CONCURRENT CHEMO RADIATION WITH CISPLATIN AND BLEOMYCIN VERSUS CISPLATIN ALONE IN LOCALLY ADVANCED SQUAMOUS CELL CARCINOMA OF ORAL CAVITY:

A RETROSPECTIVE STUDY

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CERTIFICATE

This is to certify that this dissertation titled, **"CONCURRENT CHEMO RADIATION WITH CISPLATIN AND BLEOMYCIN VERSUS CISPLATIN ALONE IN LOCALLY ADVANCED SQUAMOUS CELL CARCINOMA OF ORAL CAVITY: A RETROSPECTIVE STUDY."** is a bonafide record of the work done by Dr.Anbarasi.K, in the Division of Radiation Oncology, Cancer Institute (W. I. A.), Chennai, during the period of his postgraduate study for the degree of M.D. (Branch X – Radiotherapy) from 2013-2015 under my direct guidance and supervision.

Date: 14 Oct 2014

Place: Chennai

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ACKNOWLEDGMENT

Bleomycin was used in combination with radiation therapy in our institute since 1970's to increase the tumor control and survival rate in locally advanced oral cavity cancer. After cisplatin established as single agent chemotherapy agent in combination with radiation for head and neck cancers bleomycin was combined with cisplatin in locally advanced oral cavity cancer. As bleomycin was not used in many institutions now for concurrent chemoradiation we had the idea to analyze the effect of combination of cisplatin and bleomycin concurrent with radiation and cisplatin alone concurrent with radiation. I would like to acknowledge my gratitude to late Dr.S.Krishnamurthy, advisor and Dr.V.Shatha, Excecutive chairman, Cancer institute (WIA) for providing me the opportunity to carry out this study.

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Dr. Anbarasi K

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ABSTRACT

CONCURRENT CHEMO RADIATION WITH CISPLATIN AND BLEOMYCIN VERSUS CISPLATIN ALONE IN LOCALLY ADVANCED SQUAMOUS CELL CARCINOMA OF ORAL CAVITY: A RETROSPECTIVE STUDY

Dr.Anbarasi.K, Cancer institute(WIA)

Background:

Oral cavity cancer constitutes two thirds of locally advanced head and neck cancer in India and majority presents in advanced stage. Despite aggressive treatment disease outcome is poor. The aim of this study is to compare concurrent chemo radiation with cisplatin and bleomycin versus concurrent chemo radiation with cisplatin alone in locally advanced oral cavity cancer.

Materials and methods:

Patients with locally advanced squamous cell carcinoma of oral cavity cancers registered at cancer institute from 2009 – 2011 were included in the study. Patients who were either taken up for initial surgery or treated with radiation therapy alone were excluded from study. Of 515 patients treated with concurrent chemo radiation, 112

patients were treated with three weekly cisplatin along with biweekly bleomycin and 104 patients were treated with 3 weekly cisplatin alone concurrent with radiation therapy.

Results:

Among the patient treated with three weekly cisplatin and biweekly bleomycin there is increased two year disease free survival (45.5% versus 35.6%) and two year overall survival(60% versus 45.2%)on comparison with patients treated with three weekly cisplatin alone.($P < 0.05$). Three weekly cisplatin alone has lesser incidence of grade III mucositis, but comparable treatment breaks haematological toxicity, vomiting, treatment response when compared to three weekly cisplatin along with biweekly bleomycin.

Conclusion:

The addition bleomycin to cisplatin concurrently with radiation therapy increases disease free survival and overall survival, with manageable toxicity.

Key words:

Cisplatin, bleomycin, oral cavity cancer, survival, radiation therapy.

I.INTRODUCTION

I.1 ANATOMY:

The oral cavity extends from the vermilion border of lips to the oropharyngeal isthmus. It is bounded by the hard and soft palate junction superiorly, the floor of mouth inferiorly, the anterior tonsillar pillar laterally and by circumvallate papillae posteriorly. The entire oral cavity is lined with mucous membrane tissue. The oral cavity divided into various subsites including lips, anterior two third of tongue, floor of mouth, buccal mucosa, gingivum, retro molar trigone and hard palate.

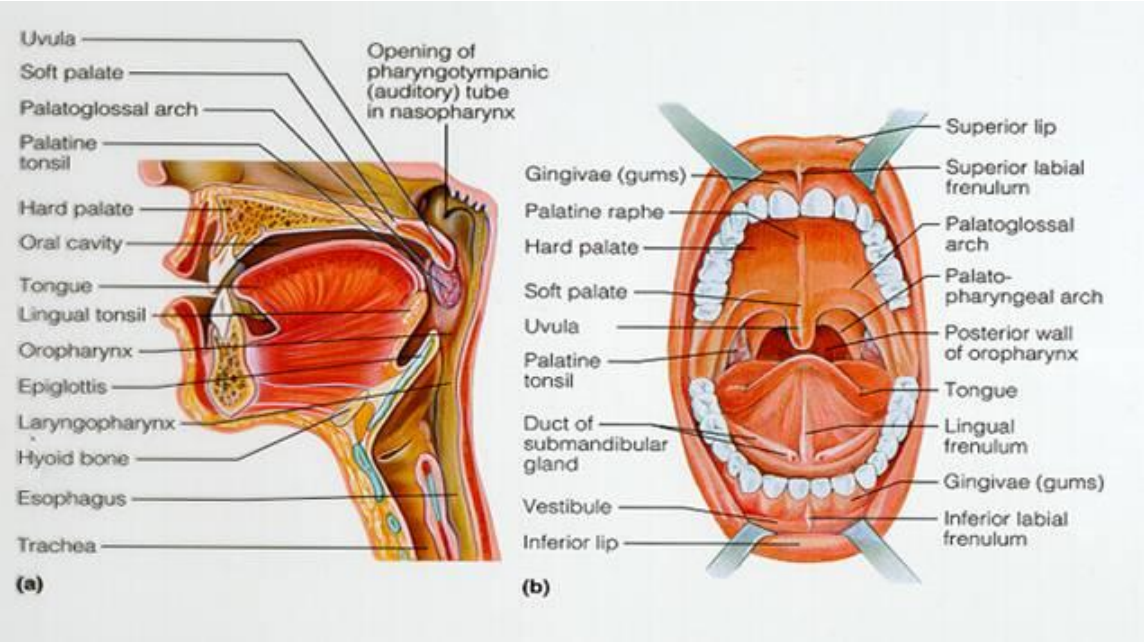


Fig 1: Anatomy of oral cavity a)lateral view b)anterior view

LIP:

The lips are divided into two parts, the upper lip and the lower lip. The lips are the transition point between facial skin and mucosal lining of the mouth and the meeting point is called vermilion border. The lips are mainly composed of orbicularis oris muscle. Both upper and lower lips are attached to gingival by labia frenula which forms by raised folds of mucous membrane. The arterial supply is from branches of facial artery namely superior and inferior labial arteries.The motor supplies are buccal and mandibular branches of facial nerve. The sensory innervation to upper and lower are the infraorbital nerve and the mental nerve respectively. The lips drain primarily into submental nodes, parotid nodes, submandibular nodes which in turn drain secondarily into jugulodigastric nodes.vascular membrane.

TONGUE:

The anterior two third of tongue is mobile and considered as a part of the oral cavity. The sulcus terminalis divides the anterior two third (oral part) of tongue from posterior one third (pharyngeal part). The oral tongue extends anteriorly from line of circumvallate papillae to undersurface of the tongue at the junction of the floor of mouth. The ventral surface of the tongue attached to floor of mouth by lingulam frenulum. The oral tongue has four areas: the tip, dorsal

surface, lateral borders and the ventral surface. The oral tongue is divided into two halves by fibrous septum.

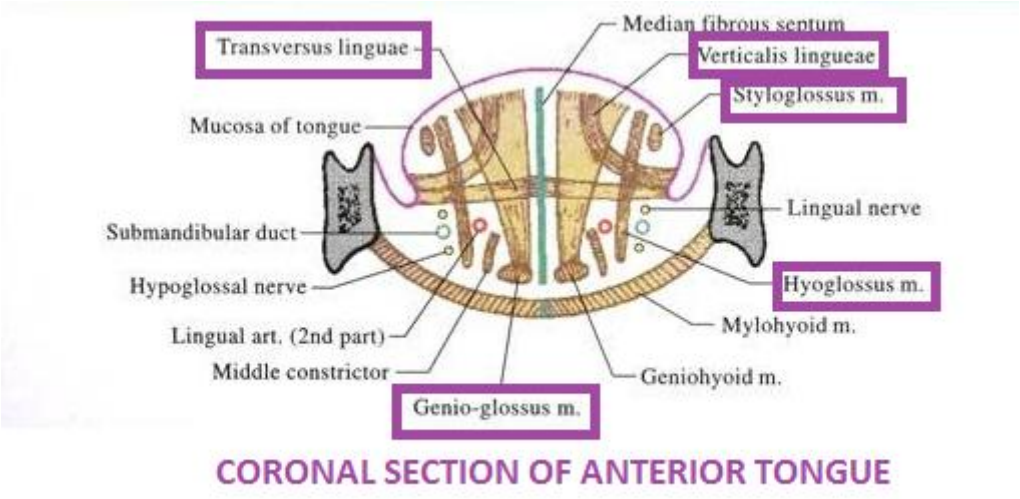


Fig 2: Coronal section of anterior tongue showing muscles of tongue and relationship

Each halves consists of four extrinsic muscles and four intrinsic muscles. The extrinsic muscles arises from the bone and onserts into tongue. Extrinsic muscles are genioglossus, hyoglossus, styloglossus and palatoglossus and all helps in altering tongue position and allows side to side movements, protrusion, retraction, elevation and depression. The intrinsic muscles originate in tongue and inserted within tongue. Intrinsic muscles are superior longitudinal muscle, inferior longitudinal muscle, verticalis muscle and transverses muscle and helps in altering shape of tongue and important for fine motions during articulation and bolus preparation.

The blood supply is primarily by lingual artery, a third branch of external carotid artery & its branches and drains into two lingual veins accompanying lingual artery and finally into internal jugular vein.

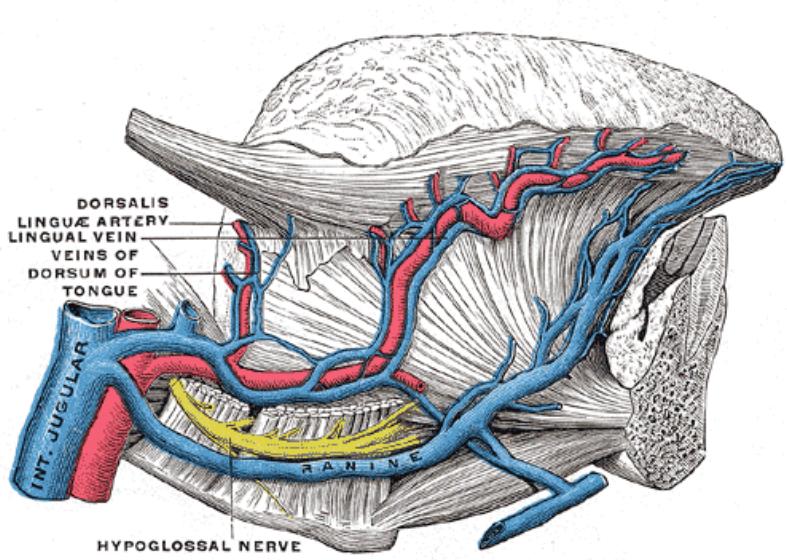


Fig: 3 Vascular and nerve supply of tongue

The motor supply of anterior two third of tongue is by hypoglossal nerve. The general sensory innervations and special sensory (taste) innervations to anterior two third of nerve are lingual nerve and chorda tympanic branch of facial nerve respectively.

The lymphatic drainage of oral tongue has three routes. The lymphatics from tip of the tongue and frenulum drain into submental nodes. Lymphatics from the

lateral part drain into submandibular nodes which in turn drains into deep cervical lymphnodes. The lymphatics from central tongue drain directly into deep cervical lymphnodes of either side.

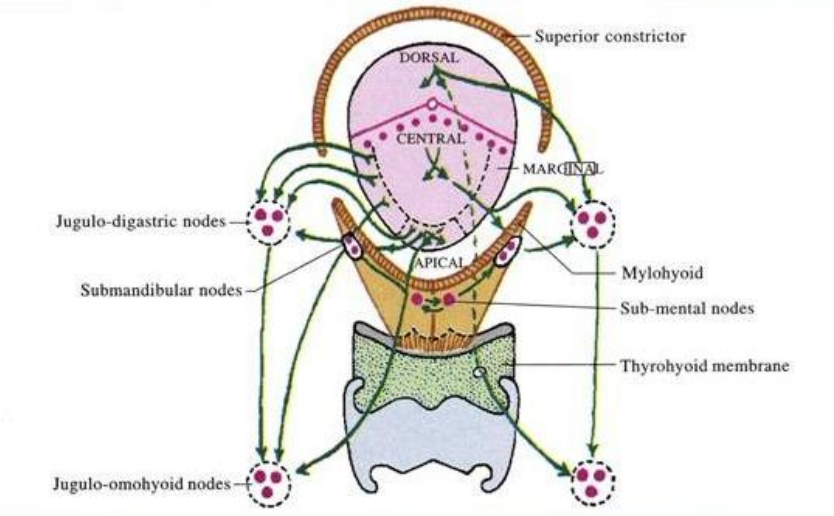


Fig:4 Lymphatic drainage of tongue

FLOOR OF MOUTH:

The floor of mouth is a semilunar space overlying hyoglossus and mylohyoid extending from inner surface of mandibular alveolar ridge to the ventral surface of the tongue. Posteriorly it extends till base of the anterior tonsillar pillar. The muscular sling formed by mylohyoid, geniohyoid and genioglossus supports the floor of mouth. The floor of mouth is divided into two halves by frenulum and contains ostia of sumandibular, sublingual and minor salivary glands. The blood supply is by sublingual artery and drains into sublingual veins and form deep lingual veins. The general sensory innervation is by lingular branch of mandibular nerve. Lymphatic drainage of floor of mouth is divided into anterior and posterior

complexes. The anterior complex drains anterior part of floor of mouth into submandibular nodes mainly and submental nodes. The posterior complex drains posterior part of floor of mouth into juguloomohyoid nodes. Bilateral drain occurs commonly.

GINGIVA:

The gingiva is composed of fibrous tissue and is mucosal lined extensions from floor of mouth and roof of oral cavity which contain the teeth. There are three types of gingiva:

1) Free or unattached or marginal gingiva- loosely tissue continous with lining of oral cavity.
2) Interdental gingival- part of gingiva that fills the space between the teeth.
3) Attached gingival- closely attached to periosteum of alveolar processes of maxilla and mandible.

The blood supply of the gingiva is by branches of superior and inferior alveolar artery. The gingiva receives sensory innervations fom mandibular and maxillary branch of trigerminal nerve. The gingiva drains into submandibular nodes mainly and submental nodes.

BUCCAL MUCOSA:

The cheeks form the lateral wall of the oral cavity. The buccal mucosa formed by mucosal lining of cheek and lips. The external surface of cheek is covered by skin. Buccinator muscle and buccal pad of covering it gives the rounded contour of cheek. The blood supply is by buccal branch of maxillary artery and drains into pterigoid plexus of veins and finally into internal jugular vein. The sensory innervation is by maxillary and mandibular branch of trigerminal nerve and motor supply is by facial nerve. The inferior and medialparts of cheek drains into submandibular nodes and superior and lateral parts drain into preauricular nodes.

RETROMOLAR TRIGONE:

The retromolar trigone is the small triangular area posterior to the third molar. The apex of triangle is formed by maxillary tuberosity and base of the triangle by the posterior third molar. The mucosa attached to hamulus of the medial pterygoid of sphenoid bone. The sensory innervation is by branches of lesser palatine nerve and glossopharyngeal nerve. The blood supply is by tonsillar and ascending palatine branches of facial artery and drains through tonsillar bed to pharyngeal plexus and to common facial vein. The lypmph from retromolar trigone drains into upper deep jugular nodes mainly and lateral pharyngeal nodes.

HARD PALATE:

The hard palate is a semilunar area forms the roof of oral cavity and floor of The nasal cavity. It extends from the superior alveolar ridge to the posterior edge of the palatine bone. The hard palate is formed by palatine process of maxilla and horizontal plates of palatine bone. The mucosa of the hard palate is closely adherent to the overlying periosteum. The blood supply is by greater palatine arteries and veins. The anterior part of hard palate is by nasopalatine nerve and ucous membrane and posterior parts innervated by greater palatine nerve. It drains into submandibular nodes or directly into superior deep cervical nodes.

I.2. OVERVIEW OF ORAL CAVITY CANCER

EPIDEMIOLOGY:

The oral cavity cancer is the sixth commonest cancer reported in the world with an annual incidence of over 300,000 cases. More than 62% of these cases are from developing countries. The age adjusted standard rates in India is over 20 per 100,000 populations whereas in United States it is 10 per 100,000 and lowest in Japan is 0.2 per 100,000. It constitutes 30% of the cancer burden in India. Tongue and cheek cancer are the most common sites and majority of them present in locally advanced stage.

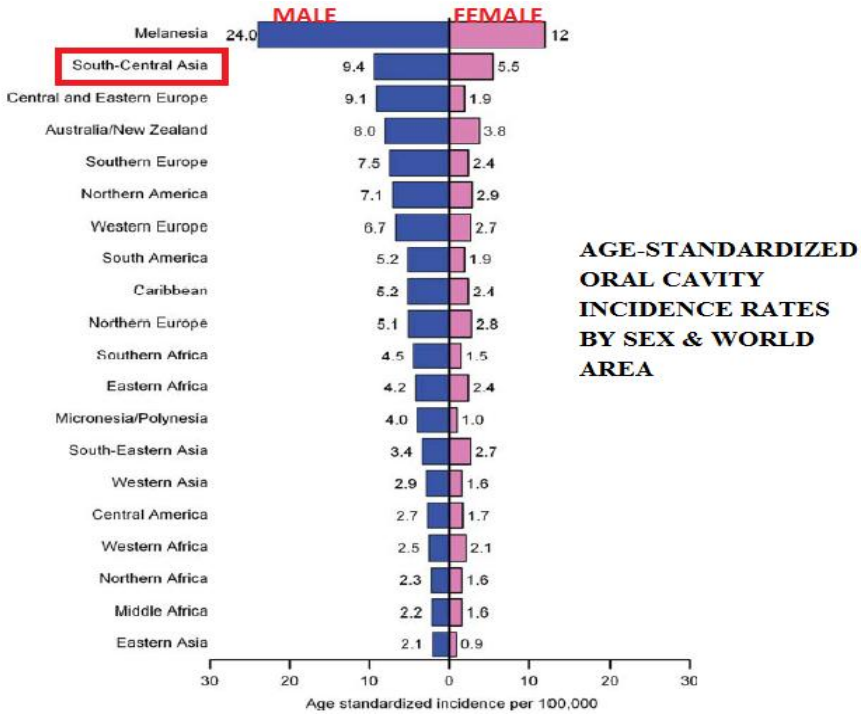


Fig:5-Age standardized incidence rates of oral cavity cancers by sex and world area

It is the most common cancer in males with an annual incidence of 45,455 and fourth common cancer in females with an incidence of 24,375.

RISK FACTORS:

Chewing tobacco products, smoking, alcohol and viral infection are the commonest risk factors of oral cavity cancers. In India chewing betal nut mixed with tobacco is the major cause of oral cavity cancer. Smoking is an independent risk factor of oral cavity cancer patients. Alcohol and smoking has synergistic action on carcinogenesis and alcohol acts an independent risk factor of oral cavity cancer. Herpes simplex virus acts as a cocarcinogen with tobacco and UV light. Around 50% of cases of oral cavity cancer has HPV infection especially HPV6 and HPV16. Ultraviolet light is a risk factors for carcinoma of lip. Other risk factors like poor oral hygiene, nutritional hygiene, continuous irritation of oral cavity also cause oral cavity cancer.

HABIT	RELATIVE RISK %
None	**1%**
Betal nut chewing	**4%**
Smoking only	**3-6%**
Betal chewing+tobacco	**8-15%**
Betal chewing+smoking	**4-25%**
Betal+tobacco+smoking	**20%**

Table 1-Relative risk of oral cavity cancers according to habits of the patients.

PREMALIGNANT LESIONS:

LEUKOLPLAKIA:

Leukoplakia is the most common premalignant lesion of oral cavity. Clinically it appears as chronic white verrucous plaque, nodular, with ulceration or erosion. It occurs anywhere in the oral cavity. 1-18% of leukoplakia develops into oral cavity cancer.

ERYTHROPLAKIA:

Erythroplakia has high chance of development of oral cavity cancer upto 51%.Clinically it appears as a chronic, red non inflammatory plaque on mucosal surface of oral cavity. As it has higher risk of malignant transformation it should be excised surgically.

SUBMUCOUS FIBROSIS:

Submucous fibrosis occurs in people who chew tobacco along with betal nut and lime. Clinically it appears as generalized white discolouration of oral mucosa with progressive fibrosis, painful muscular atrophy and restrictive fibrotic bands. If it is severe it leads to trismus, dysphagia and xerostomia. 5-10% chance of malignant transformation present.

PATHOLOGY:

Ninety percent of oral cavity cancers are squamous cell carcinoma. Remaining ten percent of the patients have non squamous histology like adenoid cystic carcinoma, adenocarcinoma, ameloblastoma, lymphoma, sarcoma and melanoma.

STAGING AND GROUPING SYSTEM:

PRIMARY TUMOR (T):

Tx	**Primary tumor cannot be assessed**
To	**No evidence of primary tumor**
Tis	**Carcinoma in situ**
T1	**Tumor 2cm or less in greatest dimension**
T2	**Tumor more than 2cm but not more than 4cm in greatest dimension**
T3	**Tumor more than 4cm in greatest dimension**
T4a(lip)	**Tumor invades through cortical bone , inferior alveolar nerve, floor of mouth or skin of face.**
T4a(oral cavity)	**Tumor invades through cortical bone into deep (extrinsic) muscle of tongue (genioglossus, hyoglossus, palatoglosus and styloglossus), maxillary sinus or skin of face.**
T4b	**Tumor involves masticator space, pterygoid plate ,or skull base and/or encases internal carotid artery.**

REGIONAL LYMPHNODES (N):

Nx	**Regional lymph nodes cannot be assessed.**
No	**No regional lymph nodes.**
N1	**Metastasis in a single ipsilateral lymph node less than or equal to 3cm in greatest dimension.**
N2	**Metastases in a single ipsilateral lymph node >3 cm, but <6 cm in greatest dimension; or in multiple lymph nodes none >6 cm in greatest dimension; or in bilateral or contralateral lymph nodes**
N2a	**Metastases in a single ipsilateral lymph node >3 cm, but not more than 6 cm in greatest dimension**
N2b	**Metastases in multiple lymph nodes none more than 6 cm in greatest dimension**
N2c	**Metastases in bilateral or contralateral lymph nodes, none 6 cm in greatest dimension**
N3	**Metastases in a lymph node >6 cm in greatest dimension**

DISTANT METASTASIS(M):

Mx	**Distant metastases cannot be assessed.**
Mo	**No distant metastases.**
M1	**Distant metastases.**

STAGE GROUPING:

STAGE	T	N	M
Stage 0	**Tis**	**N0**	**M0**
Stage I	**T1**	**N0**	**M0**
Stage II	**T2**	**N0**	**M0**
Stage III	**T3** **T1-T3**	**N0** **N1**	**M0** **M0**
Stage IV A	**T4a** **T1- T3**	**N0- N2** **N2**	**M0** **M0**
Stage IV B	**T4b** **Any T**	**Any N** **N3**	**M0** **M0**
Stage IV C	**Any T**	**Any N**	**M1**

TREATMENT:

The treatment approaches of the carcinoma of oral cavity include surgery, radiation therapy, combination of surgery and radiation therapy and concurrent chemo radiation therapy. For patients with stage I and II either modality surgery or

radiation has high chance of cure. Surgery is the preferred modality of treatment of oral cavity cancer among head and neck cancers because

- Oral cavity carcinomas can be approached easily by surgery as it is easily accessible and without major surgical dissection.

- For early cancers organ function can be well preserved even after excision. The maxillodental bony structures interfere with the dose received by tumor and at radical dose of radiation late complications like osteoradionecrosis require major surgery.

- Most of the oral cavity cancers are well differentiated and less sensitive to radiation therapy and chemotherapy.

- Histology other than squamous cell carcinoma like sarcoma and minor salivary gland tumor are less responded to radiation therapy.

- Due to involuntary mobility of tongue and air–tissue interfaces within mouth more acute toxicities during radiation delays the treatment.When the oral cavity carcinomas can be resectable without compromising functional loss surgery is the preferred treatment.

In locally advanced carcinoma concurrent chemo radiation is the alternate treatment of choice. The small tumors can effectively treated by brachytherapy and the result is same as that of surgery. The advanced cancers generally require combined modality approach. The treatment of oral cavity cancers is highly individualized and depends on stage of disease, subsite involved, and physician preferences and varies from institution to institutions. There are no definite guidelines for the treatment of oral cavity cancers. Patients who are unable to withstand major surgery can be treated with radiation therapy and concurrent chemotherapy. The choice of treatment depends on the cosmetic changes, complications and functional consequences that the treatment is expected to cause. The overview of treatment option by tumor site, local control and five year survival rates by tumor site and disease stage summarized in the following tables.

Overview of Treatment Options by Tumor Site

	Treatment Options			
Tumor Site	*Stage I*	*Stage II*	*Stage III*	*Stage IV*
Lip	S, R	S, R	SR, R, C	SR, R, C
Tongue	S, SR, R	R, SR	R, SR, C	SR, R, C
Buccal mucosa	S, R	R, S, SR	S, R, SR, C	S, R, SR, C
Floor of mouth	S, R	S, R, SR	S, R, C	S, R, C
Lower gingiva	S, R	S, R	RS, SR, C	S, R, SR, C
Retromolar trigone	S, R, RS	S, R, RS	SR, C, R	SR, C, R
Upper gingiva	S, SR, R	SR	R, SR, C	SR, C, R

C, chemotherapy with or without surgery, with or without radiation therapy as part of clinical trial; R, radiation therapy; RS, radiation therapy followed by surgery; S, surgery; SR, surgery followed by radiation therapy.

Table 2- shows overview of treatment options of oral cavity cancers by tumor site.

Local Control and 5-Year Survival Rates by Tumor Site and Disease Stage				
	Local Control Rate (%)/5-Year Survival Rate (%)			
Tumor Site	*Stage I*	*Stage II*	*Stage III*	*Stage IV*
Lip	90/90	90/90	NS/NR	NS/NR
Anterior tongue	96-100/100	85-93/70	50-65/35	40-45/NS
Buccal mucosa	90+/NS	90/NS	NR	NR/NS
Floor of mouth	88-100/NS	70-90/NS	70/NS	30-50/NS
Retromolar trigone	90-100/NS	90/NS	90/NS	60-89/NS
Upper gingiva	NR	NR	NR	NR
Lower gingiva	90+/NS	90+/NS	<90/NS	30-55/NS

Table 3-Local control rates and 5 year survival rates by tumor site and stage.

SURGERY ALONE:

Surgical resection of primary is the treatment for early T1 lesions and T2 lesions of cheek, tongue, lip and floor of mouth followed by supraomohyoid dissection or extended dissection.

RADIOTHERAPY ALONE:

Radiotherapy alone can be given by brachytherapy or external beam radiation for T1 and T2 lesions of lip, tongue, cheek and floor of mouth. Patient who are unfit for surgery and chemotherapy can also treated with radiation therapy alone.

COMBINED RADIATION THERAPY AND SURGERY:

Combined modality treatment achieves optimal results in locally advanced carcinoma of oral cavity. Initial surgery followed by radiation therapy is recommended in

- ❖ Large primary lesion
- ❖ Deeply infiltrative
- ❖ Necrotic
- ❖ Bone invasion
- ❖ Deep muscle invasion
- ❖ Cervical lymph node metastasis
- ❖ Soft tissue involvement of neck

The aims of postoperative radiation are

- ❖ To prevent and reduce local recurrence in case large tumors
- ❖ To prevent regional recurrence when there is multiple lymph node metastasis or soft tissue invasion in neck.
- ❖ To control occult disease those are not dissected surgically.

Postoperative radiation therapy is usually given to surgical bed of primary and neck and dose of 60 – 66Gy delivered in 1.8Gy – 2Gy per fraction daily and five fractions a week. Postoperative radiation starts after complete wound healing usually 2 to 3 weeks after surgery.

COMBINED RADIATION THERAPY AND CHEMOTHERAPY AND SURGERY:

Surgery followed by chemotherapy and radiation therapy is indicated in

- ❖ Positive margins or gross residual disease
- ❖ Extra capsular extension.

RTOG 9501 and EORTC 22931 are the two landmark studies demonstrated better local control and survival in patients with positive margins and extra capsular extension when treated with concurrent chemo radiation compared to radiation alone.

COMBINED CHEMOTHERAPY AND RADIATION THERAPY FOR LOCALLY ADVANCED DISEASE:

Though chemotherapy for oral cavity cancers started in earlier 1960's, it was given only in recurrent and metastatic conditions. Later chemotherapy was given

concurrently with radiation in locally advanced oral cavity cancer in order to improved cosmesis, function, local control and overall survival. Several meta-analyses compared concurred chemo radiation with radiation alone in locally advanced head and neck cancers and it was found that there is absolute benefit in overall survival by 5-8%. However the number of patients included in oral cavity cancers is small in many randomized studies. In 1961 Lo et al in a randomized study found that there is increased in over survival of oral cavity cancer patients who received 5-Fluorouracil concurrently with radiation compared to radiation alone. Other studies did not find the significant benefit in overall survival in oral cavity cancers when treated with single agent chemotherapy concurrent with radiation. Whereas multiagent chemotherapy is given in oral cavity cancers shows improved local control and overall survival when given concurrently with radiation therapy. The chemotherapeutic agents used concurrently with radiation were cisplatin, paclitaxel, 5-fluorouracil and bleomycin.

I.3.CONCEPT OF CONCURRENT CHEMO RADIATION.

INTRODUCTION:

The combined modality approach in cancer treatment is now acknowledged as better treatment in head and neck cancer as it increases the overall survival and better organ conservation. Chemotherapy is given as neoadjuvant, concurrent or adjuvant to radiation therapy.

- Chemotherapy is given before radiation to reduce the size of tumor and so field of radiation.
- Chemotherapy also given along with radiation considering it may sensitize cells to radiation and to reduce the subclinical disease at distant sites.
- Chemotherapy delivered after radiotherapy to reduce distant subclinical disease and to reduce patient's toxicity.

In late 1950s the fact that the anticancer drugs could affect the responses to radiation was first appreciated during the use of dactinomycin D in the clinical evaluation of wilm's tumor where there is increased radiation reaction in normal tissue in addition to increased damage to the tumor. These lead to systematic study

of interactions of antitumour drugs and radiation responses on normal tissue. The review of results of these studies showed that there enhanced response to radiation of normal tissue when anticancer drugs given concurrently with radiation. In 1966 McGrath and Williams proved that ionizing radiation produce damage in DNA. It is well known that anticancer drugs cause DNA damage. When these two interacts it is expected to cause more DNA damage.

The rationales of combining chemotherapeutic drugs with radiation therapy are

- Organ preservation resulting in improved cosmesis and function,
- Acts as radio sensitizer , improving local control and survival,
- Act systemically and eradicate distant micro metastases.

THERAPEUTIC RATIO:

Both radiation therapy and chemotherapeutic agents are cytotoxic to tumor and normal tissue cells. To be therapeutically beneficial radiotherapy, chemotherapy or both should increase tumor control and lesser damage to normal cells. But there is lack of specificity and is a major limitation in their use when applied either as individual treatments or in combination. Radiation inflicts damage

to tumor and normal tissues in the radiation treatment field.Chemotherapy systemic action can affect any tissue in the body.

So toxicity is often increased when two modalities are combined. In general, both the antitumor efficacy and the severity of normal tissue toxicity produced by either radiation or drugs are increased as their dose is increased. This dose-effect relationship is sigmoidal and enables estimation of the therapeutic index (ratio), which is defined as the ratio between the doses (radiation, drug) that produce the same level (probability) of antitumor efficacy and normal tissue damage. To be therapeutically beneficial, the therapeutic ratio must be positive (>1); that is, individual agents or their combination must be more effective against tumors than normal tissues.

In 1979 Steel and Peckham(1) explained the theoretical types of interaction of cytotoxic chemotherapy and radiotherapy that improves therapeutic ratio.

1) Spatial cooperation
2) Independent toxicity
3) Enhancement of tumor response
4) Protection of normal tissues.

SPATIAL COOPERATION:

Spatial cooperation was the initial rationale of combining chemotherapy and radiotherapy in which radiotherapy acts locoregionally and chemotherapy acts against micro metastases outside radiation field and both the action are independent of each other. This cooperation requires the drug which would not increase normal tissue toxicity as an additive effect and it should be used with effective doses of both modalities.For example in hematological malignancies like leukemia radiation therapy is used to treat sanctuary site like brain and chemotherapy is used systemically.

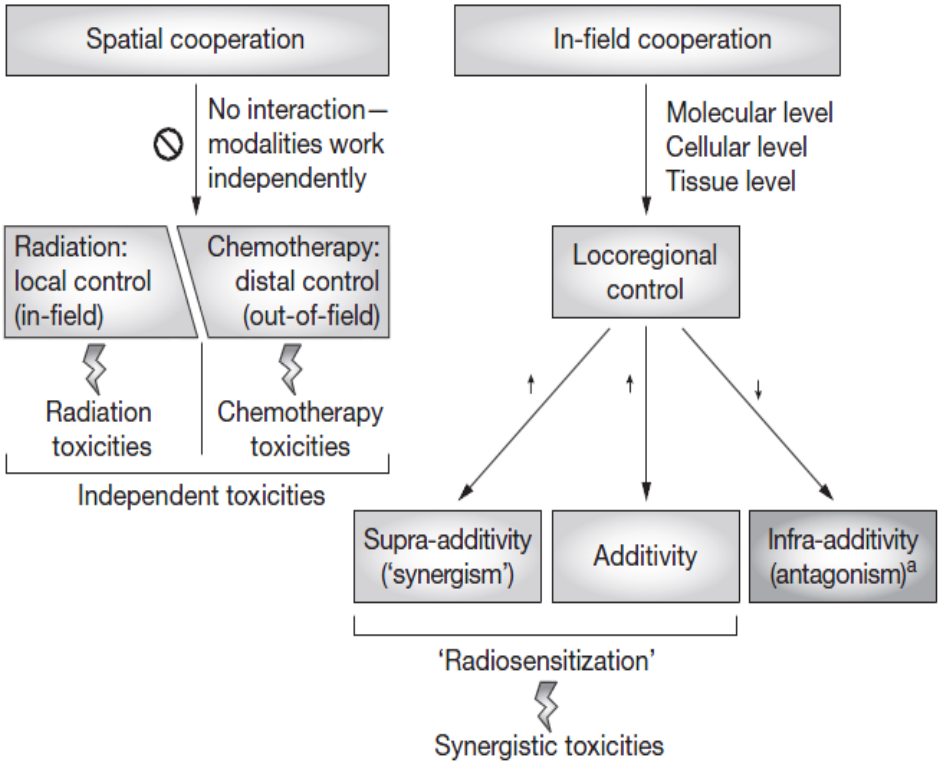


Fig 6 shows interaction of chemotherapy and radiation therapy

INDEPENDENT TOXICITY:

Toxicity to normal tissues is the major dose limiting factor for both radiation therapy and chemotherapy. Independent toxicity is an important rationale to increase the therapeutic ratio. If radiotherapy and chemotherapy given at full dose the response to tumor (cell kill) is more than the treatment with single modality alone even in the absence of interaction between chemotherapy and radiation therapy. To achieve this, the chemotherapeutic drug and radiation should not have overlapping toxicities. For example- In treatment of early stage hodgkins lymphoma combination of chemotherapy and radiation (mantle field) is highly effective for long term cure with increased long term effects. Now combing different chemotherapy agents the radiation field (involved field) and dose is reduced to get same efficacy. Retaining antitumor efficacy and minimizing normal tissue toxicity requires knowledge of individual drug toxicity, mechanism of action and pharmacokinetics of individual drug to combine with radiation.

ENHANCEMENT OF TUMOR RESPONSE:

It refers to the capacity of chemotherapy which may enhance tumor damage when interacts with radiation or sometimes radiation make cells sensitive to chemotherapy probably by interacting with chemotherapy at molecular, cellular , metabolic or pathophysiologic level. This interaction can also cause additional

damage to normal tissue apart from enhancement of tumor damage. Therapeutic gain can be achieved only when enhancement caused by interaction between chemotherapy and radiation therapy is selective to tumor rather than critical normal tissues.

PROTECTION OF NORMAL TISSUE:

This strategy is to increase tolerance to normal tissues so that higher doses of radiation therapy can be safely delivered to the tumor. In vivo testing showed that amifostine protect the salivary gland function during head and neck radiotherapy. The increased toxicity to normal tissue with radiation in setting of concurrent chemo radiation can be achieved by advances in radiotherapy treatment like conformal planning (3DCRT, IMRT), use of protons and use of radioprotectants like amifostine.

MECHANISMS OF CHEMOTHERAPY AND RADIOTHERAPY INTERACTIONS:

There are seven possible major interactions between radiotherapy and chemotherapy at cellular and molecular level. Usually more than one type of interactions occurs during concurrent chemo radiation and depends upon the type of chemotherapy used.

1 INITIAL RADIATION DAMAGE:

DNA is the critical target for both radiation therapy and chemotherapy. Radiation induces lesions in DNA like base damage, single strand breaks, double strand breaks , alkali labile sites and DNA-DNA cross links. All these lesions except double strand breaks can be easily repaired. Double strand break is either misrepaired or unrepaired leading to chromosomal aberrations and finally leads to cell death. When chemotherapy (e.g. - halogenated pyramidines) given concurrently it become incorporated into DNA and make more susceptible for radiation damage. Certain drugs (e.g.-cisplatin) interfere in cellular repair mechanism and enhance the sensitivity of tumor cells to radiation. Cisplatin integrates with DNA in close proximity to SSB caused by radiation and makes difficult to repair the DNA.

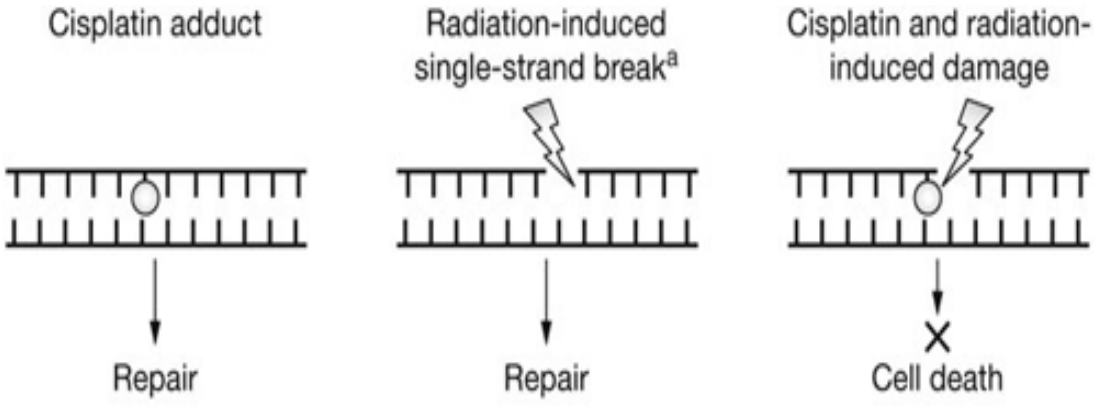


Fig 7 shows mechanism of interaction of cisplatin and radiation on DNA.

2 INHIBITION OF DNA REPAIR PROCESS:

After radiation DNA damage repair will occur. Chemotherapy cause inhibition of post radiation induced damage repair process. As DNA synthesis and DNA repair process have common pathways chemotherapy which inhibits DNA synthesis will inhibit DNA repair. In patients chemotherapeutic agents which alter the nucleoside or nucleotide mechanism will inhibit the repair of radiation induced DNA lesions and acts as potent radiosensitizer. E.g. Nucleoside analogs, thymidine analogs,halogenated pyramidines, cisplatin etc.

3 CELL CYCLE INTERFERENCE:

In 1963 Terasima and Tolmach first reported the radiation response is influenced by cell cycle. The cells in S phase are most resistant and cells in G2 & M phase are most sensitive to radiation. Chemotherapeutic agents are used to overcome this variation in radiosenstivity when given along with radiation. The drug whichselectively destroys cells in S phase or drugs which accumulate cells in G2 & M phase is used.

E.g.

1) Taxanes inhibit tubulin depolymerisation and cause cells to arrest in radiosensitive G2-M phase of cell cycle.

2) Nucleoside analogs like fludarabine incorporates with cells at S phase and cause cell damage at S phase. This also causes accumulation of cells in radiosensitive G2M phase of cell cycle.

Process affected	Mechanism[a]	Drug examples
Increased radiation damage[a]	Incorporation of chemotherapy drug into DNA/RNA	5-FU: incorporation into DNA, increasing susceptibility to RT damage Cisplatin: cross-links with DNA or RNA (intrastrand and interstrand); works for both hypoxic and oxygenated cells[51]
Inhibition of DNA repair process[a]	Interference with the DNA repair process after radiation	Halogenated pyrimidines (e.g. 5-FU, bromodeoxyuridine, iododeoxyuridine) Nucleoside analogs (e.g. gemcitabine, fludarabine) Cisplatin Methotrexate Camptothecins and doxorubicin Etoposide Hydroxyurea Carmustine, lomustine
Cell-cycle interference (cytokinetic cooperation and synchronization)[a]	Most cytotoxic chemotherapies as well as radiation are cell-cycle-specific, and proliferating cells are most susceptible Accumulation of cells in the G2 and M phases (the most radiosensitive phases) Elimination of radioresistant cells in the S phase	Taxanes lead to cell-cycle arrest via tubulin stabilization Nucleoside analogs (e.g. gemcitabine, fludarabine), etoposide, methotrexate, hydroxyurea
Enhanced activity against hypoxic cells[a]	Reoxygenation second to tumor shrinkage. Hypoxic cells are 2.5–3.0 times less radiation-sensitive than normoxic cells[18,44] Chemotherapy can help to eliminate hypoxic cells	Most chemotherapeutic agents; described in particular for paclitaxel[45] Tirapazamine, mitomycin (selective killing of hypoxic cells); nitroimidazoles (resensitize hypoxic cells to radiation)
Radiotherapy enhancement by preventing repopulation[a]	Systemic therapy can slow or stop rapid proliferation, which could otherwise be the basis for repopulation phenomenon	Most chemotherapeutic agents, in particular: Antimetabolites with activity in the S phase inhibit repopulation (e.g. 5-FU, hydroxyurea) EGFR inhibitors, which impede cell proliferation between RT fractions[100]
Inhibition of prosurvival and 'poor prognosis' markers[a]	Targeted therapies (best demonstrated for EGFR inhibition) block signaling pathways that might be responsible for radioresistance and poor prognosis	EGFR inhibitors—shown for anti-EGFR antibody, PKI-166 (small-molecule TKI), and EGFR antisense,[129–131] but on the basis of clinical experience likely to be a class effect[49,132]
Hyperradiation sensitivity[b]	HNSCC cells resistant to standard-fraction CRT can be resensitized to CRT by using smaller fraction sizes (<1 Gy) more frequently	Effect demonstrated for taxane-based CRT including paclitaxel as well as docetaxel[29,50] Low-dose fraction radiation

Table 4 shows different mechanism of interaction oh chemotherapy and radiation therapy.

4 EFFECT ON HYPOXIA:

It is well known that oxygen influence on sensitivity of cells to radiation. Hypoxic cells have shown increased resistance to radiation. Chemotherapy can abolish these hypoxic cells by multiple mechanisms.

1) Destroying tumor in oxygenated areas so that reoxygenation occurs. (E.g. paclitaxel, EGFR inhibitors).
2) Selectively destroying hypoxic cells. (e.g. Mitomycin C, Tirapazamine)
3) Radiosenstizing hypoxic cells. (e.g. Misonidazole)

5 PREVENTING REPOPULATION:

There is a homeostasis between cell production and cell loss in normal tissues. In tumor tissue there is increased cell proliferation. Chemotherapy and radiotherapy causes increased cell loss and there is compensatory cell repopulation. Fractionated radiation therapy reduces repopulation. When chemotherapy is given along with radiation therapy repopulation is reduced by decreased cell proliferation. Example: Chemotherapy agents that acts on S phase (e.g. 5-flurouracil) and agents that inhibit proliferation and their pathways (e.g. EGFR inhibitors) were good in reducing tumor repopulation . In addition it also inhibits

normal tissue regeneration which adds to treatment toxicity and it is the major limitation for concurrent chemo radiation.

6 INHIBITION OF POOR SURVIVAL MARKERS:

Agents that block signaling pathway like EGFR inhibitors and antiangiogenic agents prevents radioresistance and aggressive tumor biology as it is the major pathway for both.

7 HYPERRADIATION SENSITIVITY:

Some cancers are resistant to standard concurrent chemo radiation. They will respond to alterations in radiation fractionation. This phenomenon is termed hyperradiation sensitivity. It is observed at radiation doses greater than 1 Gy. This radioresistance can overcome by paclitaxel, docetaxel , low dose radiation preclinically. This mechanism is currently investigated in clinical trial.

CHEMOTHERAPEUTIC AGENTS USED CONCURRENTLY WITH RADIATION IN HEAD AND NECK CANCERS:

The chemotherapeutic agents can be used in chemo radiotherapy as single agent or multi agents. As the number of agents increases there is increased radiosensitization and with increased toxicity.

Table Overview of single-agent-based chemoradiotherapy platforms for head and neck cancer.

Agent and studies	Doses	RT regimen	Survival data (*n*)	Comments
Cisplatin				
Adelstein *et al.* (2003)	100 mg/m^2 every 3 weeks as CRT	70 Gy (once-daily fractionation)	37% 3-year OS (97)	Commonly used standard of care. Superior to radiation alone. Still long-term survival remains poor. Many centers moved to combination CRT platforms based on results in phase II trials
Al-Sarraf *et al.* (1998)	100 mg/m^2 every 3 weeks as CRT	70 Gy (once-daily fractionation)	76% 3-year OS (78)	Commonly used standard of care. Superior to radiation alone. Still long-term survival remains poor. Many centers moved to combination CRT platforms based on results in phase II trials
Carboplatin				
Jeremic *et al.* (1997)	25 mg/m^2 daily as CRT	70 Gy (once-daily fractionation)	55% 2-year OS (53)	Limited data as a single agent. Carboplatin is less-well established as a radiosensitizer. Comparative data show no survival difference when compared with cisplatin but superior to RT alone
5-FU				
Browman *et al.* (1994)	1200 mg/m^2/day, 72 h infusion during weeks 1 and 3 of RT	66 Gy (once-daily fractionation)	63% 2-year OS (88)	Commonly used in combined regimens (e.g. FHX or cisplatin/5-FU). Superior to radiation alone
Paclitaxel				
Lovey *et al.* (2003)	2 mg/m^2 three times weekly as CRT	66–70 Gy (once-daily fractionation)	46% 2-year OS (26)	Commonly used in combination (e.g. cisplatin/paclitaxel or TFHX)
Docetaxel				
Calais *et al.* (2004)	20 mg/m^2 weekly as CRT	70 Gy (once-daily fractionation)	47% 3-year OS (63)	Commonly used in combined regimens (e.g. cisplatin/ docetaxel or DFHX)
Cetuximab				
Bonner *et al.* (2006)	400 mg/m^2 loading, then 250 mg weekly	70 Gy (variable RT, including once-daily, hyper-fractionation and concurrent boost)	62% 2-year OS and 55% 3-year OS (211)	Possible treatment standard, especially in the elderly and patients with poor performance status. Superior to radiation alone. Role compared with cytotoxic CRT remains unclear owing to control arm (RT only)

Table 5 Oveview of single agent chemoradiotherapy trials in head and neck cancers.

OVERVIEW OF MULTI-AGENT CHEMORADIOTHERAPY PLATFORMS IN HEAD AND NECK CANCERS

Drug combination (*n*)	Chemotherapy regimen	RT employed	Survival data and comments
Cisplatin and 5-FU			
Brizel *et al.* (1998) (56)	Cisplatin 12 mg/m^2 daily on weeks 1 and 6; 5-FU 600 mg/m^2 CI days 1–5 on weeks 1 and 6	70 Gy (hyperfractionation)	55% 3-year OS. Trend towards improved survival; improved locoregional control
Taylor *et al.* (1997) (219)	Cisplatin 60 mg/m^2 every 2 weeks; 5-FU 800 mg/m^2 CI days 1–5	70 Gy on days 1–5 (once-daily fractionation) week on/week off	60% 3-year OS
Adelstein *et al.* (2006) (219)	Cisplatin 20 mg/m^2/day CI days 1–4 during weeks 1 and 4; 5-FU 1,000 mg/m^2 CI days 1–4 during weeks 1 and 4	70 Gy (once-daily or hyperfractionation)	65.7% 5-year OS
Cisplatin and paclitaxel			
RTOG 97-03 Garden *et al.* (2004) (60)	Cisplatin 20 mg/m^2 weekly; paclitaxel 30 mg/m^2 weekly	70 Gy (once-daily fractionation)	67% 2-year OS. Improved survival in comparison with cisplatin and 5-FU. Equivalent survival in comparison with FHX platform
Carboplatin and paclitaxel			
Carter *et al.* (2003) (52)	Carboplatin AUC = 1 weekly; paclitaxel 40 mg/m^2 weekly	69.6 Gy (hyperfractionation)	63% 2-year OS. Preliminary results, but several previous trials
FHX			
RTOG 97-03 Garden *et al.* (2004) (64)	5-FU 800 mg/m^2 CI days 1–5 every 2 weeks; hydroxyurea 1 g twice daily on days 0–5 every 2 weeks	70 Gy on days 1–5 (once-daily fractionation) week on/week off	69% 2-year OS. Improved survival in comparison with cisplatin/5-FU. Equivalent survival in comparison with cisplatin/paclitaxel
TFHX			
Vokes *et al.* (2003) (69)	Paclitaxel 100 mg/m^2 every 2 weeks; 5-FU; 600 mg/m^2 CI days 1–5 every 2 weeks; hydroxyurea 500 mg twice daily on days 0–5 every 2 weeks	70 Gy on days 1–5 (hyperfractionation) week on/week off	77% 2-year OS and 70% 3-year OS. Combined with induction chemotherapy
Cisplatin and cetuximab			
Pfister *et al.* (2006) (22)	Cisplatin 100 mg/m^2 during weeks 1 and 4; cetuximab 400 mg/m^2 during week 1, then 250 mg/m^2 weekly	70 Gy (concomitant RT boost)	76% 3-year OS. Trial ended early because of two deaths and adverse effects. Two randomized trials with this regimen are ongoing (RTOG and the University of Chicago, Chicago, IL)
IFHX			
Cohen *et al.* (2005) (69)	Gefitinib 250 mg daily; 5-FU 600 mg/m^2 CI on days 1–5 every 2 weeks; hydroxyurea 500 mg twice daily on days 0–5 every 2 weeks	70–72 Gy on days 1–5 (hyperfractionation) week on/week off	89% 2-year OS. Combined with induction chemotherapy

Table 6: Ovetview of multi-agent chemoradiotherapy trials in head and neck cancers.

TOXICITIES OF CONCURRENT CHEMO RADIATION:

RTOG SCORING CRITERIA:

	Grade (0)	Grade (1)	Grade (2)	Grade (3)	Grade (4)
SKIN	No difference from baseline	Faint or Follicular, or dull erythema/ epilation/dry desquamation/ impaired sweating	Tender or bright erythema, patchy moist desquamation/ moderate oedema	Confluent, moist desquamation other than skin folds, pitting oedema	Ulceration, haemorrhage, necrosis
MUCOUS MEMBRANE	No difference from baseline	Injection/ may experience mild pain not requiring analgesic	Patchy mucositis leading to inflammatory serosanguinitis discharge/ moderate pain requiring analgesia	Confluent fibrinous mucositis/ severe pain requiring narcotic	Ulceration, haemorrhage or necrosis
HEMATOLOGIC WBC- (X1000	≥ 4.0	4.0 - 3.0	3.0 - 2.0	2.0 - 1.0	<1.0
PLATELETS (X 1000)	>=100	75 - <100	50 - <75	25 - <50	<25 or spontaneous bleeding
HEMOGLOBIN (GM %)	>11	11-9.5	<9.5 - 7.5	<7.5 - 5.0	-------

Table 7- RTOG acute toxicity criteria

COMMON TOXICITY CRITERIA:

	GRADE 0	GRADE 1	GRADE 2	GRADE 3	GRADE 4
EMESIS	none	1 episode in 24 hours;IVfluids indicated in < 24 hours.	2-5 episodes in 24hours	>6 episodes in 24 hours; IV fluids, or TPN indicated > 24 hrs	Life-threatening consequences

Table 8- CTC for emesis

The toxicities during concurrent chemo radiation depends on chemotherapy agents used, dose per fraction, fractionation used. Apart from toxicities due to individual chemotherapy the other toxicities are graded as per RTOG toxicity criteria. The toxicities include

- Dermatitis
- Mucositis
- Emesis
- Neutropenia
- Anemia
- Thrombocytopenia

I.4.CISPLATIN AND RADIATION:

CISPLATIN

HISTORY:

In 1845 Michele Peyrone first described the compound named cis-platin (Cis-diamminedichlroplatinum(II) and historically it was called as Peyrone's chloride. Later in 1893 the structure of cisplatin was first synthesized and found as a part of coordination theory revolution by the swiss chemist Alfred Werner who was also called as father of coordination theory. After decades of obscurity cisplatin becomes popular in early 1960 when Barnett Rosenberg (11)from Michigan state university discovered the cytostatic effect of cisplatin. Barnett began series of experiments when electrolysis of platinum electrodes produces platinum salts which inhibit the cell division of bacteria E.coli but cell continues to grow upto 300 times the normal length and this made cisplatin to test against cancer in mice. It also found that only cis-platin has anticancer effects but not trans-platin. Cisplatin enter into clinical trial in 1971 and got FDA approved for treatment in testicular and ovarian cancer in 1978.

STRUCTURE AND SYNTHESIS:

The chemical formula for is cis-[Pt(NH3)2(Cl)2]. It is a yellow crystalline crystal with molecular weight 300. The synthesis of cisplatin has improved much as earlier synthesis contains lot of impurities and rapid synthesis of cisplatin came into practice after it is first described by Dhara(12) in 1970. Now majority of synthesis is based on Dhara's synthesis. In this K2(PtCl4) added to KI and converted into potassium tetraiodo palatinate. To this NH3 is added and forms yellow compound *cis*-[PtI2(NH3)2] and AgNO3 is added to form precipitated AgI and Pt(OH2)2(NH3)2]2. The later compound is treated with excess KCl and resulting in final product cisplatin as yellow powder.

DHARA'S SYNTHESIS OF CISPLATIN

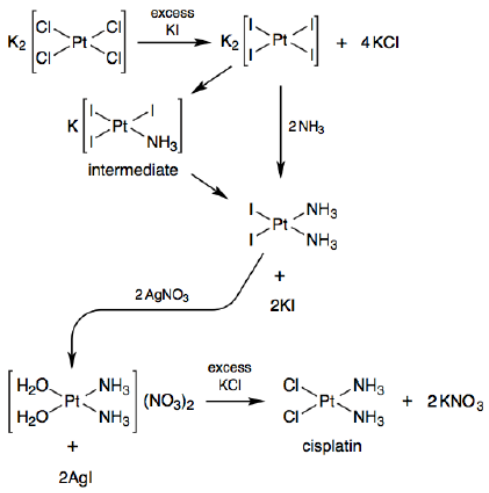


Fig 8: synthesis of cisplatin.

MECHANISM OF ACTION:

Cisplatin enters blood stream along with saline solution and drug remains intact due to high concentration of chloride. It enters cell by passive diffusion or active uptake by copper binding proteins. Inside cell chloride ions concentration is low and chloride ligand is replaced by water to form reactive charged species so that it cannot move out of the cell.

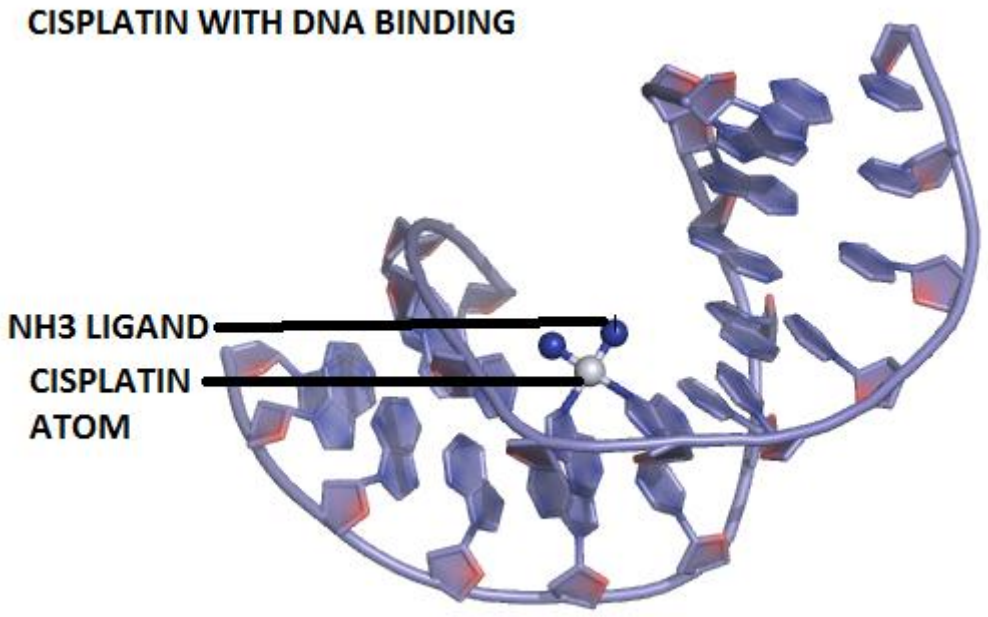


Fig 9: Binding of cisplatin with DNA.

The resulting [PtCl(H2O)(NH3)2]+ compound allows platinum to bind guanine bases of DNA forms [PtCl(guanine-DNA)(NH3)2]+ and cross linking occurs when it react guanine causing displacement of chloride ligand. Cisplatin

crosslinks with DNA in different ways and cause DNA damage and finally leads to apoptosis.

Cisplatin interacts with proteins which also contribute in cellular mitosis although this is not primary mode of mechanism. Cisplatin interacts with RNA in similar fashion as with DNA. RNA can be replaced easily and only <10% RNA is damaged at lethal dose of cisplatin.

DNA CROSS LINKS

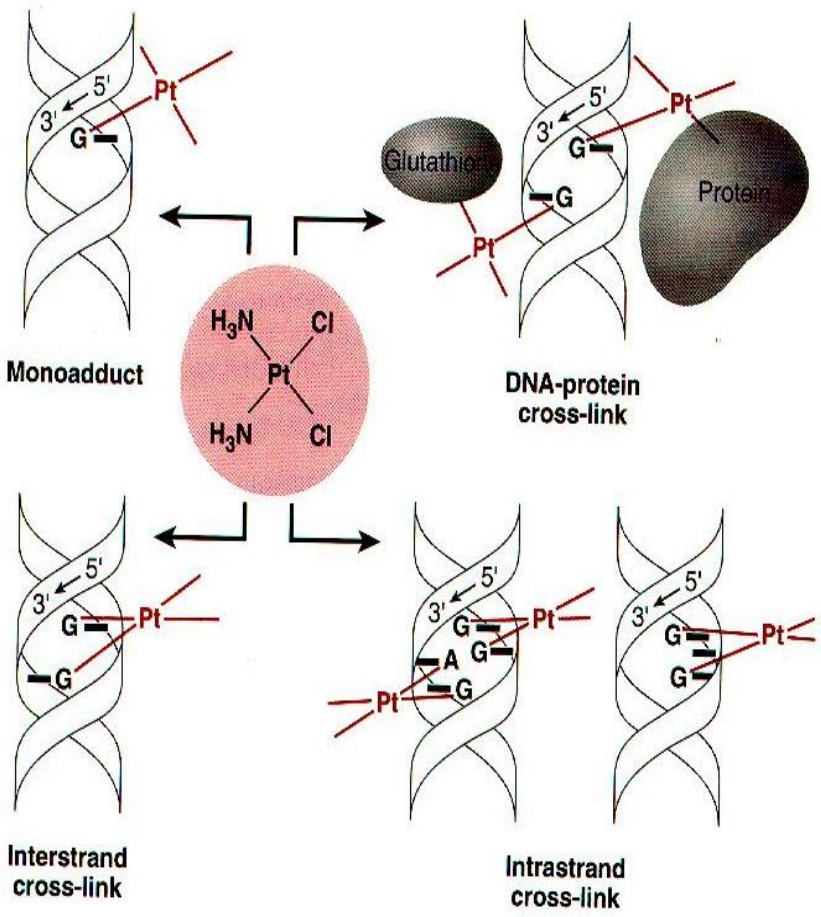


Fig 10- DNA cross links caused by cisplatin

RESISTANCE:

The major mechanism of acquired cisplatin resistance is reduction in accumulation of cisplatin inside cancer cells due to barriers across cell membrane. The other mechanisms are faster repair of DNA adducts , increased detoxification of drug, inhibition of apoptosis, increased DNA repair enzymes and deficiency of MMR enzymes(hMLH1, hMSH2).

ABSORPTION:

Cisplatin not absorbed orally. Cisplatin absorption when administered systemically is rapid and complete after administered intraperitoneally.

DISTRIBUTION:

Volume of distribution is 0.28L/Kg and distributed widely in body with increased uptake in liver, kidneys and intestine. More than 90% are bind to plasma proteins.

METABOLISM:

The plasma clearance of platinum occurs rapidly during first four hours following intravenous administration and proceeds slowly due to covalent binding to serum proteins like albumin, transferring and gamma-globulin. The half life of cisplatin is 30 to 100 hours depending on rate of infusion.

EXCRETION:

Cisplatin is eliminated via urine. After first 2-4 hours of intravenous administration about 15-25% of cisplatin rapidly excreted and intact form. In first 24 hours 20-82% is excreted as protein bound.

TOXICITY:

1. Nephrotoxicity- dose limiting toxicity
2. Nausea and vomiting
3. Myelosuppression
4. Hypomagnesimia and hypocalcemia
5. Neurotoxicity

6. Ototoxicity
7. Metallic taste of food and loss of appetite
8. Hypersensititvity reactions
9. Transient elevation of LFT
10. Ocular toxicity like optic neuritis and papilledema are rare.

INTERACTION OF CISPLATIN AND RADIATION:

Cisplatin were given along with radiation therapy since early 1970's. The first published paper work based on radiobiological aspects was by Wodinsky et al in 1974 where there is increased survival in mice when treated with combined cisplatin and radiation. In 1976 Richmond et al and powers demonstrated increased DNA damage in bacterial spores and E.coli when cisplatin concurrent with radiation is given and found cisplatin as a radio sensitizer. Cisplatin mediated radiation sensitization mechanism were summarized by Wilson et al. Both cisplatin and ionizing radiation has common target DNA. Radiation acts on DNA and cause potentially lethal damage and lethal damage. Cisplatin inhibit the repair of potentially lethal damage caused by radiation through free electron scavenging capacity and cause cell kill through additive mechanism. Radiation produces free radicals which produces toxic platinum intermediates and finally resulted in cell

killing. Radiation therapy also causes increased cellular uptake of cisplatin. All these interaction leads to increased cell cycle arrest and apoptosis. Douple and Richmond explore the optimal sequencing of cisplatin and radiation through their work in mouse mammary tumor model. They found that tumor regression was increased when cisplatin was given one hour before radiation compared to other sequence.

Cisplatin enhances the effect of radiation on cell kill when given concurrently through several mechanisms:

1. Increased formation of toxic platinum intermediates in the presence of radiation induced free radicals
2. Inhibition of DNA repair
3. Radiation induced increase in uptake of cisplatin intracellularly
4. Cell cycle arrest

Radiosensitization of cisplatin is important in the treatment of hypoxic cell fraction. If hypoxic cells are treated by radiation alone there is lack or inadequate formation of free radicals which causes sublethal damage and potentially damage. When cisplatin is given concurrent with radiation in inhibits the recovery of the hypoxic cells from the sublethal damage which cause radiosensitization and

increasing cell kill. The formation of toxic cisplatin products in the presence of radiation also targets intracellular and extracellular sites in radiation field. It also addresses systemic disease if adequate dose is given.

I.5.BLEOMYCIN AND RADIATION:

BLEOMYCIN:

HISTORY:

The Japanese scientist Hamao Umezawa(2) and his coworkers during their study of water soluble antibiotics found kanamycin, alboverticillin and phleomycin. Phleomycin is the first copper containing antibiotic found in nature and was unique in its blue color. In 1956 after few years of phleomycin discovery, it was found to inhibit Ehrlich carcinoma with a high therapeutic index and to inhibit DNA synthesis. As it showed irreversible nephrotoxicity in dog, it was not tested clinically. After searching for phleomycin containing antibiotic again Umezawa and his coworkers discovered bleomycin in 1966. Bleomycin was isolated from cultures of streptomyces verticillus. It was similar to phleomycin in blue color and copper containing antibiotic but different from phleomycin in stability and UV spectrum. Bleomycin is a stable substance and also stable in aqueous solution.

Bleomycin did not show any nephrotoxicity like phleomycin but it caused reversible hepatotoxicity. Clinical study of bleomycin conducted and Ichikawa et al found anticancer effect on squamous cell carcinoma followed by Kimura et al and krakoff et al found is effect on malignant lymphoma. Bleomycin was also active against ecrlich ascitic and solid tumors as well as sarcoma. After Umezawa published his discovery, in 1969 Nippon kayaku launched bleomycin in Japan. It got US FDA approval in 1973.

STRUCTURE & SYNTHESIS OF BLEOMYCIN:

Bleomycin was isolated as a mixture of 13 gylycopeptides from filtrates of Fungus streptomyces verticillus. After 12 years of bleomycin was isolated, the conclusive structure of bleomycin and the copper complex (10) the natural form produced by fermentation were finally determined in 1978. Various bleomycins like glycopeptides from streptomyces verticillus differ in terminal carboxy amine moiety. Biogenetically all of these amines are methionine, histididne and arginine.

Terminal Amines of Natural Bleomycins

Bleomycin	Terminal amine	Bleomycin	Terminal amine
1*	NH_2-$(CH_2)_3$-S-CH_3 (O)	A2'-c	NH_2-$(CH_2)_3$ 
Demethyl-A2*	NH_2-$(CH_2)_3$-S-CH_3 (X^-)	A5	NH_2-$(CH_2)_3$-NH-$(CH_2)_4$-NH_2
A2	NH_2-$(CH_2)_3$-S-$(CH_2)_2$ (+)	A6	NH_2-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$--NH_2
A2'-a	NH_2-$(CH_2)_4$-NH_2	B2	NH_2-$(CH_2)_4$-NH-C-NH_2 (NH)
A2'-b	NH_2-$(CH_2)_3$-NH_2	B4	NH_2-$(CH_2)_4$-NH-C-NH-$(CH_2)_4$-NH--C-NH_2 (NH) (NH)

*Derived from bleomycin A2 spontaneously.

Table 9: Tertiary amines of natural bleomycin

Apart from natural amine from which bleomycin produced , new bleomycin can be produced by culturing streptomyces verticillus in a medium containing special amine which is not present in nature.

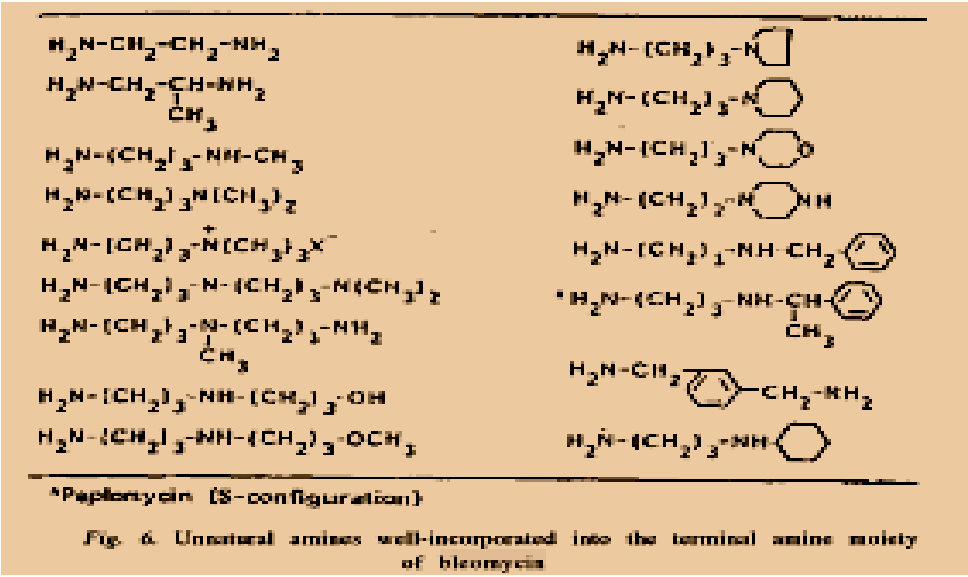


Fig. 6. Unnatural amines well-incorporated into the terminal amine moiety of bleomycin

Table 10- Unnatural amines well incorporated into terminal amine moiety of bleomycin]

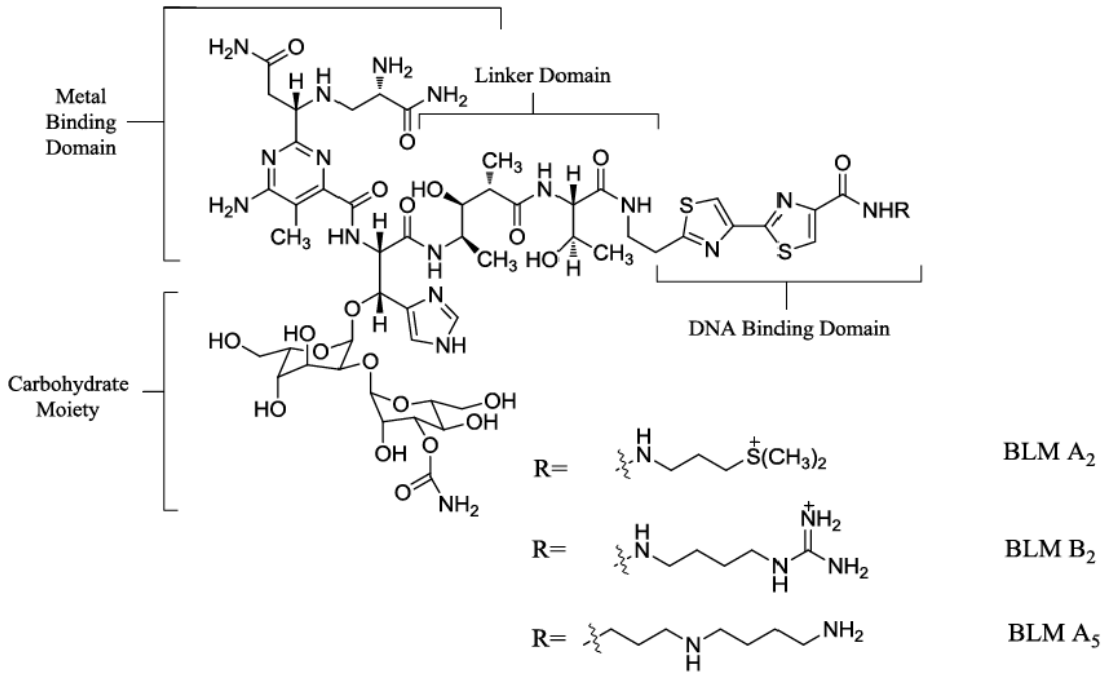
In 1982, the first total synthesis of bleomycin was published. On the basis of this bleomycin is divided into four distinct domains.

1) Metal binding domain formed by the pyrimidobalmic acid subunit along with the adjacent *â*-hydroxyl histidine . This domain act as the coordination site required for Fe(II) complexation and molecular oxygen activation responsible for DNA cleavage.
2) Bithiazole domain also called DNA binding domain located at the carboxy terminus of bleomycin . This is linked to variety of functionalized side chains (BLM A2, BLM B2, BLMA5, BLMA6) which contains either inherent inherent posistive charge or a polyamine chain that is positively charged in physiological conditions. This structural property allows interaction between positively charged bleomycin and the negatively charged phosphate of nucleic acid thus results in the binding of bithiazole domain into the minor groove of DNA and subsequent cleavage.

3) The linker domain-(2S,3S,4R)-4amino-3-hydroxy-2-methylpentanoic acid subunit provides the connectivity between metal binding domain and bithiazole domain and its length correlates with efficacy of DNA cleavage by bleomycin.

4) The carbohydrate domain is essential for full activity of bleomycin. It is also likely to participate in cell recognition by bleomycin and in cellular uptake.

BLEOMYCIN- THE FOUR DOMAINS AND TERMINAL AMINE MOIETY



MECHANISM OF ACTION OF BLEOMYCIN:

Bleomycin(6) inhibits eukaryotic and prokaryotic cell proliferation and prevents replication of DNA virus. In intact cells it mainly affects DNA synthesis and to lesser extent RNA and protein synthesis. It acts preferentially acts on cells that are actively dividing cells particularly blocks G2 and M phase of cell cycle.

INTERACTION OF BLEOMYCIN WITH DNA:

Bleomycin has dual properties of binding to DNA and chelating various metal ions, particularly iron and copper. Bleomycin-mediated DNA degradation requires the presence of a redox-active metal ion such as Fe^{2+} or Cu^{+}, as well as molecular oxygen. Bleomycin binds through metal binding domain with Fe^{2+} derived from hemoglobin or Cu^{2+} from plasma. It forms a complex with a molecule of oxygen to form bleomycin-Fe^{2+}-O_2 and when mixed with DNA an activated complex containing dioxygen and two molecules of DNA bound iron-bleomycin is formed. During activation, the reduced Fe^{2+} oxidized to Fe^{3+} and results in the production of free radicals that cause DNA strand breakage. In overall reaction bleomycin acts as ferrous oxidase which catalyses oxidization of Fe^{2+} to Fe^{3+} and reduction of oxygen. Fe^{2+} bleomycin can be regenerated from Fe^{3+} bleomycin by an NADH dependent system present in the nucleus. This redox recycling resulted in increased DNA damage.

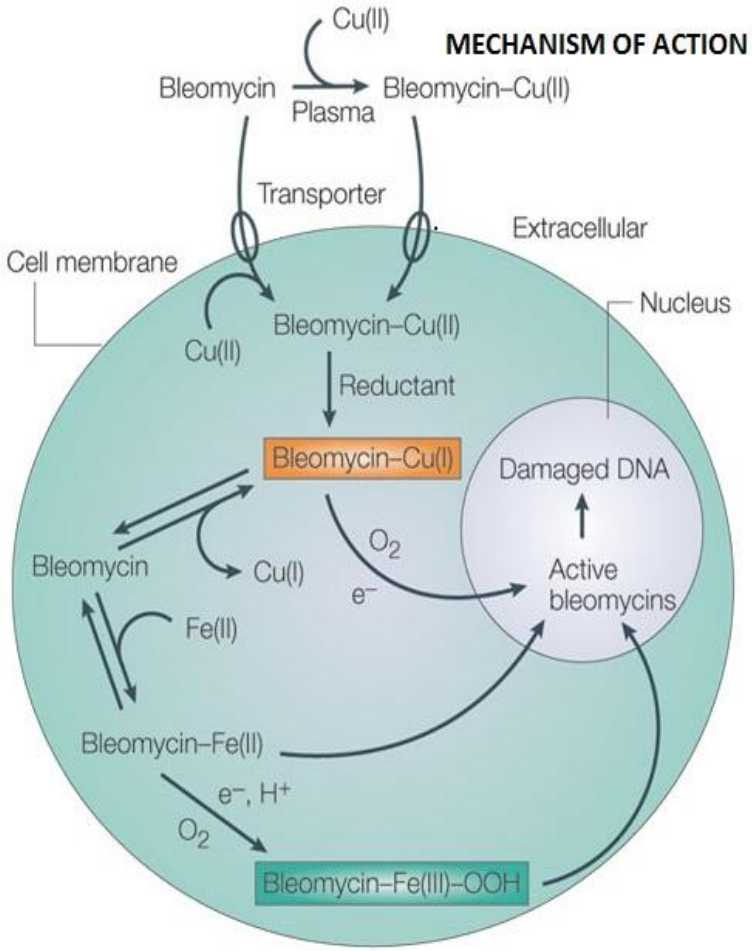
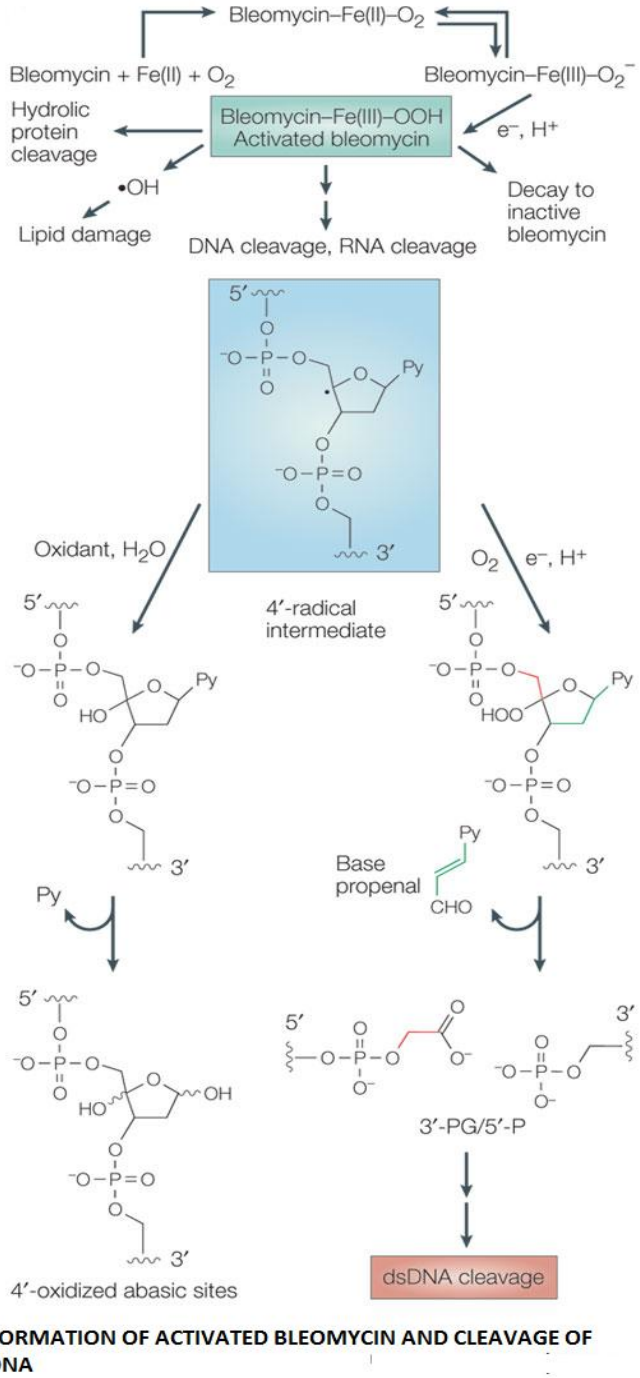


Figure 11- Mechanism of action of bleomycin

The activated drug bleomycin-Fe3+-O2 can intercalate with DNA to produce atleast four types of DNA lesions. The extent of DNA lesion depends upon the oxygen content of the cell. In the absence of oxygen bleomycin abstracts a hydrogen atom from deoxyribose at C4' position and moves it to guanine. This converts deoxyribose to an unstable sugar compound to form a mutagenic lesion,

purinic/ apyridiminic (AP) site, where the DNA strand intact but it lacks a base.



FORMATION OF ACTIVATED BLEOMYCIN AND CLEAVAGE OF DNA

Fig 12-Formation of activated bleomycin and cleavage of DNA

In the presence of oxygen, bleomycin can damage sugar moiety which results in lack of template formation by DNA polymerase and bleomycin induced single strand breaks occurs at 3'end with a fragment of sugar. This latter lesion 3'-phosphoglycolate to be removed in order to promote cell division as it blocks DNA repair synthesis.

The remaining portion of the fragmented sugar exists in the free base propenal form and form base adducts when it reacts with DNA. For example, the base propenal bears a malondialdehyde moiety, reacts with guanine to form adduct pyrimidopurinone of deoxyguanosine. Bleomycin-Fe2+-O2 complex creates an AP site on one strand and a directly opposed single strand break on the complementary strand which produces bi- stranded DNA lesions at certain sequences like CGCC. The bi-stranded lesions converted into double stranded break when there is spontaneous cleavage of the AP site by primary amines.

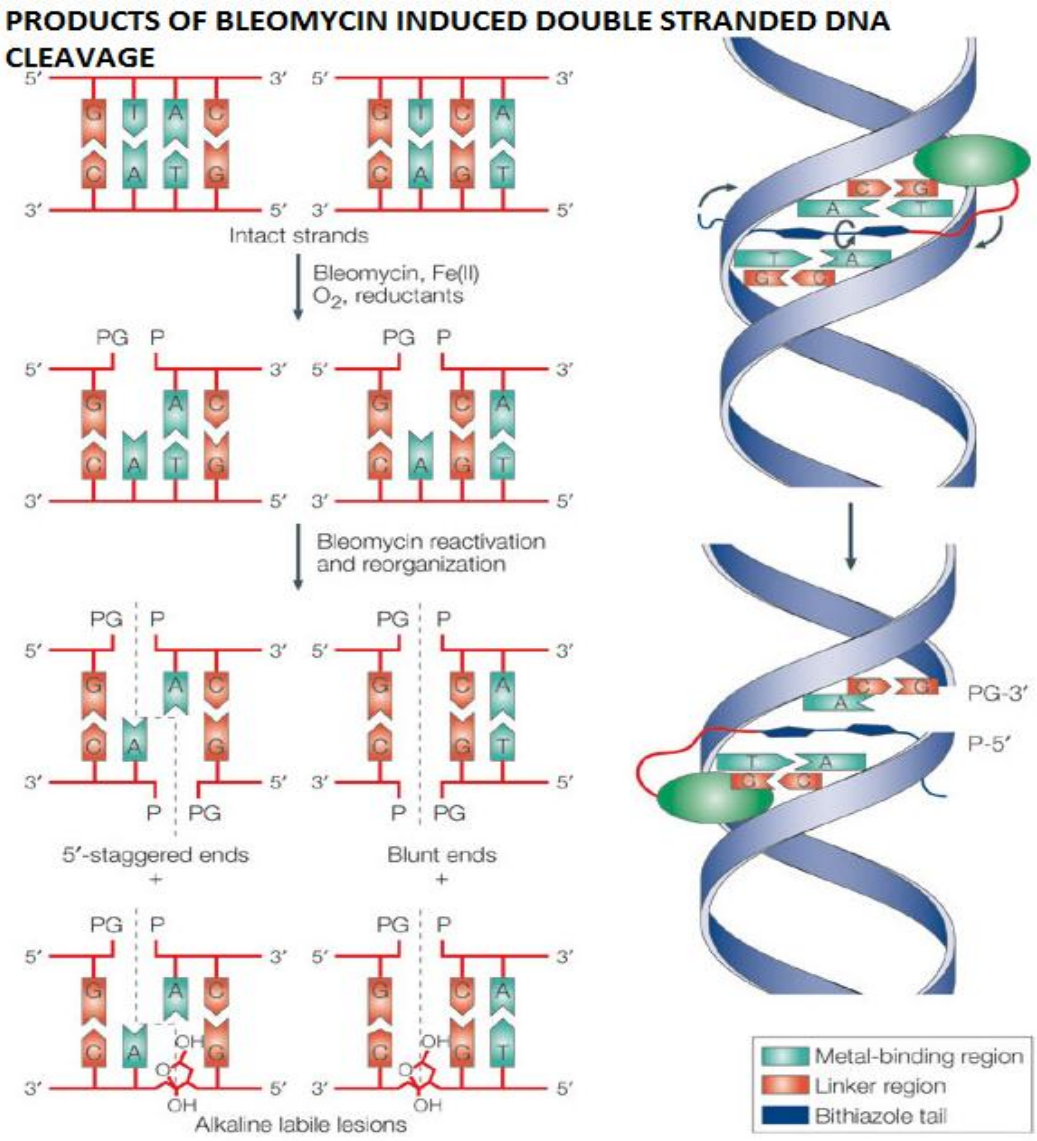


Fig 13-Products of bleomycin induced double stranded DNA cleavage

DNA cleavage by bleomycin is sequence selective fashion at a subset of 5'GC3' and 5'GT3' sites. Bleomycin mediated DNA cleavage has two primary pathways

1) Frank strand scission, which produces base propenals and results directly in DNA cleavage.
2) Via modified nucleotides that result in alkali labile lesions and subsequent cleavage sites in the presence of a base.

INTERACTION OF BLEOMYCIN WITH DNA

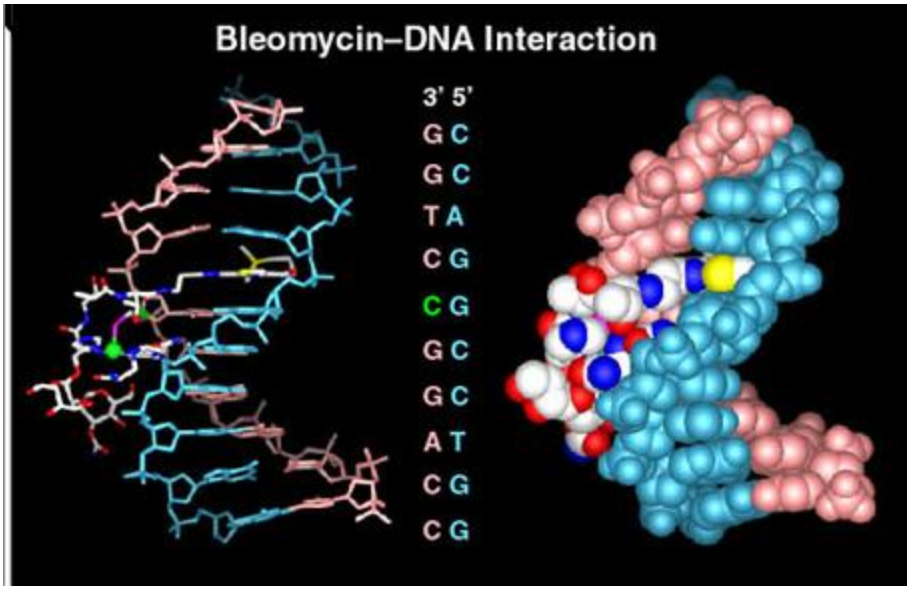


Fig 13-Interaction of bleomycin with DNA

OTHER BLEOMYCIN INDUCED CELLULAR DAMAGE:

In addition to DNA cleavage bleomycin complex cleaves varieties of RNA. By oxidation bleomycin cleaves mRNA, rRNA and tRNA. Bleomycin causes release of reactive oxygen species and cause cell and nuclear membrane peroxidation, lipid peroxidation, altered intracellular prostaglandin metabolism and

carbohydrate oxidation. This also contributes to cell death and damage but this effect can be inhibited by antioxidants.

RESISTANCE:

Several mechanisms of resistance to bleomycin have been described. Factors include increased drug inactivation, decreased drug accumulation, increased DNA repair and to lesser extent hypoxia. The major mechanism of resistance to bleomycin is due to bleomycin hydrolase, an aminopeptidase converts bleomycin into inactive form by replacing terminal amine of bleomycin with hydroxyl which is essential for iron binding and cytotoxic activity. The low levels of bleomycin hydrolase in lungs and skin explains increased toxicity of bleomycin in these organs. The normal tissue with high level this enzymes such as liver, bone marrow and spleen exhibit less susceptibility of bleomycin toxicity.

PHARMACOKINETICS:

ABSORPTION:

Bleomycin is well absorbed when administered parenterally as it is poorly absorbed across GI tract. Following are the parental routes of administration that

include intramuscular, intravenous, subcutaneous, intrapleural, intraperitoneal and attain peak plasma concentration in 30 to 60 minutes.

Routes of administration	Bioavailability
Oral	5%
Intravenous	100%
Intramuscular	100%
Subcutaneous	70%
Intraperitoneal	45%
Intrapleural	45%

Table11- Routes of administration of bleomycin and its bioavailability

Serum peak plasma concentration after administration of 15,000IU bleomycin following intramuscular and intravenous administration were 1 IU/mL and 3.3IU/mL respectively.

DISTRIBUTION:

The mean volume of distribution of bleomycin is 17.5L/m2 or 0.35L/kg. It is widely distributed throughout the body with peak concentrations of activated form

in skin, lungs, kidneys and bladder and inactivated form in liver and spleen. It does not cross the blood brain barrier. In mice high concentration of bleomycin found in skin, lung, kidney, lymphatics, peritoneum and tumor if present. The highest concentration is found in amniotic fluid, placenta but lesser concentration fetus of pregnant mice.

PROTEIN BINDING:

Bleomycin has less than 10 percent of plasma protein binding capacity.

METABOLISM:

Bleomycin undergoes extensive metabolism by cytosolic enzyme cystiene proteinase, Bleomycin hydrolase. The enzyme is distributed widely in most tissues.

EXCRETION:

The major route of elimination is through kidneys. With 24 hours 60-70% of an administered dose of bleomycin excreted in urine. In patients with normal renal function half life is 2-4 hours. In patients with renal dysfunction excretion of the drug is prolonged. For patients with creatinine clearance >35mL/minute the serum terminal elimination half life is 115 minutes. For patients with creatinine clearance >35mL/min serum terminal half life increases. In patients with renal dysfunction

40-50% of dose reduction is recommended. Toxicity is increased in patients with renal dysfunction if dose is not reduced.

TOXICITY:

1) The most common side effect of bleomycin is mucocutaneous toxicity. It includes mucositis, erythema, striae, rash, hyperpigmentation and vesiculation. Less common manifestation of skin includes dermatitis, hyperkeratosis, skin peeling and thickening, pruritis and alopecia. Skin toxicity correlates with cumulative dose of bleomycin and mostly manifest when cumulative dose of 150-200U is reached.

2) Fever and chills occurs in 25-5o% of patients following 2-8hours of drug administration and lasts up to 48 hours.

SIDE EFFECTS	INCIDENCE
SKIN TOXICITY	44%
FEVER AND CHILLS	31%
NAUSEA AND VOMITING	26%
STOMATITIS	16%
ALOPECIA	11%
PULMONARY TOXICITY	10%
FATAL PULMONARY TOXICITY	1%
ANAPHYLACTOID REACTION	<1%

Table 11- Incidence of side effects of bleomycin

3) Nausea, vomiting and tiredness occur in few patients.

4) The dose limiting toxicity of bleomycin is pulmonary toxicity and occurs in 10% of patients. It occurs as interstitial pneumonitis, subacute or chronic pneumonitis which progresses into pulmonary fibrosis and become fatal in 1% of patient. Routine chest Xray, DLCO should be taken to monitor bleomycin therapy. Reduction of 40% value of DLCO compared to pretreatment values warranted to stop further bleomycin therapy. The early symptom of bleomycin induced pulmonary toxicity is dyspnea, cough and low grade fever. Pulmonary function teat shows restrictive pattern. Pulmonary toxicity is age and dose dependent. Older persons more than 70 years of age and cumulative dose >400U associated with increased risk of pulmonary toxicity.

5) Anaphylactic reactions are rare but occurs in <1% of patients with lymphoma. It manifests as hypotension, chills, fever, dyspnea. Cardiovascular toxicity like raynauds phenomenon, cerebral infarction, myocardial infarction rarely reported.

6) Myelosupression is rare.

INTERACTION OF BLEOMYCIN AND RADIATION:

Bleomycin was one of the first drugs found to sensitize the effects of radiation. As bleomycin is effective against squamous cell carcinoma it is used along with radiation therapy as a single agent or along with other chemotherapeutic agents.

BLEOMYCIN IN VITRO

In 1972 Matsuzawa et al first report the radiosensitizing effect of bleomycin in vitro. There is reduction of the shoulder on radiation survival curve when bleomycin is used and indicates that bleomycin may inhibit sublethal damage together with direct radiosensitivity. Bleomycin acts as similar fashion as radition with increased sensitivity in G2 and M phase so that their interaction is additive. But bleomycin has little effect on hypoxic cells. Other studies also confirmed the same effect of bleomycin with radiation.

IN VIVO:

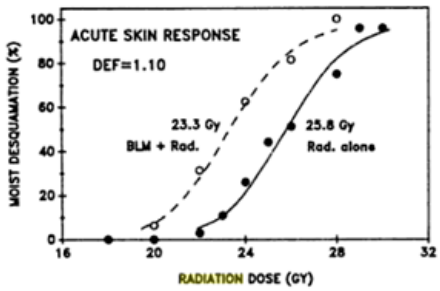
In 1972 Juul Jorgenson first demonstrated the interaction of ionizing radiation and bleomycin in murine carcinoma in vivo and showed that combination of bleomycin and radiation is synergistic when given simultaneously in mouse

model. This study leads to the introduction of bleomycin and ionizing radiation therapy into clinical trials.

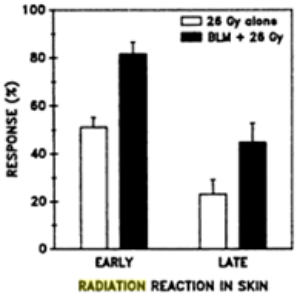
The sensitivity of bleomycin combination with radiation is differed in various experimental systems due to varying hypoxic fractions of cells. Effect depends upon the sequence and interval between applications of bleomycin and radiation suggestive of both direct effect of bleomycin on enhancing radiation and additive independent effect of two treatment modalities.

IN NORMAL TISUES:

Interaction of bleomycin and ionizing radiation was tested in several normal tissues mostly of ectodermal origin like skin, lip, mucosa, intestines, lung and several tissues.



Effect of bleomycin treatment for dose response realationship for radiation induced acute skin damage .
Increase in Dose effect factor(DEF).



Similar increase in late skin damage

When bleomycin and radiation acts on normal skin and lip mucosa there is increased in acute skin reaction and also increase in late damage than radiation alone. Late damage in the form of fibrosis and skin contracture and indicates severe risk of bleomycin and radiation combination. Hematopoietic tissue is not affected by bleomycin alone and also combined bleomycin radiation treatment. There is enhancement of radiation reaction in intestinal crypt cells due to interaction between radiation and bleomycin mainly and also due to direct effect of bleomycin alone. Bleomycin has direct effect on lung and also enhanced response when lung radiation is combined with it. Enhancement of radiation is increased by bleomycin when it gives simultaneously with radiation. If bleomycin is administered with long interval from radiation it reduces the radiation response.

Krishnamurthy et al describes that combination of bleomycin and radiation Causes

- Revascularisation and reoxygenation of the shrinking tumor when combinations were used.

- Barranco and Humphery established that fractionated dose of bleomycin has superior killing of tumor cells both in vitro and vivo and hence when bleomycin and radiation given in fractionated doses there is increased cell killing.

- Bleomycin inhibits the sublethal injury and fractionated bleomycin and radiation has increased cell killing.

- Rapidly dividing cells destroyed more compared to slow or undivided cells by radiation. Due to synchrony induced by bleomycin at S/G2 and synergism by bleomycin + radiation at G2-M phase enhances the cell killing.

REVIEW OF LITERATURE

EFFECT OF BLEOMYCIN RADIOTHERAPY COMBINATION IN MANAGEMENT OF HEAD AND NECK SQUAMOUS CELL CARCINOMA: Cancer volume 48, Issues 5, pages1106-1109, September 1981. Pankaj M. Shah et al.(19)

Fifty nine patients with squamous cell carcinoma of head and neck were included in the study. Of 59 patients included in the study 23 patients were treated with combined bleomycin and radiation therapy and 36 patients were treated with radiotherapy alone. Bleomycin were used in the dose of 15mg I.V on alternate days upto total dose of 150mg and radiation therapy is given within half an hour of bleomycin. Radiation therapy was given to all patients 200cGy per fraction daily six days per week upto TD-40-60Gy. Increased mucositis is reportedin bleomycin and radiation arm compared to radiation alone arm (82.6% vs 50%). However mucositis requiring treatment interruptions in bleomycin combined with radiation arm is 21.7 % compared to radiation alone arm 11.1%. Though there is slight increase in bleomycin radiation group (65.2%) compared to radiation alone arm(58.3%), it is not statistically significant.

COMBINED BLEOMYCIN TREATMENT AND RADIATION THERAPYIN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK REGION PARVINEM et al. Acta radiation oncolgica 1985 nov-dec:24(6): 487-9.(14)

Patient with squamous cell carcinoma of head and neck were included in the study. Patients randomized into bleomycin and radiation (25) and radiation alone (21). Bleomycin was given in the dose of 7-15mg I.M. 1 hour before radiation during first and third week up to total dose of 75mg or 150mg. Cobalt radiation therapy is given to both primary and lymph nodes in dose of 200cGy per fraction per day and five days in a week. After 30-32 Gy depending upon positive cases patients were taken for surgery in both groups. Among the operated patient there is significant difference in absence of viable cells in postoperative specimen among bleomycin and radiation group. In patients who have clinical complete response radiation completed up to 55-60Gy. No difference in toxicity causing treatment breaks due to mucositis. No statistically significant differences found in recurrence and survival between both groups.

COMBINED USE OF BLEOMYCIN WITH RADIATION IN THE TREATMENT OF CANCER CANCER VOLUME 63, 1978, pp 169-78 M.Abe et al(20)

During 1971-74 from 18 institutions of Kansai district a cooperative study was conducted to determine the effect of bleomycin and radiation in treatment of oral cavity, esophagus and bronchogenic carcinoma. Of total 189 patients, 67 patients were oral cavity cancer randomized to bleomycin combination with radiation and radiation alone by envelope method. Bleomycin was given in the dose of 15mg intravenously twice weekly upto total dose of 90mg over 3 weeks. Radiation therapy was given in the dose of 200cGy per fraction per day for five days per week upto 30Gy. At the end of 30Gy patient was treated with surgery or radiation therapy depending upon participating doctors. Data showed that there is significant increased tumor regression and complete response in patients with bleomycin and radiation therapy compared to radiation alone. The 18 month survival rate for the combined bleomycin radiation group is increased by 20% compared to radiation alone.

COMBINED THERAPY OF ORAL CANCER BLEOMYCIN AND RADIATION: A CLINICAL TRIAL.Clinical radiology 1977 28, 427-429.V.SHANTA & S.KRISHNAMURTHI

One hundred and fifty seven patients of squamous cell carcinoma of oral cavity were randomized into bleomycin plus radiation and radiation alone. Of 157 patients 87 patients received bleomycin and radiation and 73 patients received radiation alone. Bleomycin were given intravenously or intra-arterialy in dose of 10-15mg twice or three times a week or intramuscularly in dose of 30mg twice a week to total dose of 150mg. Cobalt radiotherapy given to the dose of 6 to 7.5Gy per week in combined bleomycin radiation group to total dose of 55-60Gy and 10Gy per week to total dose of 65Gy in radiation alone group. Significant favorable response is reported in combined bleomycin radiation arm (77%) compared to radiation alone (20.9%). There is significant increase in five year disease free survival in combined bleomycin radiation group (65.5%) against radiation alone (23.5%).

BLEOMYCIN AND RADIATION THERAPY IN SQUAMOUS CELL CARCINOMA OF THE UPPER AERO-DIGESTIVE TRACT: A PHASE III CLINICAL TRIAL IJROBP NOV-1985, VOL 11, 1877-86 VERMUND H et al. (15)

A prospective randomized and stratified clinical trial conducted in group of 222 patients with squamous cell carcinoma of the upper aero digestive tract. Paired randomization technique is use prospectively to compare radiation therapy alone to bleomycin and radiation therapy. Of 222 patients, 65 patients had larynx cancer, 85 patients had oral cavity cancer, 23 patients had Oropharynx cancer, 12 patients had nasopharynx cancer, 19 patients had hypopharynx cancer and 18 patients had sinuses cancer. 70% patients had stage III & IV cancer. Only seven percent of the patients were below 50 years of age and most of them were elderly. Twenty seven percent of the patients were more than 70 years age. 111 patients received radiation alone and 111 patients received both bleomycin and radiation. Radiation delivered in dose of 200cGy/fraction, five days in a week to TD-70Gy. Bleomycin was given in dose of 5mg intramuscularly per day an hour before radiation. Patient who had residual disease received interstitial radiation or surgery. Palliative chemotherapy is given for late recurrences. The response of tumor and tumor control were similar

for radiation alone and combined bleomycin and radiation. Two year and five year survival percentages were equal in both groups.

COMBINED RADIATION THERAPY AND 5-FLUROURACIL FOR ADVANCED SQUAMOUS CELL CARCINOMA OF THE ORAL CAVITY AND OROPHARYNX: A RANDOMIZED STUDY. Lo TC et al.

In 1961, 136 patients with oral cavity cancer and oropharyngeal cancer were randomized into combined radiotherapy and 5-FU and radiotherapy alone. In combined 5-FU and radiotherapy there is increased local control and survival. But it is statistically significant in oral cavity cancer only.

META-ANALYSIS OF CHEMOTHERAPY IN HEAD AND NECK CANCER(MACH-NC): An update of 93 randomized trial and 17,346 patients; PIGNON et al

Four categories according to tumour location: oral cavity, oropharynx, hypopharynx and larynx.16,192 patients were analysed, with a median follow-up of 5.6 years. The benefit of the addition of chemotherapy is consistent in all tumour locations, with hazard ratios between 0.87 and 0.88. Magnitude of benefit higher for platinum based chemo (0.75) than for other chemo (0.86) [p=0.01].

The interaction test between chemotherapy timing and treatment effect was only significant for oropharyngeal ($p < 0.0001$) and laryngeal tumours ($p = 0.05$). The 5-year absolute benefits associated with the concomitant chemotherapy are 8.9%, 8.1%, 5.4% and 4% for oral cavity, oropharynx, larynx and hypopharynx tumours, respectively.

II.1 AIM OF THE STUDY

To compare the acute toxicity, treatment breaks, clinical response and its impact on survival between cisplatin and bleomycin versus cisplatin alone concurrent with radiation therapy in locally advanced squamous cell carcinoma of oral cavity from the Tertiary cancer centre, South India between january 2009 and decembeer 2011.

II.2.OBJECTIVES OF THE STUDY

PRIMARY OBJECTIVES:

1. To compare the clinical response of primary and regional lymph nodes between the patients treated with cisplatin and bleomycin versus cisplatin alone concurrent with radiation for locally advanced squamous cell carcinoma during the period from January 2009 to December 2011.

2. To compare the disease free survival and overall survival between the patients treated with cisplatin and bleomycin versus cisplatin alone concurrent with radiation

SECONDARY OBJECTIVES:

1. Acute toxicity is defined and graded as per RTOG toxicity criteria .Acute toxicity of the treatment such as myelosupression, vomiting, mucocutaneous toxicity, pneumonitis were compared between the patients treated with cisplatin and bleomycin versus cisplatin alone concurrent with radiation.

2. Treatment breaks defined as interruptions of radiotherapy treatment of more than five days continuously due to side effect of treatment and comparison made between two treatment groups and its impact on survival rate is analyzed.

3. Detailed analysis on age groups of person affected, cumulative dose of cisplatin and its impact on response and survival is compared.

II.3.MATERIALS AND METHODS

STUDY DESIGN:

Retrospective analytical study

STUDY SETTING:

Cancer institute is the tertiary cancer speciality centre in world and founded by Dr.Muthulakshmi Reddy on June 18, 1984 in Chennai, India. It includes a 423 bedded hospital, a research centre on oncology, preventive oncology division and college of oncological sciences that conducts various courses like post graduate in radiation oncology, surgical oncology, medical oncology, psycho oncology, medical physics and nursing oncology. Over 50 new patients and 500 follow up patients has been daily in cancer institute.

STUDY POPULATION:

Patients with locally advanced oral cavity carcinoma registered in cancer institute from 2009 to 2011 treated with concurrent chemo radiation were reviewed. The chemotherapy agents used in concurrent chem. Radiation were weekly cisplatin, carboplatin, cetuximab, three weekly cisplatin alone and three weekly cisplatin along with biweekly bleomycin. Of 515 patients treated with

concurrent chemoradiation all the 216 patients treated with three weekly cisplatin with or without bleomycin concurrent with radiation were included in the study.

STUDY PERIOD:

January 2009 to December 2011.

SAMPLE SIZE: 216

Total of five hundred and fifteen patients were treated with concurrent chemo radiation using different chemotherapeutic agents like cisplatin, carboplatin, bleomycin and cetuximab. Of these all 216 patients treated with three weekly cisplatin with or without bleomycin were included in the study. Of 216 patients, 112 patients were treated with cisplatin and bleomycin concurrent with radiation and 104 patients were treated with cisplatin alone concurrent with radiation therapy.

STUDY TOOL:

Index registry of the year 2009, 2010 and 2011 from tumour registry and case details from case record, chemotherapy chart, radiotherapy chart, bed side chart and investigations were reviewed to collect data concerning age, sex, habits,

comorbids, disease site, TNM stage, radiotherapy dose/fractionation, chemotherapy details, toxicity, response, failure rate, survival status for patients treated with cisplatin and bleomycin and cisplatin alone concurrent with radiation.

INCLUSION CRITERIA:

1. Pathologically proven squamous cell carcinoma of oral cavity.
2. Clinical stage T1-2, N1-3 or T3-4, N0-3 including no distant metastasis.
3. Performance status ECOG 0-2.
4. Age >18 years
5. Absolute neutrophil count (ANC) > 1,500 cells/mm3; Platelets > 100,000 cells/mm3; Hemoglobin > 10.0 g/dl; Note: The use of transfusion or other intervention to achieve Hb >8.0 g/dl is acceptable.
6. Adequate hepatic function defined as follows:
    - ❖ Bilirubin < 2 mg/dl within 2 weeks prior to registration;
    - ❖ AST or ALT < 3 x the upper limit of normal within 2 weeks prior to registration.

7. Adequate renal function, defined as follows:

   Serum creatinine < 1.5 mg/dl within 2 weeks prior to registration or creatinine clearance (CC) ≥ 50 ml/min within 2 weeks prior to registration determined by 24-hour collection or estimated by Cockcroft-Gault formula

8. Adequate pulmonary function –Baseline PFT/ DLCO
9. Informed consent

EXCLUSION CRITERIA:

1. Histopathologically other than squamous cell carcinoma.
2. Cancers of oropharynx, hypopharynx, larynx, nasopharynx and unknown primary of cervical lymphadenopathy.
3. Stage T1-2,N0
4. Distant metastasis or adenopathy below clavicles.
5. Previously treated with surgery.
6. Simultaneous primaries.
7. Prior invasive malignancy unless disease free for 3 years.
8. Prior systemic therapy or radiotherapy for the study cancer.
9. Prior allergic to cisplatin and bleomycin.
10. Not willing to sign informed consent.

PRE TREATMENT ASSESMENT:

1. Complete history

2. Complete physical examination.

3. Examination of oral cavity.

4 .Indirect laryngoscopy, Direct laryngoscopy and pharngoscopy.

5. Complete blood work up- hemogram, serology, RFT, LFT, electrolytes, creatinine clearance.

7. Chest X-ray, ECG, Echocardiogram.

8. US kidneys

9. Pulmonary function test- DLCO

10.CT scan of head and neck in selected case.

TREATMENT PROTOCOL:

Terasima et al in an experimental study of use of bleomycin in mouse fibroblastic cells and lymphatic leukemia cells, survival response as a result of varying concentrations of bleomycin is reported. Survival response is interpretated in a curve by an upward concave curvature, consists of a steep portion as well as a more gradual portion and it is due to resistance induced by bleomycin. This resistance can be overcome by removal of bleomycin and completely disappeared

after four hours. Based on this finding it is considered that administration of bleomycin in smaller dose with increased frequency rather than larger dose with lesser frequency of same cumulative dose. Takao Ohnuma et al in his study prove that weekly twice schedule of bleomycin was tolerated well in patients. Clinically there is small difference in effectiveness of combined bleomycin treatment based on timing of bleomycin administration combined with radiation. The scheduling of administration of bleomycin before or after radiation was not defined clearly. Based on previous clinical experiences this modified dose of radiotherapy fractionation and bleomycin dose was adopted in our institute to enhance the local control in oral cavity cancer.

GROUP A:

Cisplatin is given in dose of 70mg/m2 every three weekly in all patients. Bleomycin is given at dose of 10 units twice a week on Tuesday and Thursday till completion of radiotherapy. Radiotherapy to primary and nodal regions is given at dose of 250cGy/fraction weekly three days on Monday, Wednesday and Friday to TD- 60-65Gy.

GROUP B:

Cisplatin is given in dose of 70mg/m2 every three weekly in all patients. Radiotherapy to primary and nodal regions is given at dose of 200cGy/fraction five days a week to TD-60-66Gy**.**

Radiation therapy:

Conventional treatment protocol:

Patients in the conventional radiotherapy group were treated with 6MV X-rays from a linear accelerator using 2 opposing lateral photon fields to treat primary, upper and middle cervical lymphnodes and one anterior field to treat lower neck nodes.

Field setup:

Superior border: Skull base.

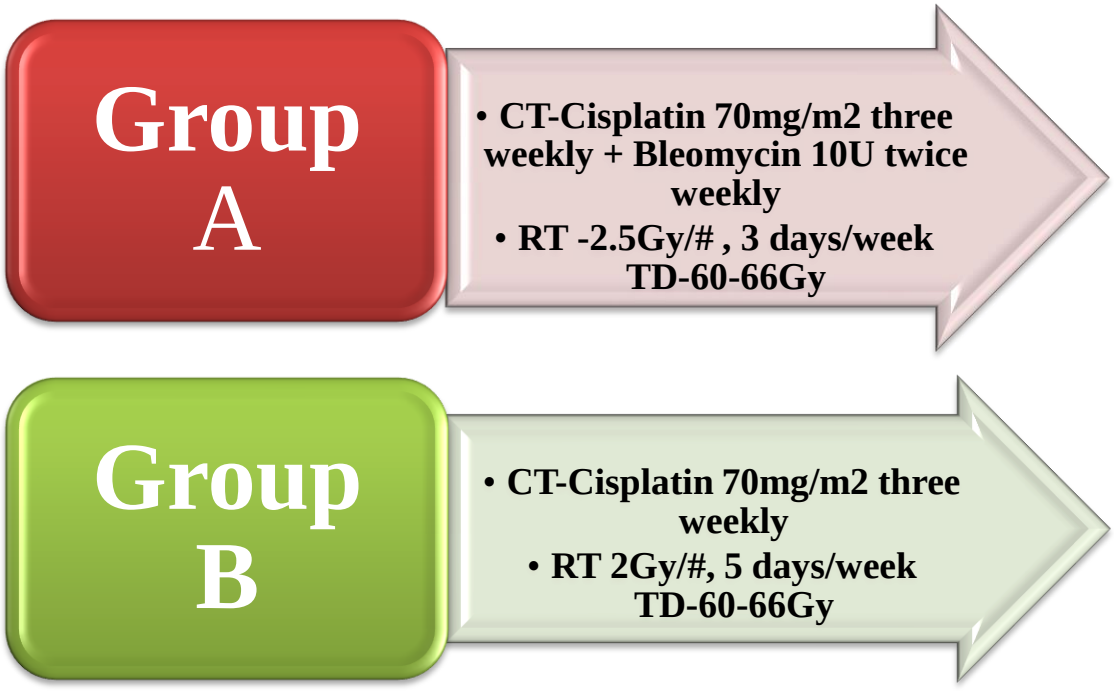
Inferior border: Bottom of hyoid bone and match with lower anterior neck field.

Anterior border: Mentum

Posterior border: posterior to spinous processes or in the presence of large nodal mass aim to cover more posteriorly.

Low anterior neck field:

Superior: Bottom of hyoid bone and match with upper neck lateral fields. Inferior:Inferior edge of the clavicular head Lateral: Two thirds of the clavicle or 2 cm lateral to the adenopathy(which ever more lateral)



Patient was evaluated with hemogram, renal function test, hepatic function test, electrolytes before every cycle of three weekly cisplatin. Total WBC count

was checked twice a week in all patients and in patients with counts less than 4000 cells per mm3 bleomycin is pended till count improves. Patients with grade III neutropenia and febrile neutropenia received growth factor support and antibiotics. In combined bleomycin group chest X ray was taken at the end of 30Gy. In Patients who had grade III mucositis, the above chemotherapy is pended tillmucositis resolves.

III.RESULTS

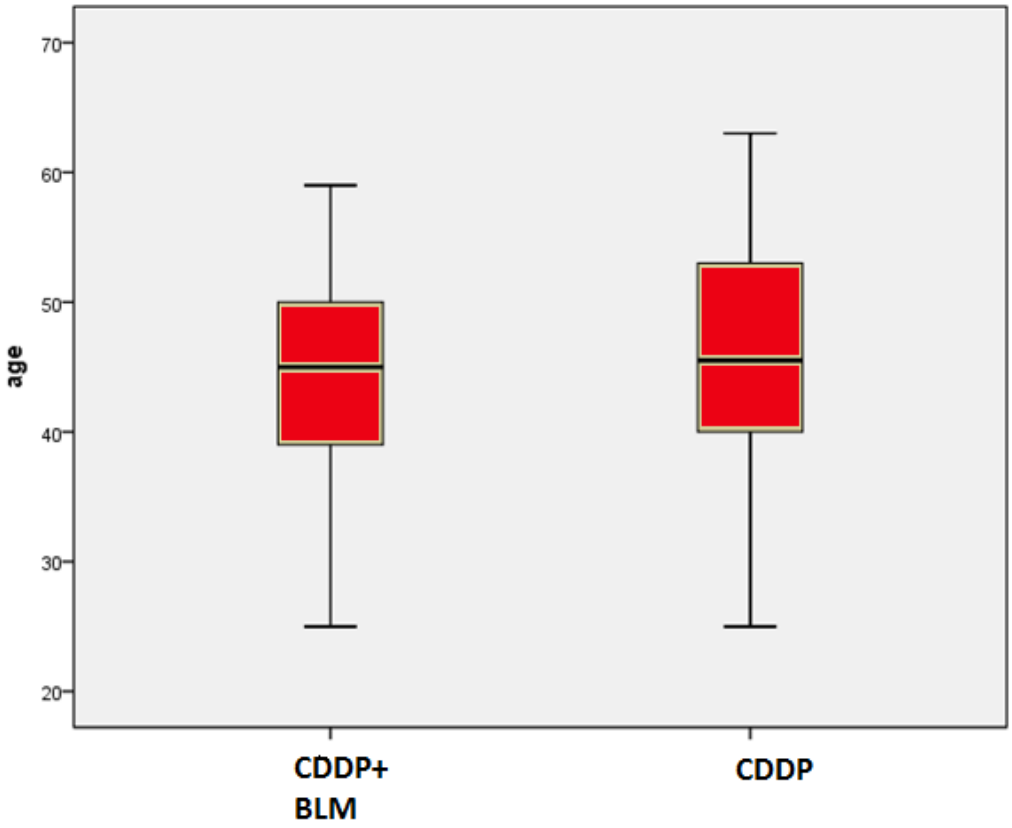
From January 2009 to December 2011 patients were treated for oral cavity cancer in our institute. Of these 216 patients were treated with three weekly cisplatin with or without bleomycin concurrent with radiation therapy were included for the study.

AGE – SEX DISTRIBUTION:

All 216 patients were in age group between 25 to 63 years. The mean and median age is 45 years in both groups. 184 were male patients and 32 patients were female patients. There are no statistically significant differences were identified in age and sex distribution between both groups.

AGE (years)	MALE n (%)	FEMALE n (%)	TOTAL 216(100%)
25-34	22(12%)	1(2.9%)	23(10.7)
35-44	68(36.9%)	7(20.5%)	75(34.7)
45-54	61(33.1%)	20(58.8%)	81(37.5)
55-64	33(18%)	4(11.8%)	37(17.1)

AGE DISTRIBUTION IN BOTH GROUPS

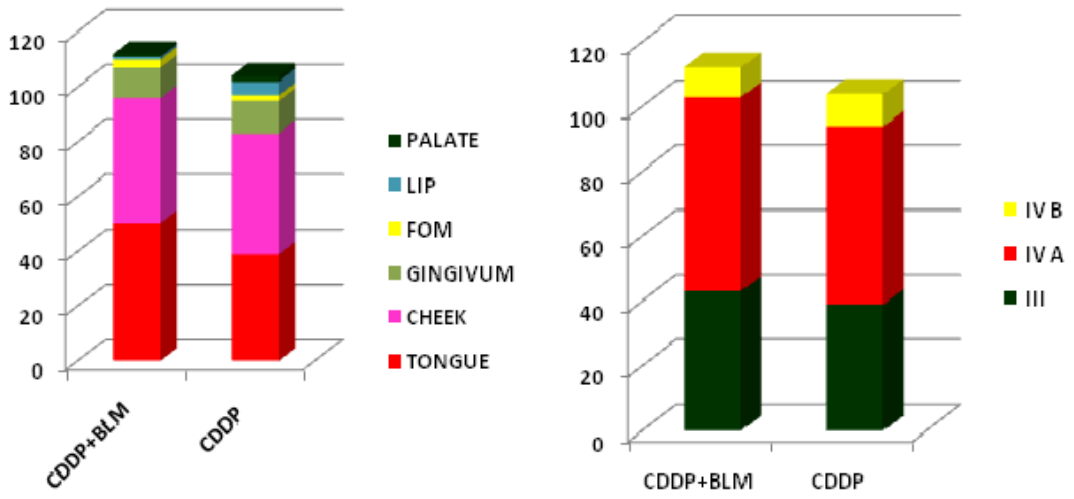


SITE – STAGE DISTRIBUTION:

Eighty percent of patients have tongue and cheek cancers. Of 216 patients 89 patients has anterior two thirds of tongue cancer, 90 patients has cheek cancer, 23 patients has gingival cancer, 6 patients has lip cancer, 5 patients has floor of mouth cancer and 3 patients has hard palate cancer. Among all patients 62 percent of patient has stage IV cancer. Of 216 patients 82 has stage III cancer, 115 has stage IV A cancer and 19 has stage IV B cancer.

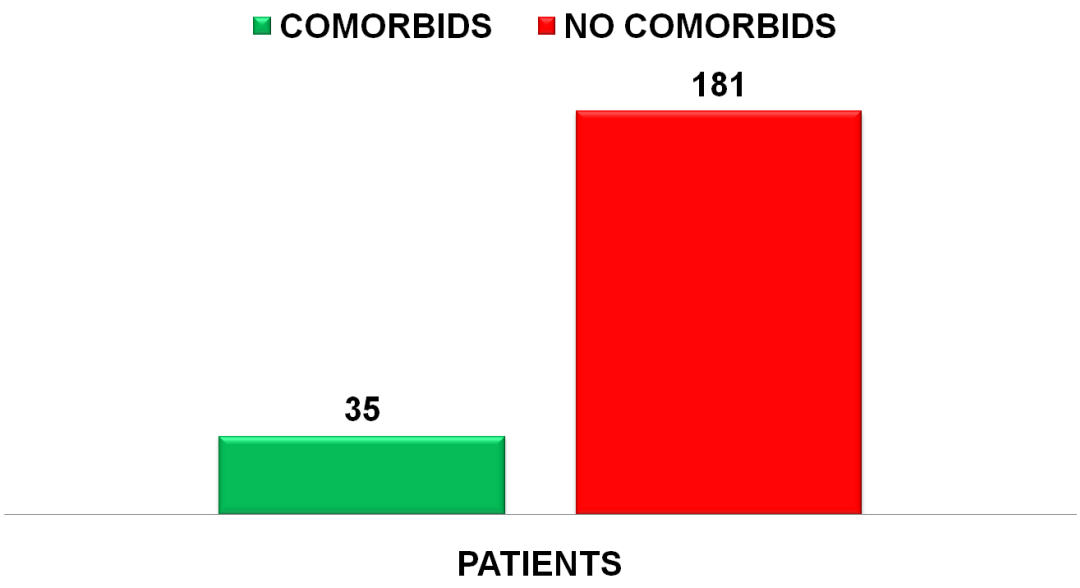
SITE	STAGE III	STAGE IVA	STAGE IVB	n (%)
TONGUE	37	51	1	89(41.2)
CHEEK	31	43	16	90(41.7)
GINGIVUM	8	14	1	23(10.7)
LIP	2	3	1	6(2.7)
FLOOR OF MOUTH	2	3	-	5(2.3)
PALATE	2	1	-	3(1.4)
TOTAL	82(37.9%)	115(53.2%)	19(8.9%)	216

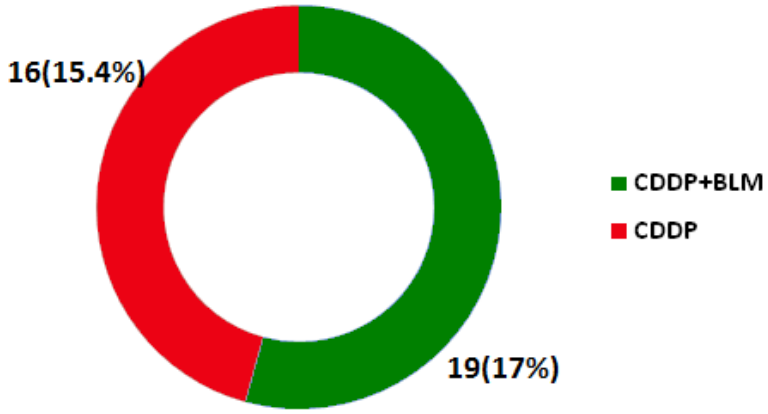
SITE-STAGE DISTRIBUTION IN BOTH GROUPS



No significant difference in site wise and stage wise distribution between both groups.

COMORBIDS:



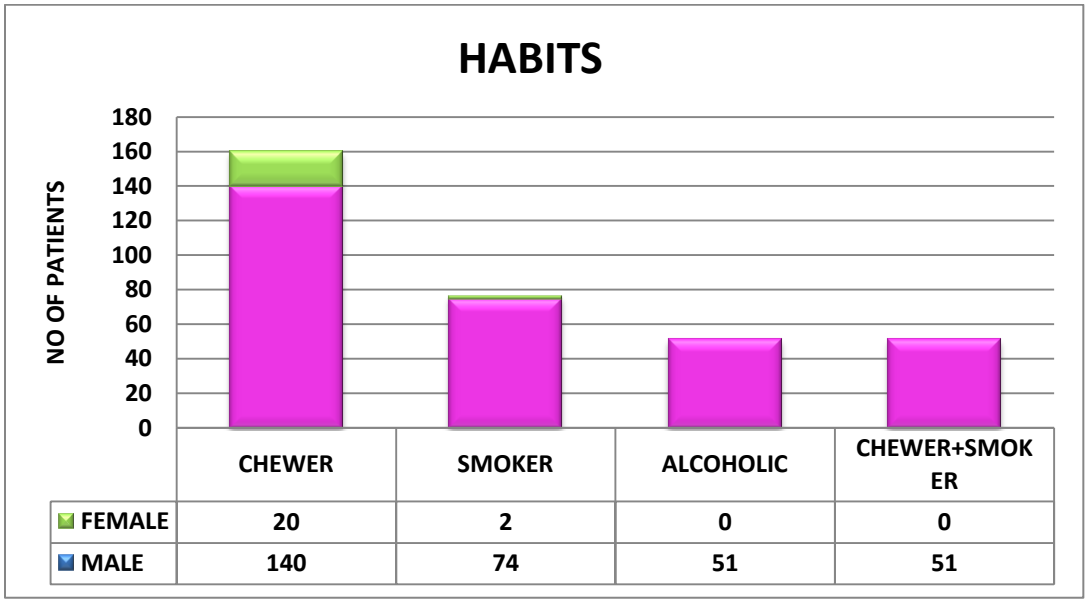


Of 112 patients treated with cisplatin and bleomycin concurrent with radiation 19 patients has associated comorbid condition in the form of diabetes,

hypertension and ischemic heart disease. Of 104 patients treated with cisplatin alone 16 patients has associated comorbid condition.

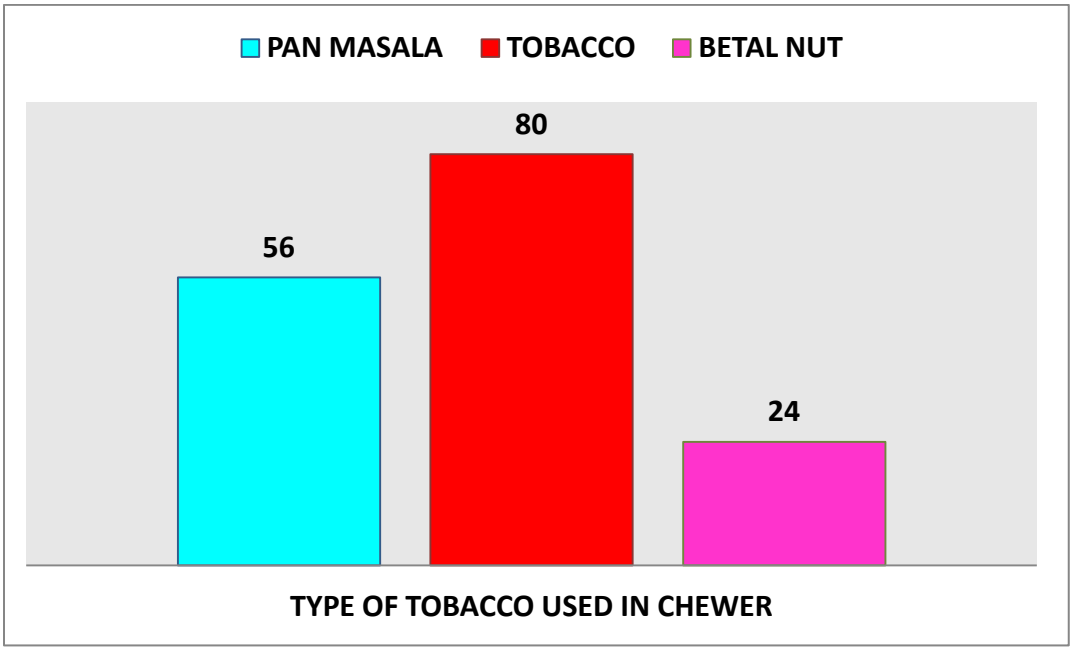
HABITS:

Tobacco chewing, smoking and drinking alcohol are major risk factors for oral cavity cancers. Of total 216 patients analysed eighty five percent of patients have history of tobacco and alcohol abuse. No statistically significant habit differences were identified between both groups.



TYPE OF TOBACCO USE IN CHEWER:

Types of tobacco used were panmasala and tobacco with or without betal nut.

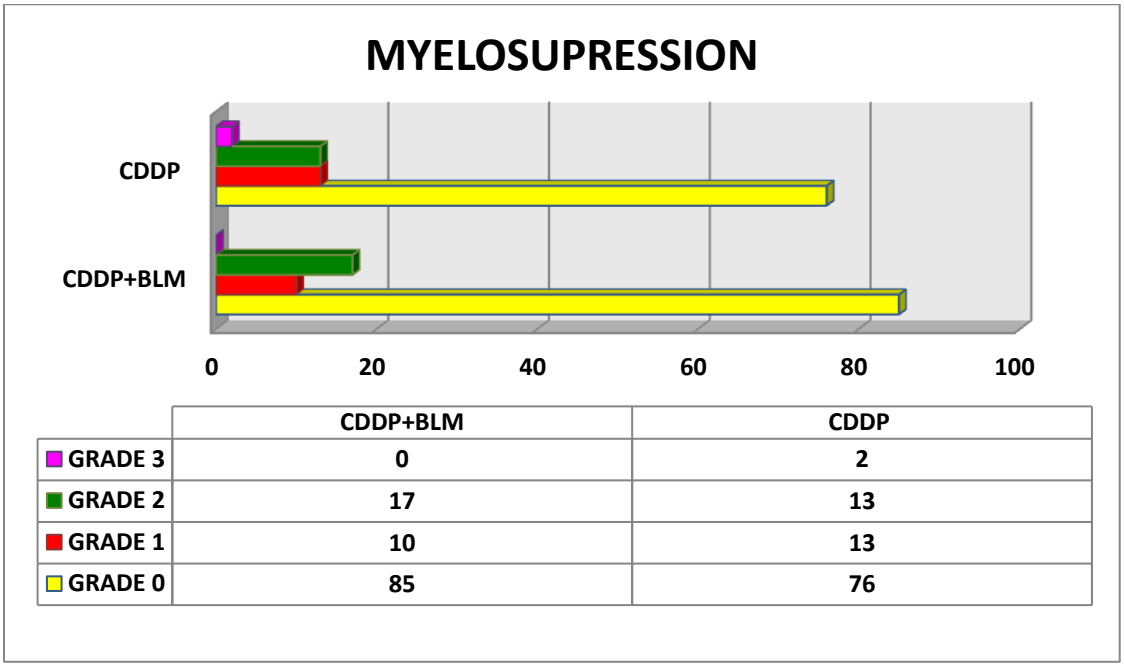


TOXICITY:

In combined cisplatin bleomycin group toxicity expected were myelosuppresion, emesis, mucositis, electrolyte imbalance, decreased renal function test, ototoxicity, pneumonitis and bleomycin induced rash. In cisplatin only group the same toxicities except pneumonitis and bleomycin induced rash were expected. Of 112 patients treated with combined bleomycin cisplatin and radiation group two patients had bleomycin induced skin rash which requires discontinuation of further use of bleomycin. Pneumonitis was not reported.

MYELOSUPRESSION:

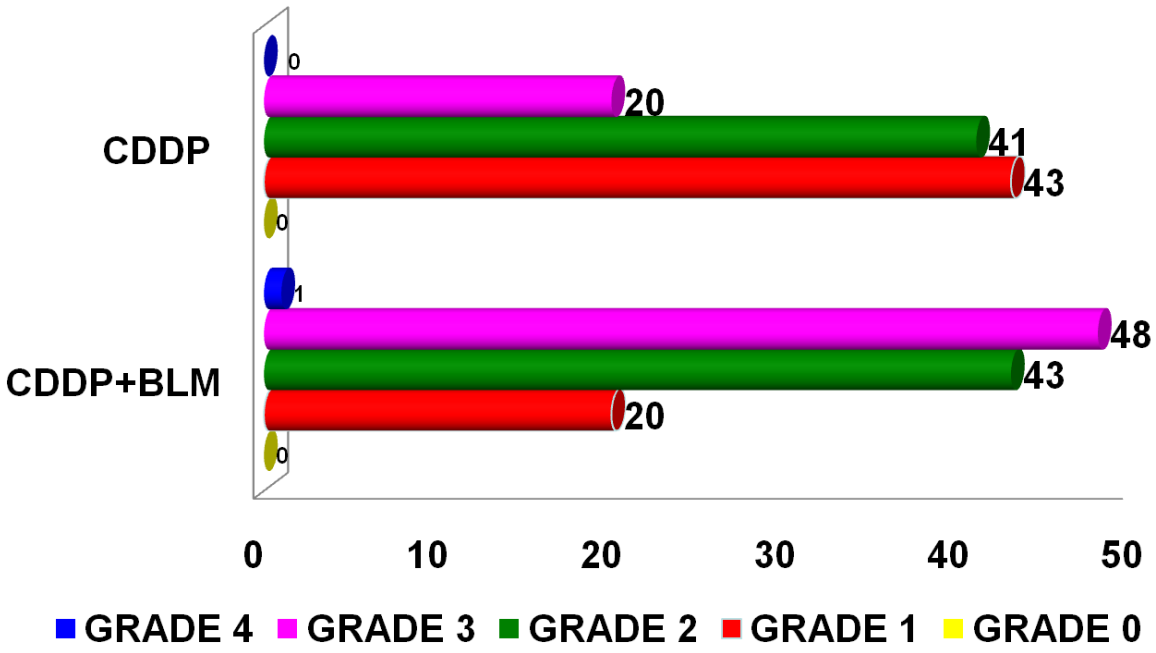
As per RTOG toxicity grading criteria myelosupression during treatment recorded. Results were comparable in both groups.



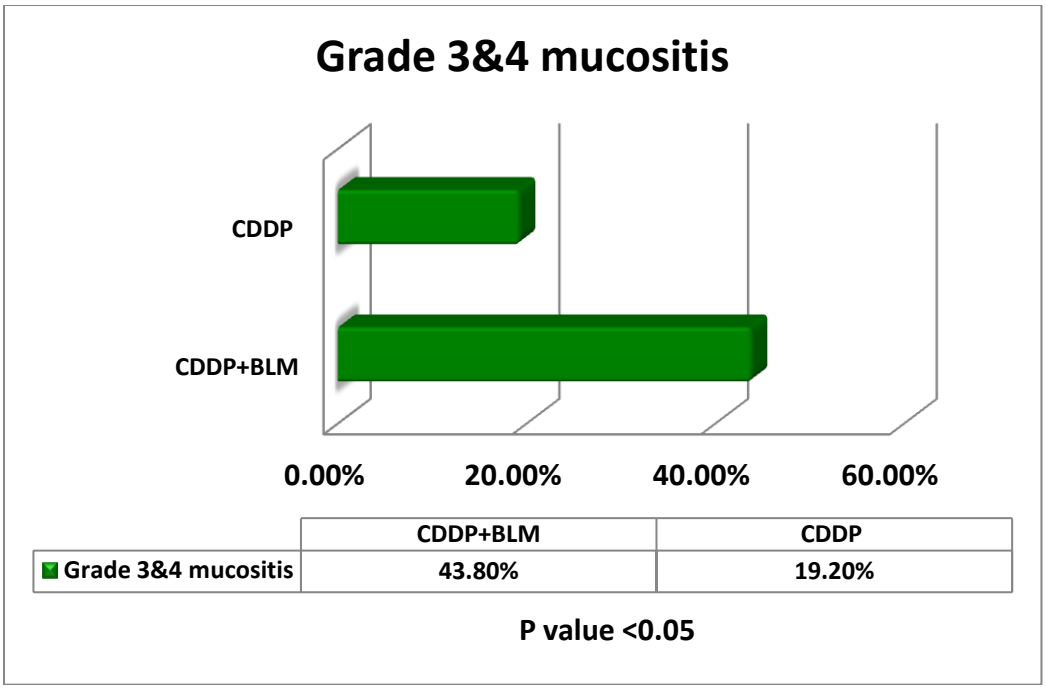
	CDDP+BLM	CDDP
GRADE 3	0	2
GRADE 2	17	13
GRADE 1	10	13
GRADE 0	85	76

MUCOSITIS:

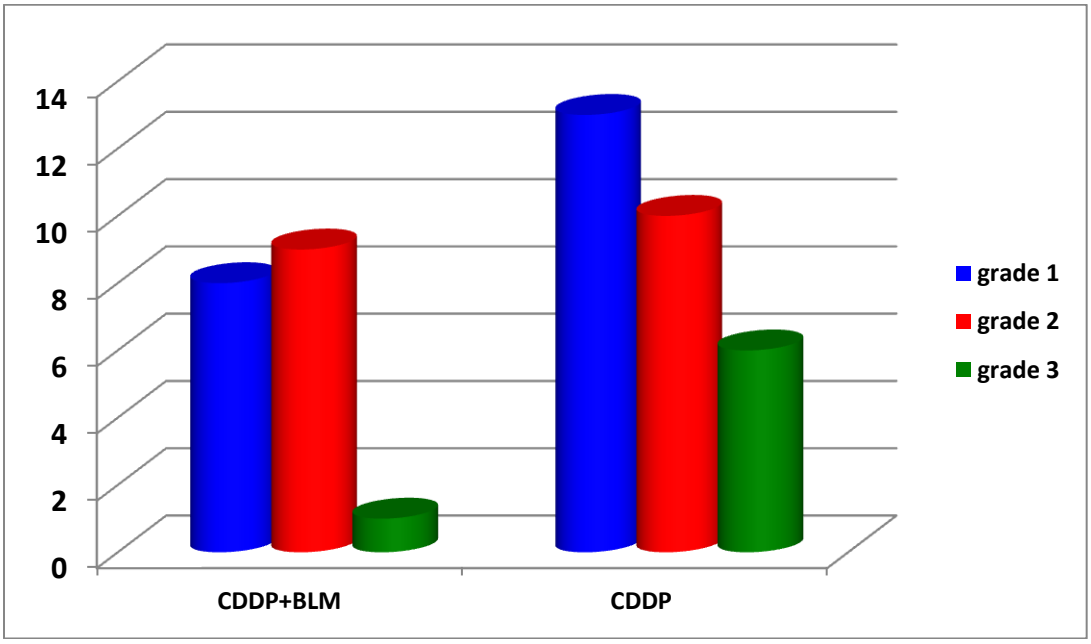
Increased mucositis was reported in cisplatin bleomycin group compared to cisplatin alone with radiation therapy. There in one grade 4 mucositis reported and was in cisplatin bleomycin group.



Of 112 patient in cisplatin bleomycin combined with radiation number of grade 3 and grade 4 mucositis was reported in 49 patients (43.8%) compared to 20 patients(19.2%) in cisplatin alone with radiation group with statistically significant value p <0.005.



EMESIS:

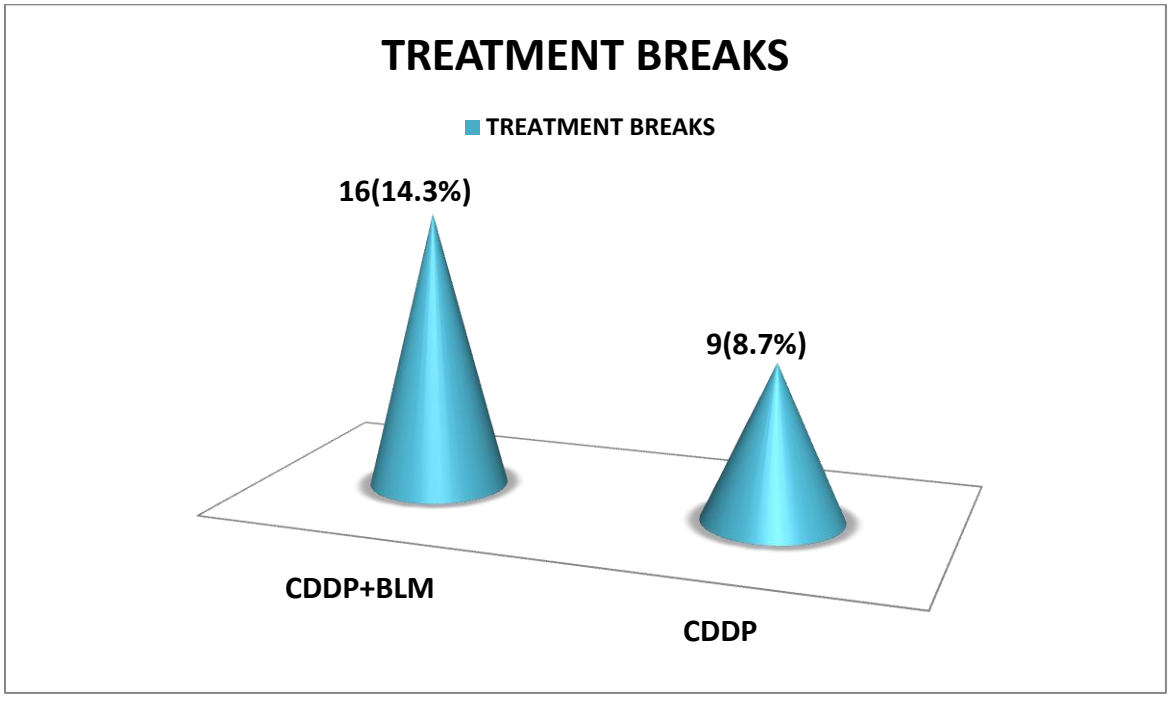


In cisplatin bleomycin group 8(7.1%), 9(8%) and 1(0.9%) patients had grade1, grade2 and grade3 respectively. In cisplatin alone group 13(12.5%),

10(9.6%) and (5.8%) patients had grade1, grade2 and grade3 respectively. Though slight increase in emesis is reported in cisplatin only group it is not statistically significant between them.

TREATMENT BREAKS:

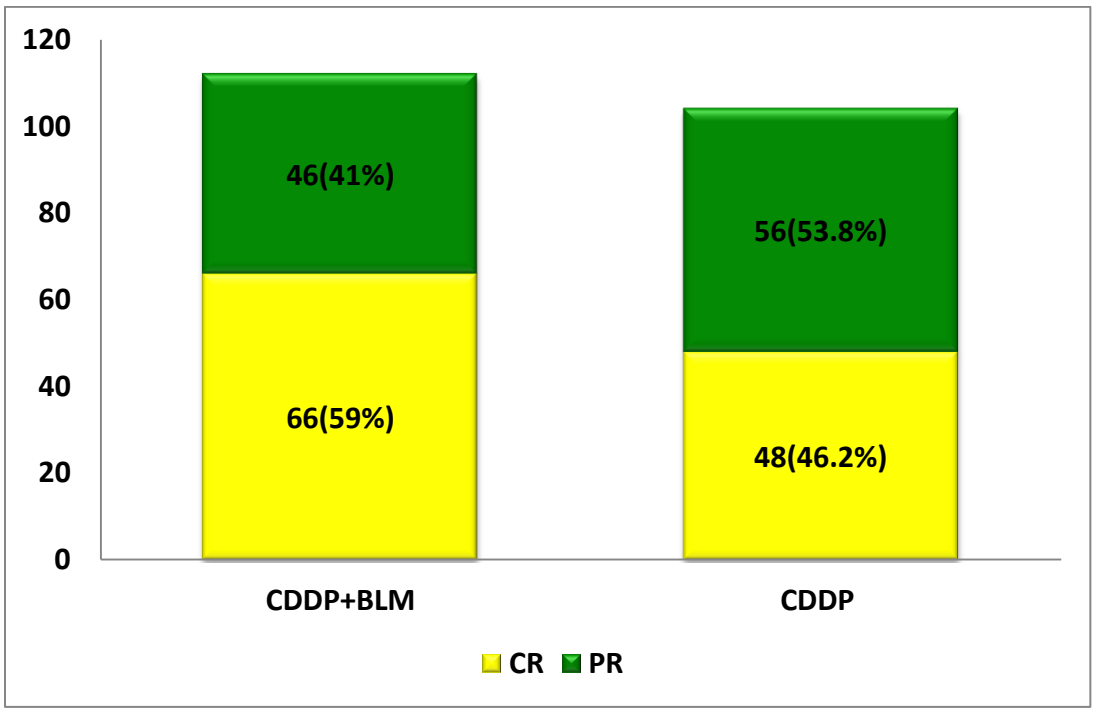
During treatment toxicity can be managed by symptomatic care and temporary stopping of radiation and chemotherapy to help in faster healing of symptoms. When radiation is pended for more than five days in a week is called as treatment break. There is increased in treatment breaks in combined bleomycin and cisplatin arm compared to cisplatin alone arm combined with radiation.



TREATMENT RESPONSE:

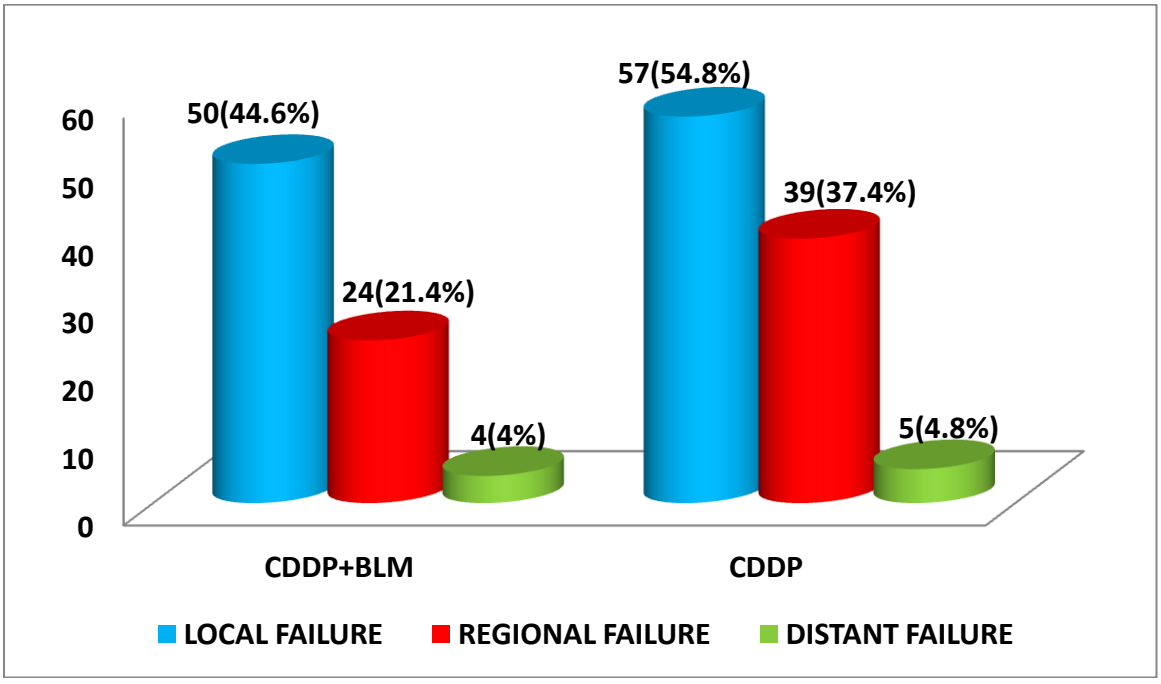
The response to treatment was assessed at 6 weeks after completion of concurrent chemo radiation. Complete regression of lesion is taken as complete response. Anything less than that is taken as partial response. In cisplatin bleomycin group there is increase in complete response 74(66.1%) compared to cisplatin alone 59(56.7%) group with p-value =0.06.

The complete response rate in patients who had treatment breaks was increased in combined cisplatin and bleomycin group compared to cisplatin alone group (64%vs11.1%)



FAILURE RATE:

Failure can be local, regional or distant. It can occur at the end of treatment or during follow up after complete response to treatment. There is increased in local failure in patient received cisplatin alone concurrent with radiation compared to cisplatin and bleomycin group (54.8% vs 44.6%) and also increased in regional failure also reported in patient received cisplatin alone concurrent with radiation compared to cisplatin and bleomycin group (37.4% vs 21.4%). The distant failure rate is comparable in both groups.



SALVAGE SURGERY:

In patients with resectable locoregional failure salvage surgery is considered as better option in order to remove cancer that are not responded to initial treatment and to improve survival. Of 216 patients treated with concurrent chemo radiation 30 patients underwent salvage surgery. In combined cisplatin and bleomycin group 20 patients underwent salvage surgery. 26% of patients who had local failure and 33.3% of patients who had regional failure are salvaged in combined cisplatin and bleomycin group. In cisplatin alone group, of 10 patients 12.2% and 18.9% of patients who had local and regional failure are salvaged. There is statistically significant increase in salvage rate in cisplatin bleomycin group compared to cisplatin alone with radiation.

	Total salvage Sx	**Local failure sal.**	**Regional failure sal.**
CDDP+BLM	20	13/50(26%)	8/24(33.3%)
CDDP	10	7/57(12.%)	7/37(18.9%)

DISEASE FREE SURVIVAL:

The two year disease free survival of combined cisplatin and bleomycin group is greater than cisplatin alone with radiation group(45.5% vs 35.5%) with statistically significant p-value<0.05.

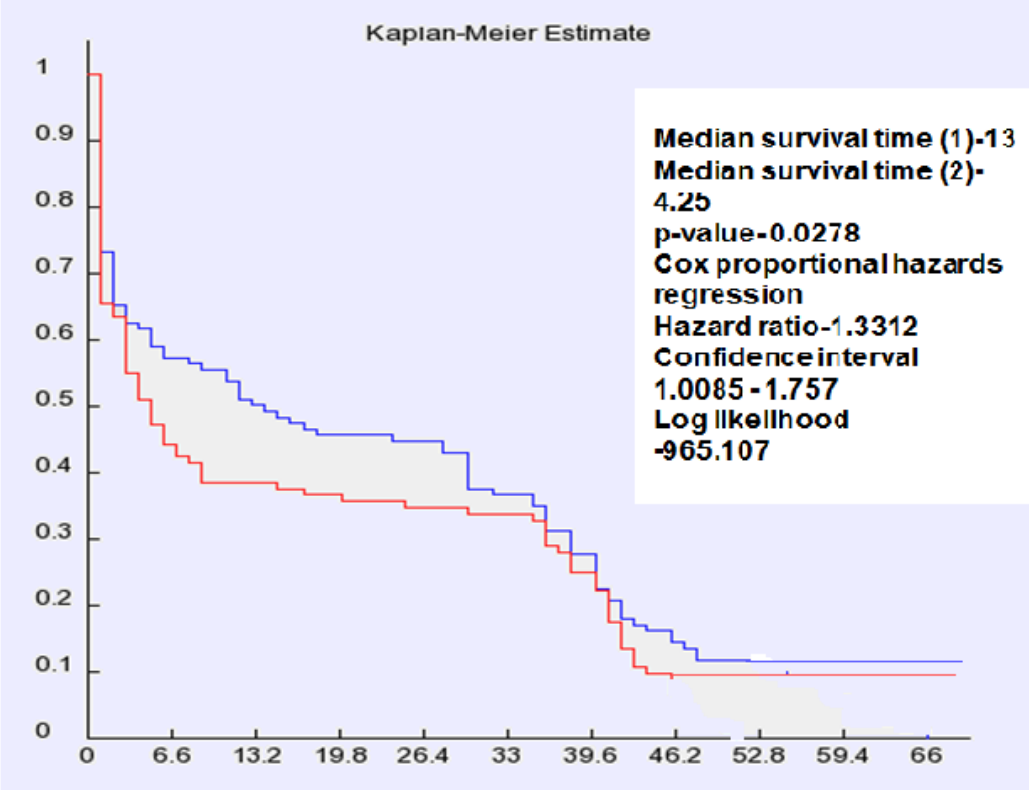


Fig- Blue line combined cisplatin+bleomycin+RT; Red line- cisplatin alone + RT

OVERALL SURVIVAL:

The two year overall survival in combined cisplatin bleomycin group is increased compared to cisplatin alone group (60.7% vs 47%) with statistically significant p value 0.0043.

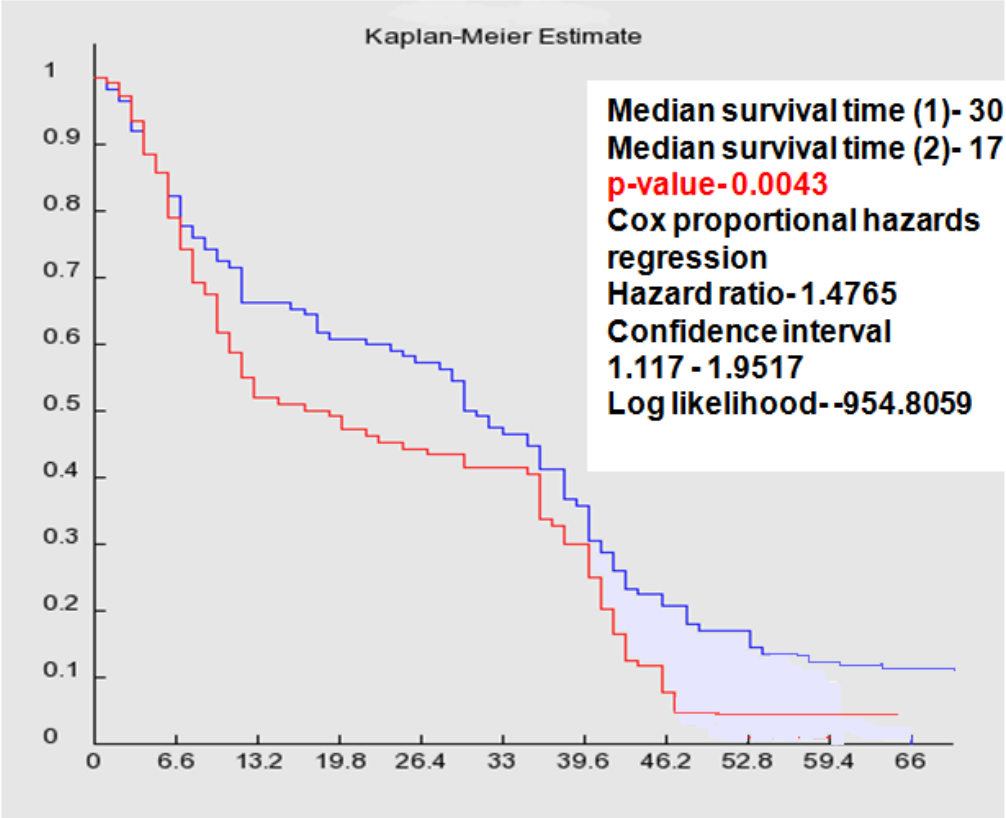


Fig- Blue line combined cisplatin+bleomycin+RT; Red line- cisplatin alone + RT

SITE WISE OVER ALL SURVIVAL:

SITE	CDDP+BLM	CDDP
CHEEK	26/46(56.5%)	18/44(40.9)
FLOOR OF MOUTH	2/3(66.6%)	1/2 (50%)
GINGIVUM	7/11(63.6%)	6/12(50%)
LIP	1/1(100%)	3/5(60%)
PALATE	1/1(100%)	2/2(100%)
TONGUE	31/50(62%)	19/39(48.7%)

STAGE WISE OVERALL SURVIVAL:

STAGE	CDDP+BLM	CDDP
III	33/43(76.7%)	28/39(71.8%)
IVA	31/60(51.6%)	20/55(36.4%)
IV B	4/9(44.4%)	1/10(10%)

TREATMENT BREAKS WISE OVERALL SURVIVAL:

The combined cisplatin and bleomycin group combined with radiation causes more treatment breaks compared to cisplatin alone group combined with radiation. Increased treatment breaks affects the tumour response and in turn survival of the patient. Inspite of increased treatment breaks in combined bleomycin cisplatin group compared to cisplatin alone group the two year overall survival was increased in combined cisplatin bleomycin group (50% vs 22.2%) with statistically insignificant p-value-0.126. In cisplatin alone group even without treatment break two year over all survival is 47%.

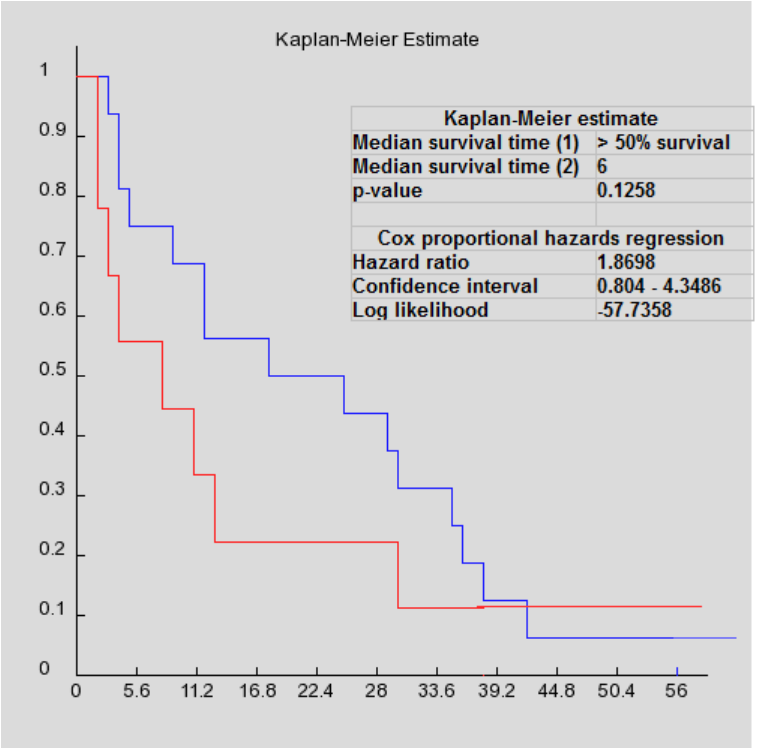


Fig- Blue line combined cisplatin+bleomycin+RT; Red line- cisplatin alone + RT

AGE WISE OVERALL SURVIVAL:

AGE	CDDP+BLM	CDDP
<40 YRS	20/38(52.6%)	10/30(33.3%)
>40 YRS	46/74(62.2%)	39/74(52.7%)

CUMULATIVE DOSE OF CISPLATIN WISE:

GROUP	<200mg/m2	>200mg/m2
CDDP+BLM	13/25(52%)	55/87(63.2%)
CDDP	06/15(40%)	43/89(48.3%)

IV.DISCUSSION

The oral cavity cancers are the most common cancer in head and neck cancers. Nearly 90 percent of these presents with locally advanced stage. Combined modality treatment is usually preferred treatment. When treated with only radiation the response to treatment and survival is poor. This poor response to radiation is attributed partly due to large tumors with more necrosis , poor blood and oxygen supple and in part due to development of central necrotic core after radiation alone as described by Krishnamurthy et al due to centripetal contraction of malignant zone. Surgery improved survival but only twenty five percent accepts mutilating surgery and in those patients who underwent surgery after radiation has severe cosmesis and functional loss. So from 1960 in our institute various combination therapy were tried to improve the response and survival of advanced oral cavity cancers. Many well designed, detailed evaluated and randomized study were conducted using several agents in our institute by Krishnamurthy et al. Radiation therapy was combined with

- Synckavit in 1967
- Methotrexate in 1976

- Bleomycin in 1980
- Metronidazole in 1981
- Bleomycin and hyperbaric oxygen in 1983
- Pepleomycin and radiation in 1987

Among all these randomized well controlled trial bleomycin combination with radiotherapy has better local control and survival rates in oral cavity cancers. Everywhere in the world cisplatin dominated as a chemotherapy agent for combination with radiation in head and neck cancers. In order to improve the local control , survival and as already bleomycin proved better combination with radiation in our institute three weekly cisplatin was combined with bleomycin and radiation therapy in treatment of locally advanced oral cavity cancers.

The aim of this study was to analyze the acute toxicites, clinical response and its impact on survival between combined cisplatin and bleomycin versus cisplatin alone concurrent with radiation in locally advanced oral cavity carcinoma treated from January 2009 to December 2011 in our institute. Of total 216 patients 114 patients were treated with combined cisplatin and bleomycin with radiation therapy and 104 patients were treated with cisplatin alone with radiation therapy. Patient characteristics like age, sex, site, stage, comorbids and associated habits

were equally distributed in both groups. There is no statistically significant difference between cisplatin plus bleomycin and cisplatin alone group in terms of patient characteristics.

Based on the clinical experience with different dose level, frequency used and its tolerability on patients when used concurrently with radiation bleomycin was given 10units twice weekly in our institute along with radiation. The incidence of toxicity is found less without compromising tumor control with this schedule. This was also supported by clinical study on tolerance to twice weekly dosage by Takao Ohnuma et al where use of bleomycin 15 units twice weekly was tolerated better. Initially bleomycin was used with conventional fractionation in our institute which results in increased mucositis and increased treatment breaks. Based on our clinical experience this modified radiation fractionation is followed in our institute to reduce the bleomycin toxicity without compromising local control.

The side effects reported were emesis, myelosuppression and mucositis in both groups. The myelosuppression was comparable in both groups. As bleomycin does not cause bone marrow suppression we did not find increased myeolosuppression when combined with cisplatin. The emesis reported in both groups was not statistically significant though there is slight increased in emesis in

cisplatin alone arm. There is increased mucositis in combined bleomycin and cisplatin group compared to cisplatin alone. The incidence of grade 3 and grade 4 mucositis was more in combined bleomycin and cisplatin group compared to cisplatin alone (43.8% vs 19%). The treatment breaks between two groups are not statistically significant though there is slight increased in treatment breaks in cisplatin and bleomycin group (14% vs 9%). The increased mucositis with use of bleomycin and radiation also reported in Pankaj Shaw et al, Cachin et al and Vermund et al where majority are pharyngeal cancers . Pankaj M shaw et al reported 80% mucositis in bleomycin group but mucositis causing treatment breaks was only 22 percent. Though there is increased incidence of mucositis there is lesser percentage of patients has treatment break in our study this is because all patients are oral cavity cancer. All patients has feeding tube to improve nutrition and good local care enables to improve mucositis easily compared to other studies where majority of patients treated were oropharyngeal cancers where local care is difficult compared to oral cavity. Parvinem et al did not report increased mucositis in patients treated with bleomycin and radiation.

There was no pneumonitis or decreased in lung function reported in combined bleomycin and cisplatin group. Two patients developed bleomycin

induced rash after receiving 30 units of bleomycin. One patient has decreased renal function in cisplatin alone group. No other side effects reported in both groups.

The local control rates in combined cisplatin and bleomycin group was 59 percent in comparison to 46 percent in cisplatin alone combined with radiation group. The increased tumor control was also reported by Krishnamurthy et al, Kapstad et al(13) and Abe et al. No difference in tumor control was reported by Cachin et al and vermund et al. This difference in tumor control by Vermund et al may be because increased number of old age persons in this study. Only seven percent of the patients were below the age of 50 years and more than twenty seven percent of patients were above seventy years of group. As per MACH NC analysis the benefit of chemotherapy in terms of survival was not observed in patients over 65 years of age. The mean age in cachin et al study was 50 years and only 83 percent were evaluable at the end of study. The use of bleomycin with conventional fractionation in these study results in increased complication where in our institute fractionation was different.

The local failure in cisplatin plus bleomycin group and cisplatin alone group was 44.6% and 54.8% and regional failure was 21.4% and 37.4% respectively. The

decrease in local and regional failure was not found in distant failure which is comparable in both groups.

Among the patients who have failed in cisplatin and bleomycin group 26% of patients with local failure and 33.3% of patients with regional failure group were salvaged successfully. Among the patients with cisplatin alone group 12% of patients with local failure and 18.9% of patients with regional failure were salvageable. The increased salvageable rate found in cisplatin bleomycin group.

The 2 year survival rates in combination cisplatin and bleomycin group was significantly better when compared to cisplatin alone group (60.7% vs 47%). The 2 year survival rate decreases as stage increased.

The increased survival benefit of cisplatin and bleomycin also seen subsite wise. In our study cheek cancer has lesser survival rate compared to other subset because there in increased number of patients with stage IV B compared to other sites.

There are studies which shows decreased overall survival in younger patients lesser than 40 years of age. Our study also shows decreased over all

survival in younger patients compared to patients in age group in more than 40 years of age. The cisplatin and bleomycin group shows better survival in younger patients in age group less than 40 years of age compared to cisplatin alone group (52.6% vs 33.3%).

It is well known that treatment breaks will affect response of the disease and survival of the patients. When the two year survival rate was compared in both groups among patients who had treatment breaks the difference in survival was statistically insignificant although increased survival was found in cisplatin and bleomycin group.

The effect of cisplatin is increased when cumulative dose of cisplatin was more than 200mg/m2. The two year overall survival was better in patients with combined cisplatin and bleomycin group who received cumulative dose of cisplatin more than 200mg/m2 compared to those treated with cisplatin alone group (63.2% vs 48.3%). In patients with cisplatin and bleomycin group who received cumulative dose of cisplatin less than 200mg/m2 the two year survival was 52% and was lesser than cisplatin alone who received lesser than 200mg/m2(52% vs 40%) and comparable to the patients who received cumulative dose of cisplatin more than 200mg/m2 in cisplatin alone group(52% vs 48.3%) .

V.CONCLUSION:

The present study shows evidence favoring use of bleomycin as an addition to cisplatin with radiation in locally advanced oral cavity carcinoma under the conditions described. The difference in tumor response, failure rates and survival curve indicates that addition of bleomycin with cisplatin at best has effect on prognosis of the patients with locally advanced oral cavity cancers. There is no serious complicates reported except acceptable and manageable oral mucositis inshows increased tumor control and increased survival in locally advanced cancer with acceptable toxicity. Hence bleomycin can be added to cisplatin to increase tumor control and survival.

However this is only retrospective study and randomized control study is needed to confirm these results.

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