamous Cell Carcinoma
NA	⇨	Not Applicable
RT	⇨	Radiotherapy
SOP	⇨	Site of Primary
StOP	⇨	Stage of Primary Tumor
OM	⇨	Oral mucositis
Obj	⇨	Objective Assessment
Subj	⇨	Subjective Assessment
HPE	⇨	Histopathological Examination