Radiation Biology

with Relevance to Dentistry

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walnutpublication.com
INDIA • UK • USA

Paperback ISBN: XXXXXXXX

First Published in January 2021

Published by Walnut Publication (an imprint of Vyusta Ventures LLP)
www.walnutpublication.com

USA

6834 Cantrell Road #2096, Little Rock, AR 72207, USA

India

#722, Esplanade One, Rasulgarh, Bhubaneswar – 751010, India

#55 S/F, Panchkuian Marg, Connaught Place, New Delhi - 110001, India

UK

International House, 12 Constance Street, London E16 2DQ, United Kingdom

Printed in India by KSRK Impressions, Jain Complex, Sirsakhurd, Dhamda Road, Durg – 491001, Chattisgarh, India

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Chapter 1: Introduction

Dr. Abhishek Gupta

Radiobiology

- Study of the effects of ionizing radiation on living systems.
- Requires studying many levels of organization within biologic systems spanning broad ranges in size and temporal scale.

Effect on Biological Molecules

- **Proteins**
  - Denaturation.
  - Inter- and intramolecular cross-linking.
  - Enzymes get inactivated leading to failure or conversion of substrate to product.
- **Nucleic acids**
  - Change or loss of base.
  - Disruption of hydrogen bonds between DNA strands.
  - Breakage of DNA strands.
  - Cross-linking of DNA strands.

Effects at Cellular Levels Intracellular

- **Nucleus**: inhibition of cell division.
- **Chromosomes**: single or double armed chromosomal aberrations
- **Cell cytoplasm:**

– Increased permeability to sodium and potassium ions.

– Swelling and disorganization of mitochondria.

– Focal cytoplasmic necrosis.

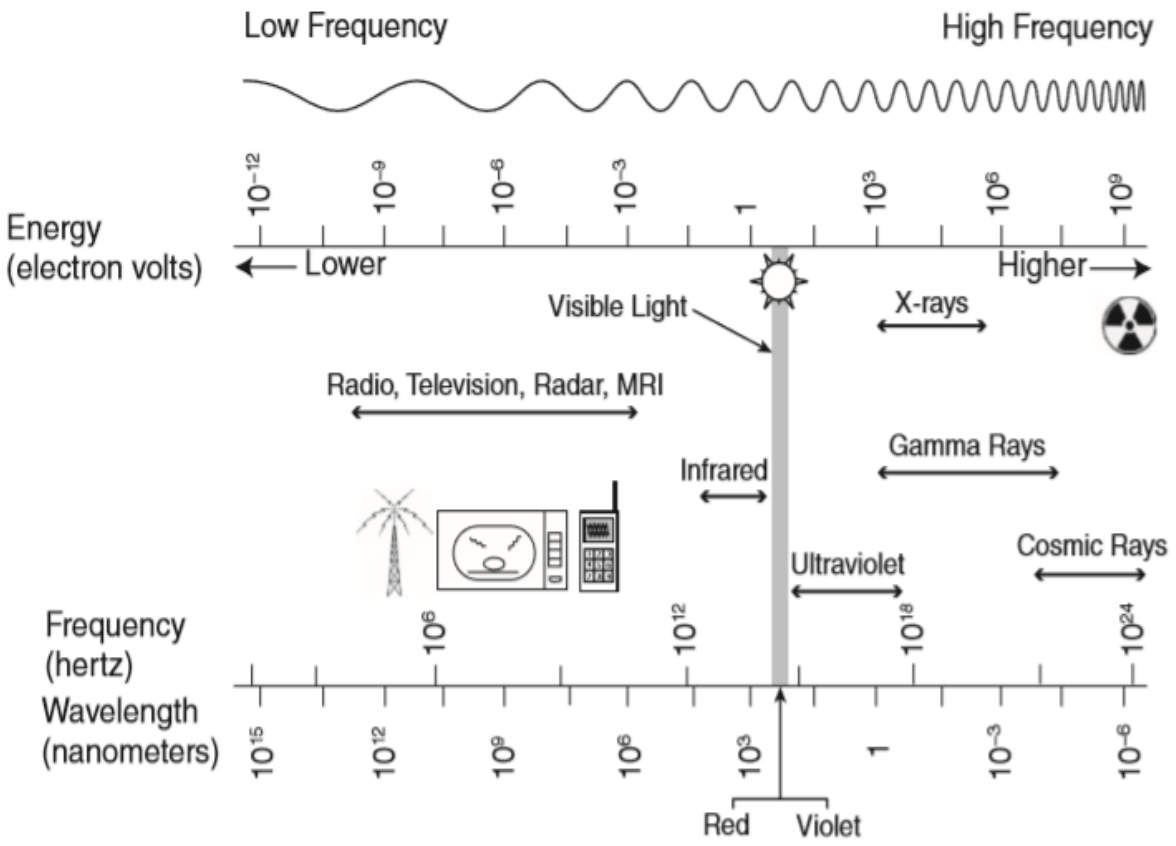
- **Effect on Cell Kinetics**
- This basically effects cell division and cell maturation process and depends upon:
  - Dose of radiation (High / Low)
  - Period of radiation (Acute / Chronic)
  - Nature of dose of radiation (Fractionalized / At one time)
  - Interval period between doses

Chapter 2: Direct and Indirect Effects

Dr. Kumari Sonam Jha

2.1: Ionization

- If an atom loses an electron, the nucleus becomes a positive ion and the free electron negative ion.
- This process of forming an ion pair is termed **Ionization**.
- To ionize an atom requires sufficient energy to overcome the electrostatic force binding the electrons to the nucleus.
- RH + x-radiation $\rightarrow$ R + H+ + e-
- Atomic number vs absorption
- The binding energy of an electron is related to the atomic number of the atom and the orbital type.
- Mass absorption coefficient of photoelectric absorption varies directly with the third power of the atomic number of the absorber (Z^3).
- Mass absorption coefficient for the Compton process is nearly independent of atomic number.
- In radiotherapy, high-energy photons in the range of 1-10 MeV are preferred because absorbed dose is nearly the same in bone and soft issues whereas low energy photons are preferred in diagnosis because of the much-desired large contrast in absorption of these tissues.



Primary types of ionizing radiation of concern in radiobiology are

A) alpha particles,

B) beta particles (electrons & posiotrons),

C) X- rays

D) gamma rays, and

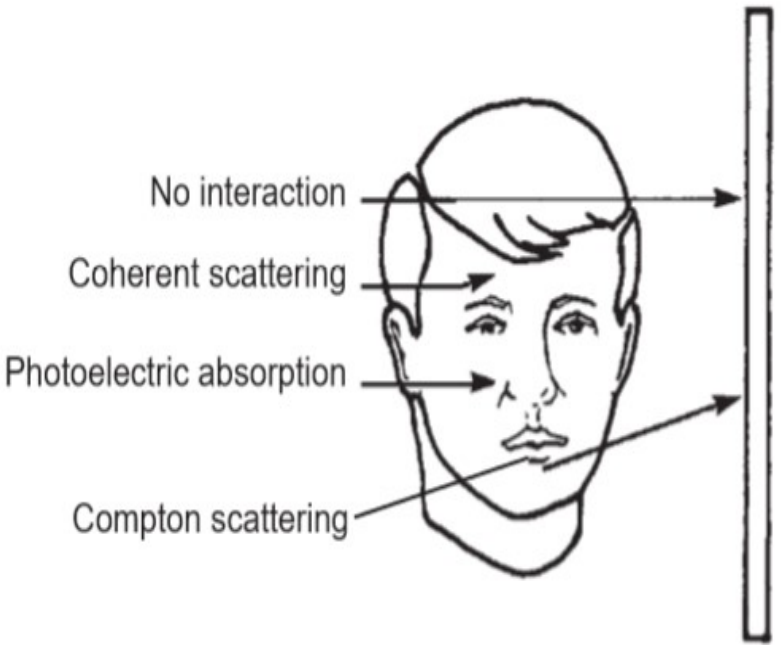
E) neutrons

A) photoelectric absorption (23 %)

B) crompton scattering (49 %)

C) pair production (electron and positron)

D) coherent scattering (7%)



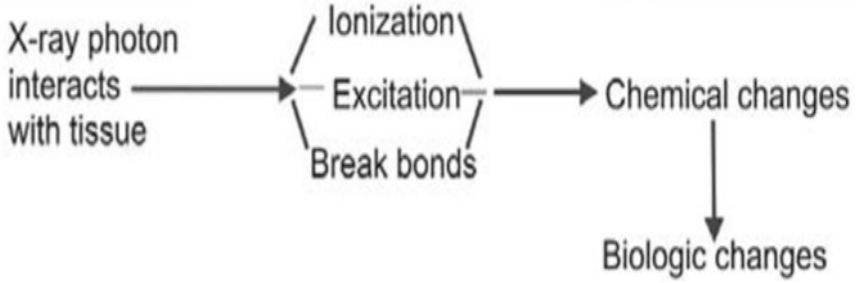
2.2: Direct Effect

- Energy of a photon or secondary electron ionizes biologic macromolecules.
- Target action theory

Target Action Theory

This states that the changes occur due to:

- Absorption of energy by biological molecules.
- Transfer of energy between unstable intermediate molecules.
- Formation of stable damaged molecules by disassociation or cross-linking



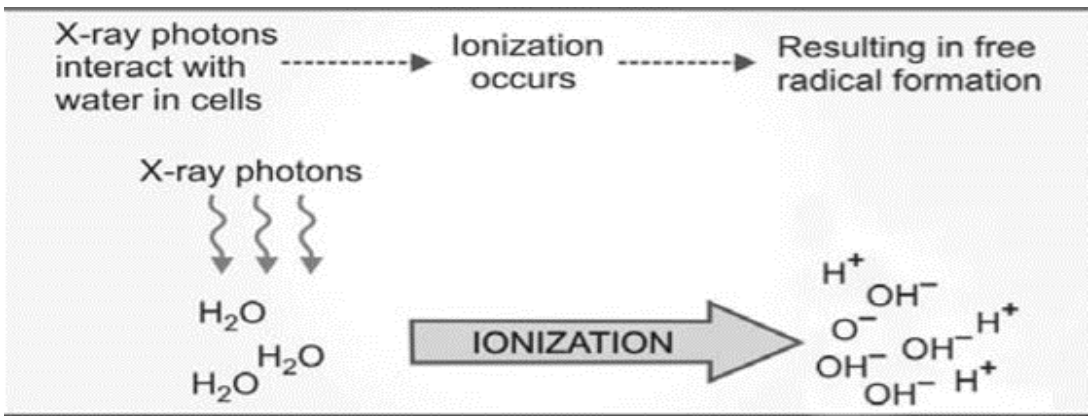
- Specific targets within the cell, probably the DNA or RNA in the nucleus take a direct hit from an incoming X-ray photon, or an ejected high energy electron, which breaks the relatively weak bonds between the nucleic acids.

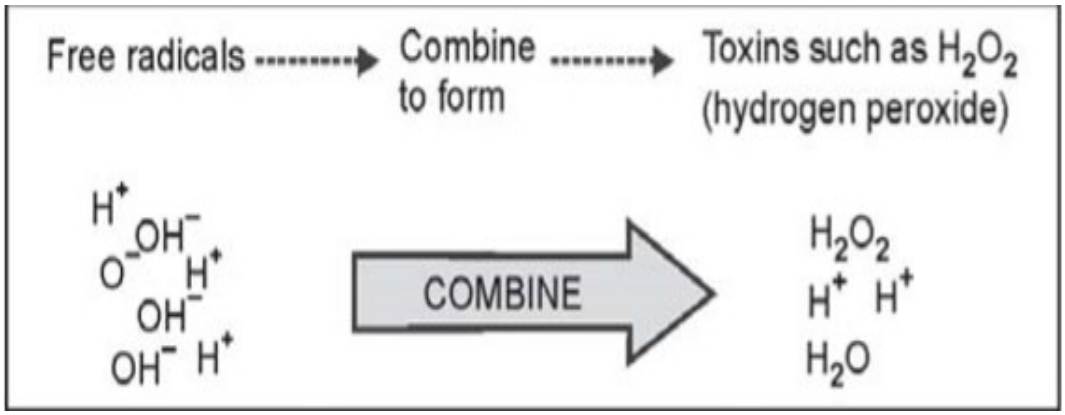
The subsequent effect includes:

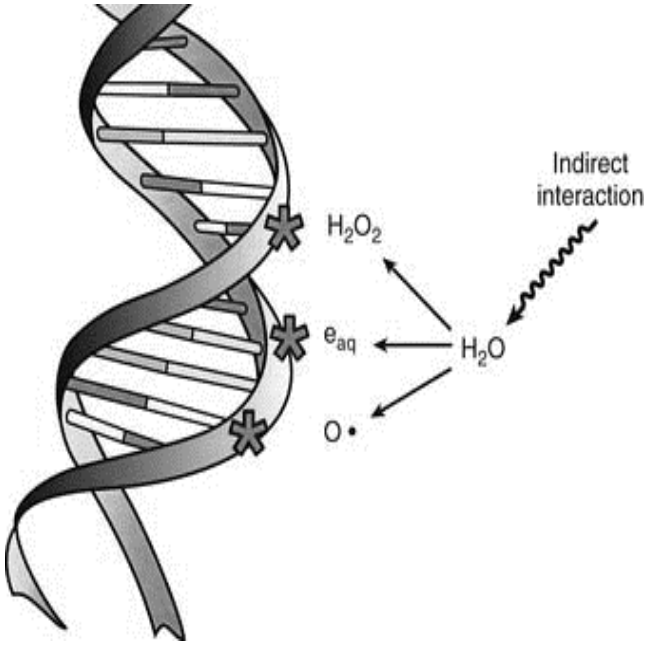
- Inability to pass on information.
- Abnormal replication.
- Cell death.
- Only temporary changes—the DNA being repaired successfully before further cell division.
- If the radiation hits some **somatic** cells, the effects on the DNA could result in radiation induced malignancy.
- If the damage is to **reproductive stem** cells, the result would be radiation-induced congenital abnormalities.

2.3: Indirect Effects (Poison Chemical Theory)

- A photon may be absorbed by water in an organism, ionizing some of its water molecules.
- The resulting ions form free radicals (radiolysis of water) that in turn interact with and produce changes in biologic molecules.







2.4: Acute Exposure

- This occurs when a large dose of radiation is absorbed in a short period of time, e.g., Nuclear accident.
- The radiation damage caused by acute radiation is much more than that caused by chronic exposure to radiation.
- This type of exposure is not seen when using Dental Diagnostic Radiations.

2.5: Chronic Exposure

- This occurs when small amounts of radiations are absorbed repeatedly over a long period of time.

Chapter 3: Deterministic and Stochastic Effects

Dr. Rohit

3.1: Deterministic Effects on Cells

Effects on Intracellular Structures:

1. NUCLEUS: sensitive site in the nucleus is the DNA within chromosomes.

2. CHROMOSOMES: useful markers for radiation injury. They may be easily visualized and quantified, and the extent of their damage is related to cell survival.

Effects on Cell Replication

- a reduction in size of the irradiated tissue as a result of mitotic delay and cell death.
- The three mechanisms of reproductive death are

    a) DNA damage,

    b) Bystander effect &

    c) Apoptosis.

Deterministic Effects on Tissues and Organs

- Radiosensitivity of a tissue or organ is measured by its response to irradiation.
- Severity of change depends on the dose and the amount of cell loss.

3.2: Short Term or Long-Term Effect

Short-Term Effects

- Effects seen in the first days or weeks after exposure
- Determined primarily by the sensitivity of its parenchymal cells.
- The extent of cell loss depends on damage to the stem cell pools and the proliferative rate of the cell population.

- Tissues composed of cells that rarely or never divide demonstrate little or no radiation-induced hypoplasia over the short term.

3.3: Long-Term Effects

- Seen months and years after exposure
- loss of parenchymal cells and replacement with fibrous connective tissue
- caused by reproductive death of replicating cells and by damage to the fine vasculature

3.4: Stochastic Effects

- Result from sublethal changes in the DNA of individual cells.
- Most important consequence: carcinogenesis.
- Heritable effects (much less likely)
- The ICRP estimates that a single brief whole-body exposure of 1Gy to 10000 people results in about 500 additional cancer deaths over the lifetime of the exposed individuals.

3.5: Carcinogenesis

- Radiation - induced gene **mutation**
- Radiation acts as an **initiator** (change in the cell: no terminal differentiation)
- Radiation acts as a **promoter**, stimulating cells to multiply.
- **Conversion** of proto-oncogenes to oncogenes.
- Susceptibility of Different Organs to Radiation -Induced Cancer

HIGH	INTERMEDIATE	LOW
Colon	Bladder	Bone surface
Stomach	Liver	Brain
Lung	Thyroid	Salivary glands
Bone marrow		Skin
Female breast		

Leukemia:

- increase the absolute risk by about 0.06% per 0.1 Gy.
- Maximum dose of 200 R is required for any damage to the marrow or blood forming organs.
- within 5 years following exposure and returning to baseline rates within 30 years.
- < 20 yrs of age are more at risk.
- Appear sooner than solid cancers

Thyroid Cancer:

- Follicular
- Children > susceptible than adults.
- Females 2-3 times more susceptible than males.
- A dose of 10 R will produce thyroid cancer

- All dental radiography gives scattered radiation to the thyroid, except cephalometry and curved surface tomography, where the thyroid is in the direction of the primary beam.

Brain and Nervous System Cancers

- Diagnostic x-ray examinations in utero and to therapeutic doses in childhood or as adults
- Average midbrain dose of about 1 Gy.
- A case-control study has shown an association between intracranial meningiomas and previous medical or dental radiography.
- The strongest association was with a history of exposure to full-mouth dental radiographs when younger than 20 years.

Salivary Gland Cancer

- Incidence increased in patients treated with irradiation for diseases of the head & neck or to diagnostic x radiation.
- An association between tumors of the salivary glands and dental radiography has been shown
- Only individuals who received an estimated cumulative parotid dose of 0.5 G y or more showed a significant correlation between dental radiography and salivary gland tumors.

3.6: Linear Energy Transfer

- LET is the energy transferred per unit path length.
- The LET is the product of the **specific ionization** and the **average energy** transferred per ion pair.
- Unit: keV/ µm
- High-LET radiation will quickly deposit its energy over a short range, while low-LET radiation will deposit its energy more slowly as it traverses a medium, resulting in a longer range.

3.7: Relative Biological Effectiveness

- The RBE is used to quantify the biological damage produced by a given type of radiation.
- Ratio of the dose of a standard radiation (e.g., 250-kV X-rays) to the dose of the radiation type of interest, such that the doses compared produce the same amount of biological damage.
- Generally, the RBE increases with increasing LET.
- For a given dose, high-LET radiations are usually more efficient at producing biological damage than low-LET radiations.
- However, the maximum RBE occurs at a LET of approximately 100 keV/μm.
- Above this value, higher LET does not contribute to more cell damage, and RBE decreases.

3.8: Exposure

Measure of radiation quantity, the capacity of radiation to ionize air.

- SI unit: **kerma**
- Kerma measures the kinetic energy transferred from photons to electrons.
- Is the sum of the initial kinetic energies of all the charged particles liberated by uncharged ionizing radiation (neutrons and photons) in a sample of matter, divided by the mass of the samples.

3.9: Absorbed Dose

- Amount of energy absorbed per unit mass.
- Absorbed dose = Ed / m
- S.I. units: Gray (1 Gy = 1 J/ kg).
- Earlier rad (radiation absorbed dose) was used.

  1Gy = 100 rad

3.10: Equivalent Dose

- Used to compare the biological effectiveness of different types of radiation to tissues.
- Is the product of the **absorbed dose (D_T)** in the tissue multiplied by a **radiation weighting factor** (W_R), often called the **quality factor?**
- Expressed as a summation to include the effects of irradiation of tissue by more than one type of radiation.
- Used for radiation protection and occupational exposure.
- S.I. unit: **sievert (Sv)**
- Earlier the unit rem (radiation equivalent man) was used, (100 rem = 1 Sv).
- For diagnostic x-ray examinations 1 Sv equals 1 Gy.

3.11: Effective Dose

- Estimate the risk of radiation in humans.
- Allows the risk from exposure to one region of the body to be compared with the risk from exposure to another region.
- It is sum of the products of **equivalent doses** to each organ/tissue (H_T) and the **tissue weighting factor** (W_T).
- The unit of effective dose is the **Sievert (Sv).**

3.12: Effective Dose in Intraoral Radiography (Microsv)

Table 1 Effective dose in intraoral radiography (μSv).

Author/source	Parameters	ICRP 60 (ave./film)
Gibbs [26]	70 kV, short round cone, E-speed, 18 films	100 (5.5)
	70 kV, long rectangular cone, E-speed, 21 films	14 (0.66)
	Long round cone, E-speed, 4 bitewings	12 (3)
UNSCEAR 2000 [35] (Health-care level I)		(13)
	70 kV, Short round cone, E-speed, 2 bitewing films	4 (2)
European Commission Issue 136 [8]		(1–8.3)
Iwai et al. [5]	60 kV, 24 cm round collimation	(9.3)
White and Pharoah [36]	Long round cone, D-speed, FMX	388 (18.47)[a]
	Long round collimation, PSP or F-speed film, FMX	171 (8.14)[a]
	Long rectangular collimation, PSP or F-speed film, FMX	35 (1.6)[a]

[a]ICRP 103, 2007.

3.13: Effective Dose in Panoramic Radiography (Microsv)

Table 2 Effective dose in panoramic radiography (μSv).

Author/source	Apparatus/parameters	ICRP 60
Danforth et al. [37]	Planmeca PM 2002®: 60 kV, 4 mA, 18 s	3.85
UNSCEAR 2000 [35] Health-care level I		7–14
European Commission Issue 136 [8]		3.85–30
Iwai et al. [5]	Veraview®: 75 kV, 8 mA	10.3
Gijbels et al. [38]	Cranex tome®: 70 kV, 4 mA, 15 s	8.1
	Cranex Excel®: 65 kV, 6 mA, 19 s	12.3
	Veraviewepocs 5D®: 70 kV, 4 mA, 8.2 s	5.5
	EC Proline®: 64 kV, 7 mA, 18.3 s	14.9
	Orthoralix 9200 DDE®: 74 kV, 4 mA, 12 s	4.7
Ludlow et al. [39]	Sirona Orthophos XG® (CCD)	14.2[a]
	Planmeca Promax® (CCD)	24.3[a]
Gavala et al. [40]	Planmeca Promax®: 66 kV, 6 mA, 16 s	17
	Planmeca PM 2002®: 66 kV, 8 mA, 18 s	23
	Planmeca PM 2002®: 60 kV,4 mA, 18 s	12
Matsuda et al. [41]	Asahi Hyper X®: 78 kV, 10 mA	12.76[b]
	PanoACT-1000®: 80 kV, 6 mA	6.66[b]
	OP 200®: 66 kV, 10 mA	8.89[b]

[a] ICRP 103, 2007.
[b] ICRP 103, 2007 excluding reminder tissues.

The average dose for **intraoral radiography**: ranged from less than 1 to around 20 microSv, depending on the film/digital sensors used, collimation, focus skin distance and tube voltage.

- Effective dose in **panoramic radiography:**
  more consistent and ranged from 4 to 30 microSv
- For **dental cone-beam CT** the effective dose varied widely among the products and the size of the field of view (FOV).
- When a limited area is exposed, the effective dose is less than 100 microSv.
- When the whole face is imaged or large FOV is selected, the effective dose ranges from 500 to 700 microSv.

Recommended Annual Limits for Human Exposure to Ionizing Radiation

RECOMMENDATION	NCRP	ICRP
Occupational Dose Limits		
Relative to stochastic effects	50 mSv annual effective dose limit and 10 mSv age (yr) cumulative effective dose limit	50 mSv annual effective dose limit and 100 mSv in 5-yr cumulative effective dose limit
Relative to deterministic effects	150 mSv annual equivalent dose limit to lens of eye and 500 mSv annual equivalent dose limit to skin and extremities	150 mSv equivalent dose limit to lens of eye and 500 mSv annual equivalent dose limit to skin and extremities
Nonoccupational (Public) Dose Limits		
Relative to stochastic effects	5 mSv annual effective dose limit for infrequent exposure and 1 mSv annual effective dose limit for continuous exposure	1 mSv annual effective dose limit and, if higher, not to exceed annual average of 1 mSv over 5 yr
Relative to deterministic effects	50 mSv annual equivalent dose limit to lens of eye, skin, and extremities	15 mSv annual equivalent dose limit to lens of eye and 50 mSv annual equivalent dose limit to lens of eye, skin, and extremities
Embryo-fetus	0.5 mSv equivalent dose limit per month after pregnancy is known	2 mSv equivalent dose limit after the pregnancy has been declared
Negligible individual dose*	0.01 mSv annual effective dose	None established

Adapted from White and Pharoah. Oral radiology – Principles and Interpretation. First south Asia Edition[1]

3.14: Collective Dose

- The dose received per person in Sv multiplied by the number of persons exposed per year i.e., man-sievert per year.
- This unit is generally used for protection purposes and in population response calculations

3.15: Radiolysis of Water

- Interaction of radiation with water causes ionization and excitation process producing:

1. short-lived H_2O^+ radical-cations
2. fast electrons, and
3. electronically-excited water molecules (H_2O^*).

$IR + H_2O \rightarrow H_2O^+ + e^-$

$IR + H_2O \rightarrow H_2O^*$

H_2O^+ ions and excited water molecules are unstable and decompose (10^{-13} s) to form OH• and H• radicals

$H_2O + H_2O^+ \rightarrow H_3O^+ + OH•$

$H_2O^* \rightarrow OH• + H•$

This can react with water forming an anion which rapidly dissociates to give a hydrogen atom (H·).

$$e^- + H_2O \rightarrow H_2O^- \rightarrow OH^- + H•$$

The thermalized electrons are solvated by dielectric interactions with neighboring water molecules to form (e-aq) free electron.

It reacts with a proton to give a hydrogen atom (H·)

$e^-_{aq} + H^+ \rightarrow H•$

In oxygenated solutions, e-aq is converted to O2-, which is a strong oxidizing agent and the precursor of hydrogen peroxide:

e-aq+ O2 → O2-

These primary water radicals (eaq, OH, H•) have high reactivity towards molecules of cells, DNA, lipids and other subcellular constituents.

In oxygenated solutions, hydrogen atoms can react with oxygen to give hydroperoxyl free radicals (HO2•):

H• + O2 → HO2•

Hydroperoxyl free radicals contribute to the formation of hydrogen peroxide in tissues:

$HO_{2\cdot}$ + H. H2O2

$HO_{2\cdot}$ + $HO_{2\cdot}$ O2 + H2O2

Relative Radio sensitivity of Various Organs

HIGH (DIVIDE REGULARLY)	INTERMEDIATE (OCCASIONALLY)	LOW (INCAPABLE)
Lymphoid organs	Fine vasculature	Optic lens
Bone marrow	Growing cartilage	Muscle
Testes	Growing bone	
Intestines	Salivary glands	
Mucous membranes	Lungs	
	Kidney	
	Liver	

3.16: Heritable Effects

- Changes seen in the offspring of irradiated individuals.
- They are the consequence of damage to the genetic material of reproductive cells.

- Probability from dental radiography is quite low, as the annual **genetically significant dose**" from dental radiography is 0.08 microSv.

3.17: Genetically Significant Dose

Dose that if received by every member of the population would be expected to result in the same total heritable injury to the population as do the actual gonadal doses received by the individuals exposed.

- Individual gonadal dose following a FMS is less than **O.01microSv** in an adult.
- The gonadal dose is so small from dental radiography that the risk of heritable defects is negligible in comparison with the somatic risk.
- studies of the children of patients who received radiotherapy show no detectable increase in the frequency of genetic diseases.

3.18: Doubling Dose

- The amount of radiation a population requires, to produce in the next generation as many additional mutations as arise spontaneously.
- Estimated to be approx. 1 sievert (Sv).
- Doubling dose for low dose-rate exposure in humans was estimated by the BEIR III Committee to be in the range of 50 to 250 rems (0.5 to 2.5 Sv).
- The corresponding estimate of the 1986 UNSCEAR report was 100 rads (1 Gy).

3.19: Dental risk implications

- Diagnostic radiation accounts for only about 11% of all exposure.
- Only about 1% of this 11%, or about 0.1% of the total exposure, results from dental radiography.
- Risk associated with dental radiographic examinations:

EXAMINATION	MANUFACTURER	MODEL	FORMAT	SV	PROBABILITY OF * IN A MILLION FATAL CANCERS
bitewing	planmeca	intra	PSP /F speed /rectangular	5.O	0.3
Full mouth series	planmeca	intra	psp/f speed /rectangular	34.9	2
full mouth series	planmeca	Intra	PSP/F speed /round	170.7	9
Full mouth series	planmeca	intra	D speed/round	388.0	21
panoramic	planmeca	promax	CCD	24.3	1.3
panoramic	serona	Orthophos XG	CCD	14.2	0.8
cephalogram	Varian medical	interray	PSP	5.6	0.3

LARGE	FIELD OF	VIEW
machine	Sv	Probabality of*/m fatal cancer
I cat NG	74.0	4
CB mercuRay	569.0-1073.0	31-59
kodak	93.0-260.0	
range	30.0-260.0	

MEDIUM	FEILD	OF VIEW
Machine	Sv	Probability of */m fatal cancer

Galileos	70.0-128.0	4-7
I cat ng	87.0	5
CB mercuRay	407.0-510.5	31
kodak	76.0-166.0	
range	48.0-510.5	

	Small field view	
Machine	Sv	Probability of */ M fatal cancer
Orthophos XD 3G	64	4
I cat classic	34.0-148.5	
Promax	30.0-674.0	27-36
preXion	189.0-388.0	10-21
Range	30.0-674.0	

	MULTISLICE CT	
machine	SV	Probability of */M fatal cancer
somaton	534.0-860.0	29-47
range	474.0-1110.0	

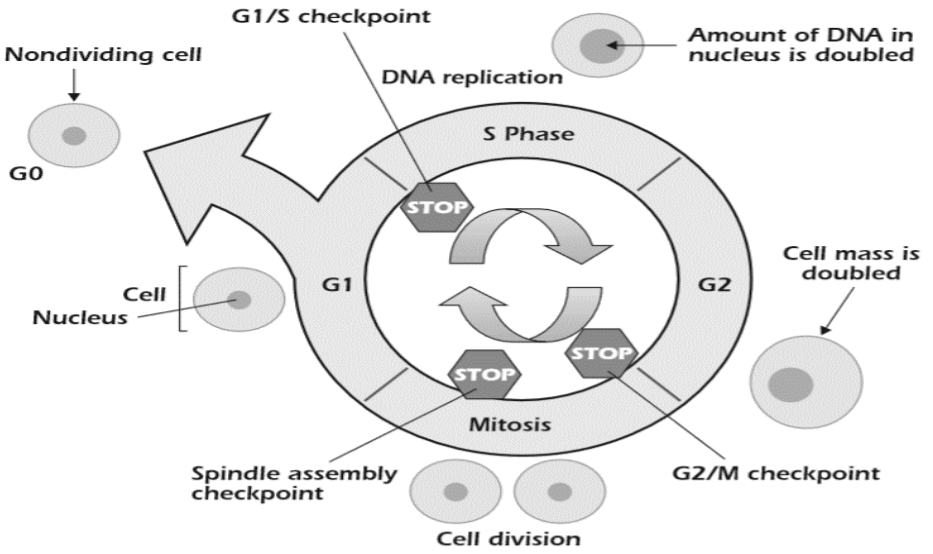
- The BEIR V committee estimates that a single, brief whole-body exposure of 0.1 Gy to 100000 people results in about 443 additional cancer deaths over the lifetime of the exposed individuals
- The ICRP 1990 estimate of 500 lifetime fatal cancers per 10000 exposed persons per Sv.
- There is a solid body of work demonstrating increased risk of tumours in individuals exposed to more than about 100 mGy
- Radiosensitive structures in the head and neck include thyroid glands, salivary glands, bone marrow (leukaemia) and brain.
- Dental exposures have been specifically linked to **meningiomas, salivary gland tumours and thyroid tumours.**

3.20: Role of Cell Cycle in Mediating Sensitivity to Radiotherapy

- Cell cycle regulation is the most important determinant of ionizing radiation sensitivity.
- A common cellular response to DNA - damaging agents is the activation of cell cycle checkpoints.

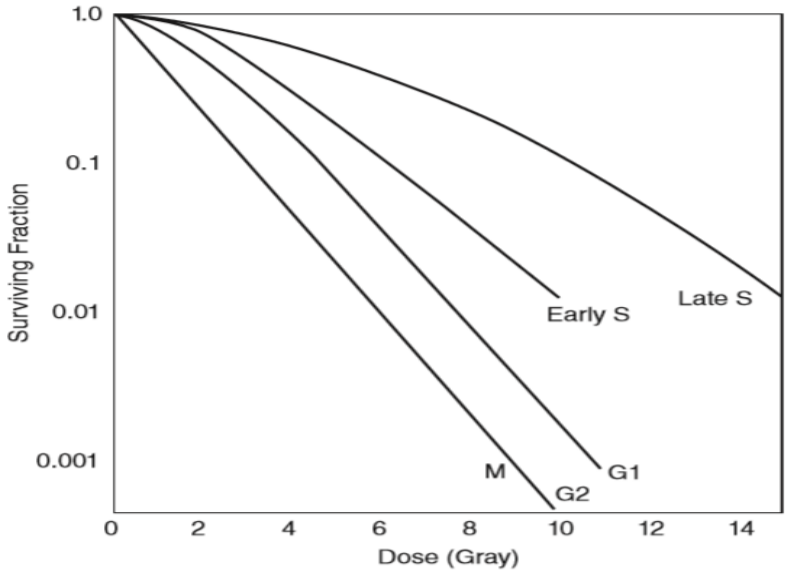
The cell cycles

- G1 - the first gap in activity, between mitosis and the S phase (most variable length)
- S - DNA synthesis phase
- G2 - the second gap in activity, between S phase and the next mitosis
- M - mitosis, identifiable by light microscopy and the most constant time
- If the cells stop progressing through the cycle, they are in G0



Variation of radio sensitivity with cell age in the mitotic cycle

- Cells are **most sensitive** at or close to M (mitosis), **G2M**
- G2 phase is usually as sensitive as M phase
- **Resistance** is usually **greatest** in the latter part of **S phase** due to repairs that are more likely to occur after the DNA has replicated
- If G1 phase has an appreciable length, a resistant period is evident in G1.



Damage Recognition and Signaling:

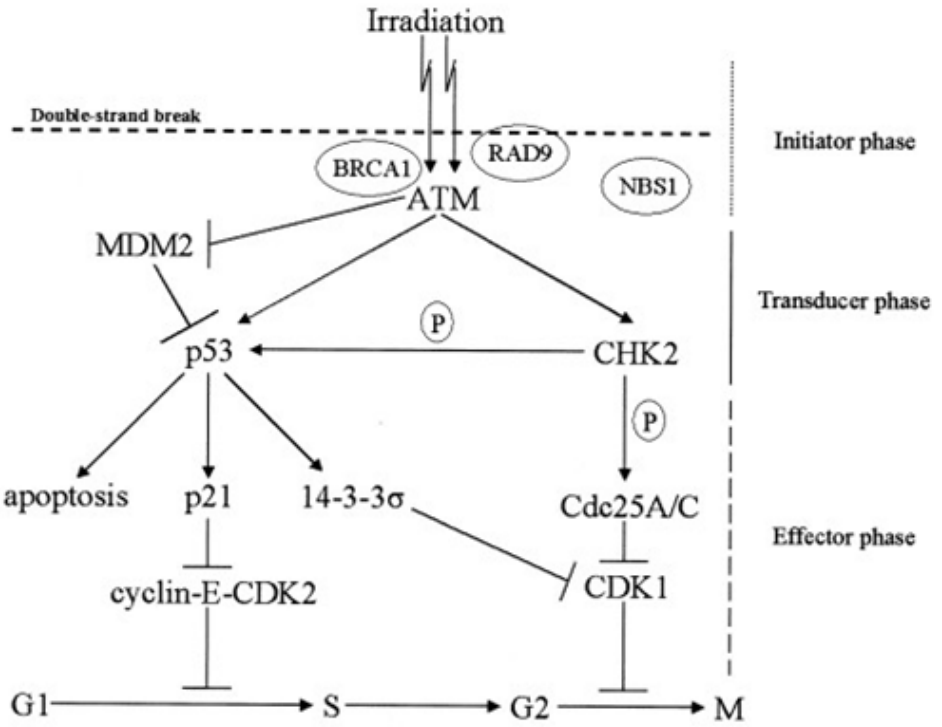
First step: ATM binding to DNA termini.

This induces kinase activity in ATM which phosphorylates and activates the **CHK kinases**

Phosphorylated p 53 is released from MDM-2 and is stabilized to induce p21,

Inhibits the cyclin-dependent kinase cyclin E-CDC-2 controlling The G1-S transition in the cell cycle.

The resultant G1 arrest after irradiation ensures that the damaged DNA is not replicated before repair



- Multiple pathways are involved in maintaining the genetic integrity of a cell after its exposure to ionizing radiation.
- The DNA damage induced by ionizing radiation initiates signals that can ultimately activate

  - either temporary checkpoints that permit time for genetic repair or

  - irreversible growth arrest that results in cell death (necrosis or apoptosis).
- One of the key proteins in the checkpoint pathways is the tumor suppressor gene **p53**, which coordinates DNA repair with cell cycle progression and apoptosis.
- Specifically, in addition to other mediators of the checkpoint response

CHK kinases, p21, p53 mediates the two major DNA damage- dependent cellular checkpoints,

- one at the G1–S transition (more direct & significant) and

- the other at the G2–M transition

Radiation Induced Molecular Check Point Genes:

Position	Primary signalling proteins	Applies to cells irradiated in	Features
G1	ATM, p53, p21	G1	Prevents entry into S
S	ATM, Chk1/Chk12, CDC25A/ CDC25C, BRCA1, BRCA2	S	Slows progression through S
G2-early	ATM, Chk1/Chk12, CDC25A/ CDC25C, BRCA1, BRCA2	G2	Prevents entry into mitosis
G2-late	ATR, Chk1, CDC25A/CDC25C	All phases	Accumulation of cells in G2

- The **G1 checkpoint** prevents the replication of damaged DNA before the cell's entry into S phase, and

- the **G2 checkpoint** prevents the segregation of aberrant chromosomes during M phase.
- Two molecularly distinct **G2/M** checkpoints can be identified

1. The first of these G2/M checkpoints occurs **early** after exposure to ionizing radiation,

- is very transient,

- is ATM dependent, and

- is dose independent.

- represents the failure of cells that had been in G2 at the time of irradiation to progress into mitosis.

- the "**late**" G2/M accumulation,

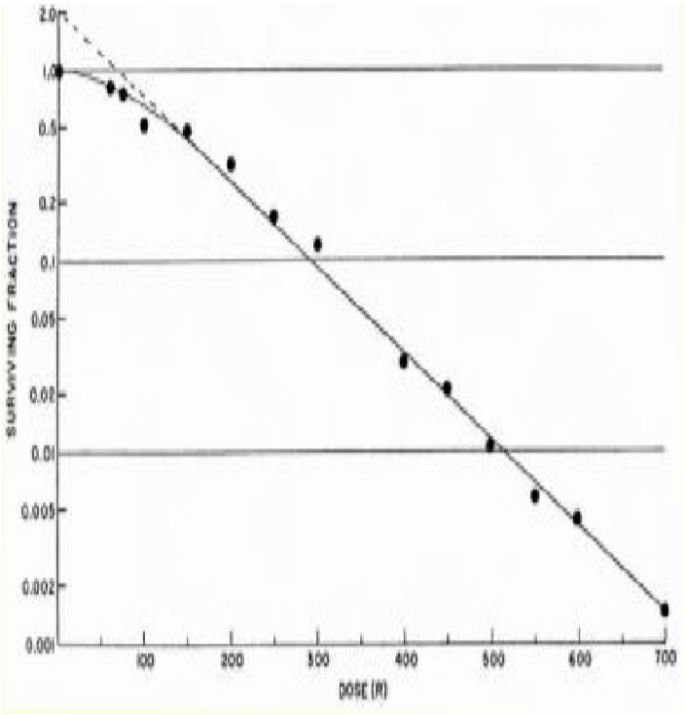
- begins to be measurable only several hours after ionizing radiation,

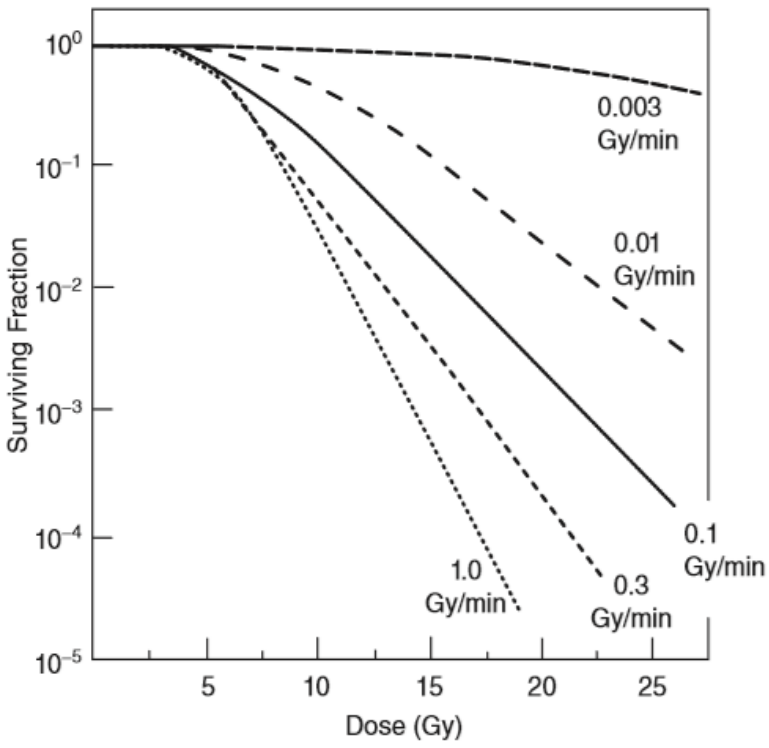
- is ATM independent,

- is dose dependent, and

- Represents the accumulation of cells that had been in earlier phases of the cell cycle at the time of radiation exposure
- Bartkowiak et al. showed that cells are extremely sensitive to the G2/M checkpoint and accumulate in a dose-dependent manner with doses as low as 0.2 Gy.
- Doses of 2 Gy have also been studied and been shown to increase considerably the G2/M phase fraction as well.
- Doses of 1 Gy have been shown to induce hyperradiosensitivity in a number of cell lines.

Cell Survival Curves:





Cellular Injury

- There are 3 ways for cellular injury to occur after ionizing radiation exposure.

1) Division delay: with dose dependent delay in cell division;

2) **Reproductive failure:** when cells fail to complete mitosis either immediately or after one or more generations; and

3) **Interphase death:** a relatively prompt death caused by the **apoptosis** mechanism.

1. Division delay

- Mitotic division is delayed
- This is the first observable effect from ionizing radiation exposure.
- This is seen in doses greater than 0.5Gy (50 rads) up to approximately 3 Gy (300 rads).
- At more than 3 Gy (300 rads), the mitotic rate does not recover and the division may never happen, thus killing the cell.

2. Reproductive failure

- Based on the dose, as dose increases, so does reproductive death
- At levels at or below 1.5 Gy (150 rads), reproductive failure is random and nonlinear.
- At doses above 1.5 Gy (150 rads), it is linear and nonrandom.

3. Interphase death

- Seen most commonly with **lymphocytes**,
- Cell death can occur many generations from the initial radiation exposure
- It is thought that this is either a natural process of aging (apoptosis), or that a critical mechanism of cell replication has been altered.
- It depends on the type of cell affected and the dose to the cell.

Radiobiological Definition of Cell Death

- Cells are generally regarded as having been "killed" by radiation if they have lost reproductive integrity, not by whether they physically survive in the population.
- Can occur by:

1. **apoptosis**,

2. **necrosis**,

3. **mitotic catastrophe or**

4. by induced senescence.

Apoptosis- programmed cell death triggered in response to cellular stress (e.g., radiation)

- Previously - called **interphase cell death**.
- microscopy:

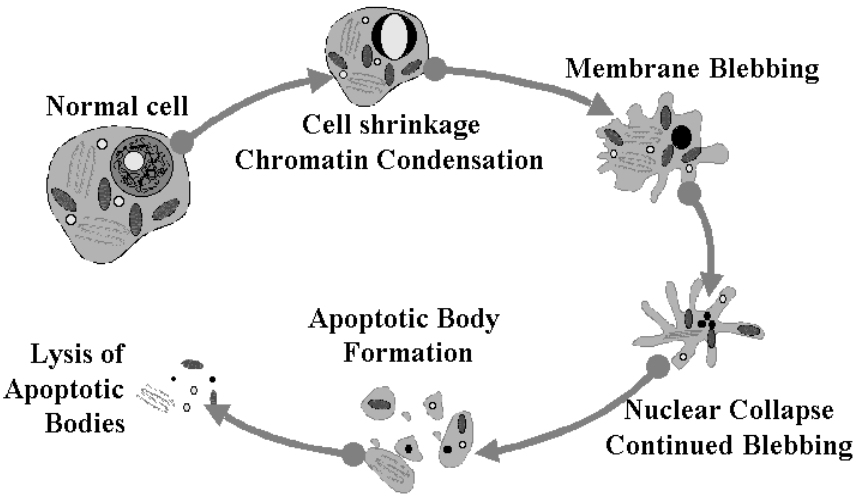
  - typical shrinkage of cellular morphology,

  - condensation of chromatin,

  - nucleosome laddering indicating chromatin degradation,

  - cell membrane blebbing,

  - activation of caspases and release of cytochrome c.

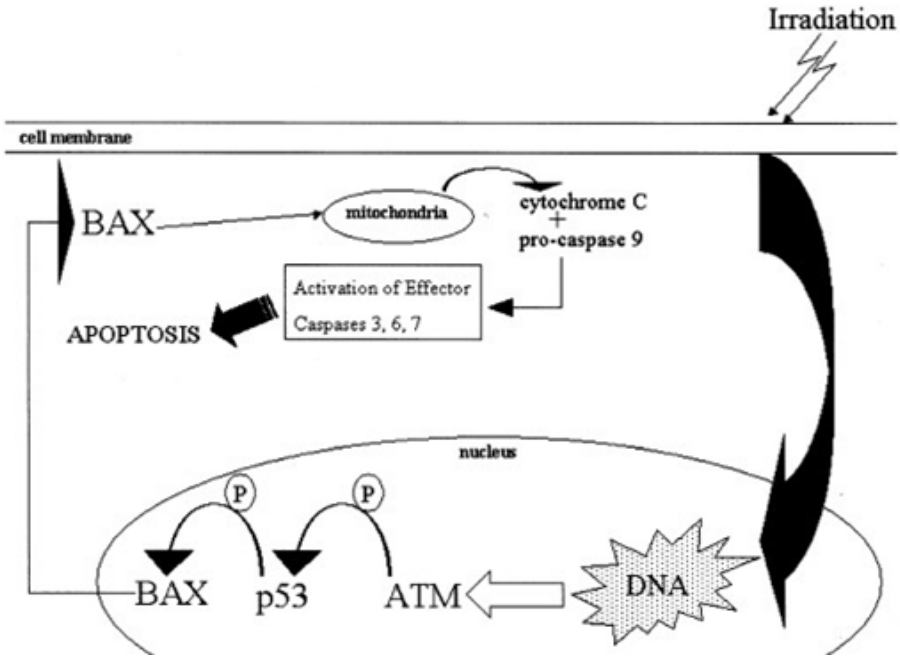


The relation of apoptosis and radiosensitivity is, however, controversial.

- Although some investigators have reported that apoptosis is an important mechanism by which RT kills cells,
- others have argued in opposite.
- Use of apoptotic assays concentrates on the first 90% of cell killing, but the outcome of treatment depends on multi-log cell kill.
- The **clonogenic cell survival assay** is, therefore, a more appropriate method to assess radiation sensitivity.
- Loss of colony-forming ability is likely to be the key event in radiation-treated tumor cells, and the appearance of morphologic and molecular evidence of apoptosis is probably downstream from this event.
- Because the cell cycle is strongly affected by irradiation, and radiosensitivity depends on cell cycle position and cell cycle progression

- **some association between apoptosis and radiosensitivity has been observed**.

- **p53** plays a role in regulating the progression through the cell cycle, it can also induce **apoptosis** in cells.
- Although the exact means by which p53 activates apoptosis is unclear, evidence has shown that p53 mediates apoptosis by way of **transcription independent and transcription-dependent mechanisms**
- p53 is known to regulate the expression of several proteins involved in the apoptotic pathway, including CD95, PIDD, PIGs, PERP, and KILLER/DR5
- p53 also interacts with BAX, BCL-XL, and BCL-2 to exert a direct apoptotic effect at the level of the mitochondria



- Additionally, **Fas**, a cell-surface protein that triggers apoptosis when it binds to its ligand, is encoded by a target gene transcriptionally activated by p53.
- Despite p53's known interaction with all of these antiapoptotic genes, none of them, however, appears to be the principal mediator of the p53 apoptotic signal.
- This leaves open the possibility that a uniqueness exists among p53 targets, and a tissue/cell-type specificity in their regulation in response to ionizing radiation.
- Radiation can induce the cleavage of the membrane-bound protein **sphingomyelin**, resulting in the formation of **ceramide**, a lipid second messenger.
- Conversely, ceramide production can be inhibited by **BCL-2, an anti-apoptotic membrane-protein**;
- in turn, ceramide itself has been implicated in the downregulation of BCL-2.
- The ceramide produced in response to ionizing radiation, therefore, appears to act as a positive regulator of **apoptosis**.

2. Necrosis

- Generally, occurs after high radiation doses.

- Passive process in which cells pass through mitosis with unrepaired DNA strand breaks, leading to lethal chromosomal aberrations (micronuclei) in nonclonogenic daughter cells.
- Characterized by:

  - a loss of membrane integrity & Increase of membrane permeability,

  - cell swelling,

  - dilation of cytoplasmic vesicles, and

  - the subsequent random degradation of DNA.

  - Shut down of cell metabolism

3. Mitotic catastrophe

- Reproductive cell death is a result of mitotic catastrophe which can occur in the first few cell divisions after irradiation, and it occurs with increasing frequency after increasing doses.
- Cells that fail to divide successfully after irradiation can also undergo apoptosis at that stage.
- abnormal mitosis (result of DNA damage or deranged spindle formation coupled to the debilitation of different checkpoint mechanisms)
- usually ends in the formation of large cells with multiple micronuclei and decondensed chromatin

Senescence or Replicative senescence (RS)

- observed when cells stop dividing, and this differs from the behavior of stem cells and tumour cells which do not show these limitations.
- Senescent cells:

  - edematous and

  - show poor cell-cell contact,

- increased polyploidy,

- decreased ability to express heat shock proteins, and

- shortening of telomeres.

Dose–Response Models

- There are many different theoretical types of dose– response models used to explain the effects of radiation exposure.
- Different models suggest different possibilities of response to radiation exposure.
- Ranging from linear–no threshold, which suggests any exposure (even background radiation) is harmful, to the possibility that low-dose radiation exposure is beneficial (**radiation hormesis**).

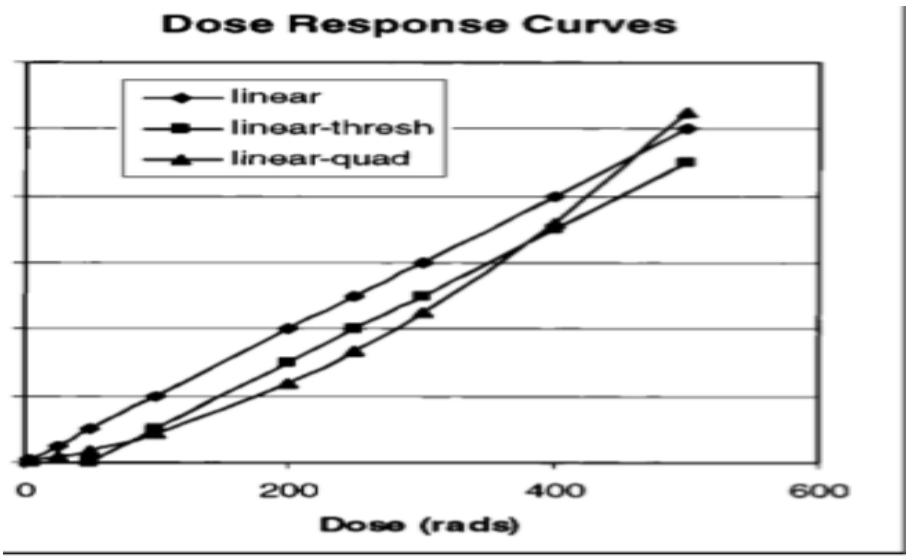
Three dose– response models used in radiation biology are:

1) Linear –no threshold,

2) Linear –threshold, and

3) Linear quadratic.

- These dose–response models are used to extrapolate high-dose effects (which are known) to the low-dose range (which has not been reliably detected)



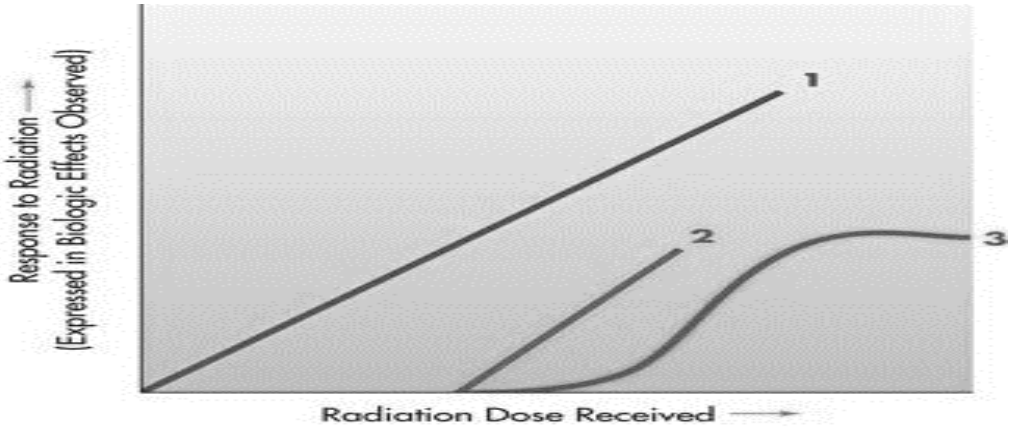
1. Linear–No Threshold Model

- Used for any known carcinogen at any level of exposure
- States that any radiation exposure, no matter how small, can induce cancer.
- The linear–no threshold dose–response model is used for regulatory purposes, whenever a xenobiotic or other carcinogenic agent is known at any dose level.
- While this is a possibility, generally no clinical effects are seen below approximately 0.5 Gy (50 rads).
- At high doses, or more than 0.5 Gy (50 rad), clinical symptoms of radiation start to appear;
- At much higher doses, radiation exposure is clearly a known carcinogen, primarily due to its mutagenic effect on cells.
- The greatest association is with leukemia.

2. Linear–Threshold Model

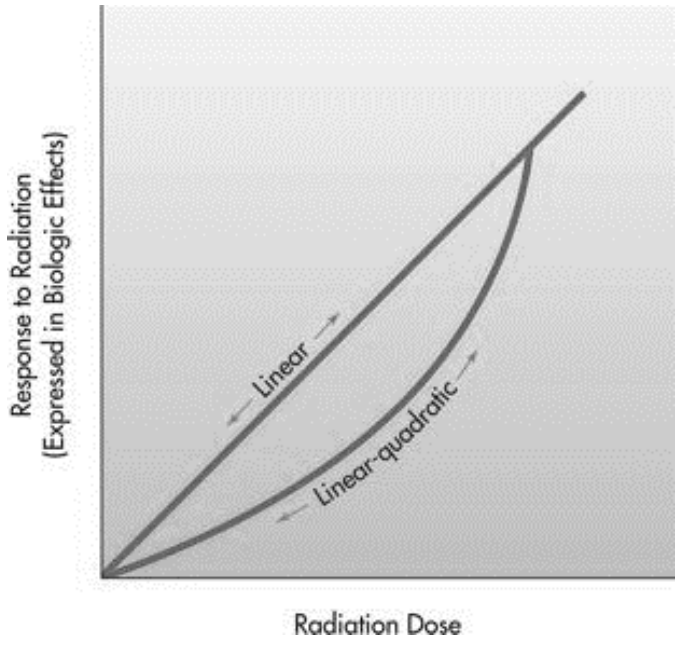
- Consists of a known threshold below which no effects are seen.
- At the threshold level, effects are noticeable and increase linearly as the dose increases.
- This is the dose–response model that may make the **most sense** to use, because it is generally accepted that there are no clinical effects seen from radiation exposure at or below 0.5 Gy (50 rads).
- Risk estimation in this dose range should be strictly qualitative accentuating a range of hypothetical health outcomes with an emphasis on the likely possibility of zero adverse health effects.
- The current philosophy of radiation protection is based on the assumption that any radiation dose, no matter how small, may result in human health effects, such as cancer and hereditary genetic damage
- Below 0.1 Sv (10 rem) (which includes occupational and environmental exposures) risks of health effects are either too small to be observed or are nonexistent."

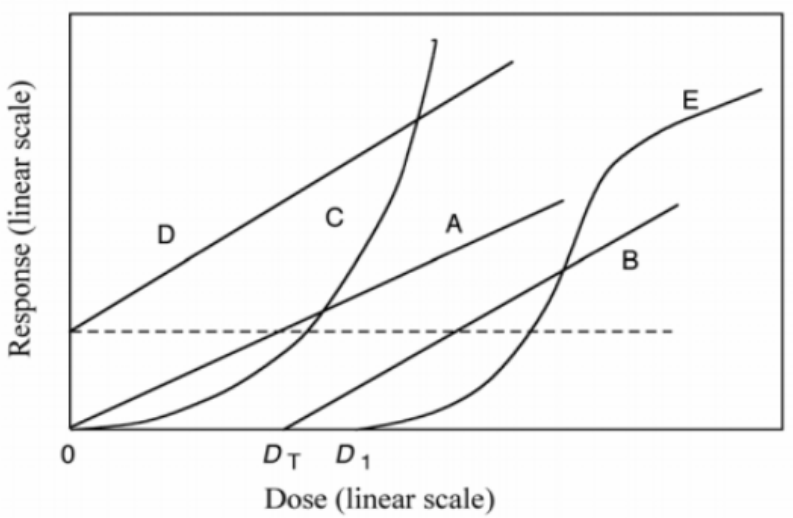
- Although this position statement suggests levels 5-fold less for clinical effects, it still indicates that a threshold does exist to a certain degree in radiation exposure.
- This statement favors a linear–threshold dose–response model with a threshold of 0.1 Sv (10 rems).



3. Linear Quadratic Model

- The linear quadratic dose–response model is used for overall human response to radiation exposure.
- Response at low levels of radiation exposure is linear, while higher doses are quadratic.
- There is no threshold in this dose–response model.
- In cell survival theories, the linear quadratic dose–response model is used to represent the multiple target/single hit theory.
- best shown as a **semilog plot** of survival against irradiation dose, generally in the dose range of 1 – 10 Gy for single cells.
- The most common model used today is the **linear-quadratic model**.





- For high LET irradiation the quadratic component is small or non-existent.
- Any dose–response model for radiation in the lower levels is extrapolated from what is known at high-dose levels.
- Thus, any lower-level response from radiation is only theorized, not proven.

The **only accepted dose–response model** by the **Nuclear Regulatory Commission (NRC**) is the linear–no threshold dose–response model, which suggests that any radiation exposure can lead to cancer induction.

Pregnancy & radiation

- No deterministic effect is seen at fetal dose below 100mGy.
- Vulnerable period for deterministic effect: **2nd to 20th week of gestation**
- The period of maximal sensitivity of the brain: **8 to 15 weeks after conception.**
- The frequency of severe mental retardation after exposure to 1 G y during this period is about 43%.
- exposure of a pregnant woman: not exceed 0.5 rem (5 mSv).
- The foetal dose from a dental X ray exam has been estimated to be between 0.3 µSv and 1µSv
- The risk for radiation induced childhood fatal cancer is about 6% per Gy.
- Exposures in the range of 2 to 3 Gy during the first few days after conception are thought to cause undetectable death of the embryo.

Acute Radiation Effects

- Acute ionizing radiation exposure is "harmless" at background or **diagnostic** levels, but is no stochastic and harmful at high-dose levels.
- At or above approximately **0.5 Gy (50 rads),** acute effects are predictable and follow a linear path.
- Staging of Acute Radiation Syndromes

Prodromal Phase:

Signs and symptoms include:

- NVD,
- hair loss above 3 Gy (300 rads),
- skin erythema above 6 Gy (600 rads)

Latent Phase: Period of no signs or symptoms

Manifest Phase:

- signs and symptoms return to prodromal levels or worse.

  - NVD returns,

  - hematologic syndrome,

  - GI Syndrome,

  - CNS syndrome

- **Recovery Phase**:

  - less than a 10-Gy (1000 rads) dose.

3.21: Chronic Radiation Effects

- Chronic effects of ionizing radiation exposure are primarily stochastic.
- The chief concern is possible cancer induction.
- However, noncancerous effects are possible, such as **cataract** formation in the eye.
- Another possible chronic stochastic effect is shortening of the life span.
- **Leukemia** has been associated as a stochastic effect of chronic radiation exposure with doses as low as 0.50–1 Gy (50–100 rads).
- Between 1–5 Gy (100–500 rads), there is a linear correlation between dose and leukemia incidence.
- Data suggest that incidence of leukemia increases at a rate of 1–2 cases per million per year per 0.001 Gy (1 rad) as a result of exposure.
- There is an average latency period of 14 y from exposure to onset of disease.
- Higher doses of ionizing radiation have also been associated with **thyroid, bone, lung, and various other cancers.**

3.22: Molecular and Cellular Radiobiology

Radiation lesions in DNA

- Single strand breaks in the phosphodiester linkage,
- Double strand breaks on opposing sites
- Displaced or base damage
- Protein-DNA crosslinks
- Protein -protein crosslinks involving nuclear proteins such as histones and non-histone proteins.
- The numbers of lesions induced in the DNA of a cell by a dose of 1-2 Gy are approximately:

  - base damages > 1000;

  - single strand breaks (ssb) ~1000;

  - double strand breaks (dsb) ~40.

Major types of DNA repair

- Double strand breaks: two primary repair pathways

Non-homologous end joining (NHEJ):

- Repair operates on blunt ended DNA fragments resulting from broken phosphodiester linkages.

1. Enzymatic "cleaning up" of the broken ends of the DNA molecule.

2. Ku70/Ku80 repair proteins to recognize the lesion termini

3. Binding of the Ku-heterodimer to DNA-Protein kinase

4. Activation of the XRCC4 ligase enzyme by this complex for final religation of the fragments.

Homologous recombination (HR)

utilizes sequence homology with an undamaged copy of the broken region

1. Starts by nucleolytic resection of blunt ends,

2. Binding of NBS/MRE11/rad50 protein complex to the DNA termini,

3. Followed by strand exchange facilitated by attachment of rad51/XRCC2 protein.

4. Then there is DNA synthesis of the missing nucleotides on the undamaged templates and ligation.

Consequences of Unrepaired DNA Damage: Chromosome Damage

- Mutations from low dose exposure influence base pairing, coding, transcription and gene expression.
- Aberrant chromosomes arise when broken ends rejoin with other broken ends to generate rings, dicentrics, translocations and other chromosome aberrations.
- Dicentric chromosome aberrations arise post replication from the joining of 2 broken chromatids in different chromosomes and can be used as a marker for radiation exposure.
- Acentric fragments and dicentrics are unstable aberrations and may not survive past the next mitosis, implicating loss of genetic material which may signal death in diploid cells.
- In polyploidy cells such losses may be of lesser consequence

Role of Cell Cycle in Mediating Sensitivity to Radiotherapy

- Multiple pathways are involved in maintaining the genetic integrity of a cell after its exposure to ionizing radiation.
- The DNA damage induced by ionizing radiation initiates signals that can ultimately activate

  - either temporary checkpoints that permit time for genetic repair or

  - irreversible growth arrest that results in cell death (necrosis or apoptosis).

- Such checkpoint activation constitutes an integrated response that involves- **sensor** (RAD, BRCA, NBS1),
  - **transducer** (ATM, CHK), and
  - **effector** (p53, p21, CDK) genes.
- One of the key proteins in the checkpoint pathways is the tumor suppressor gene p53, which coordinates DNA repair with cell cycle progression and apoptosis.
- Specifically, in addition to other mediators of the checkpoint response (CHK kinases, p21), p53 mediates the two major DNA damage- dependent cellular checkpoints,
  - one at the G1–S transition (more direct & significant) and
  - the other at the G2–M transition
- The cell cycle phase also determines a cell's relative radiosensitivity, with cells being
- most radiosensitive in the **G2-M phase**,
- less sensitive in the **G1 phase**, and
- least sensitive during the **latter part of the S phase**.
- This understanding has, therefore, led to the realization that one way in which chemotherapy and fractionated radiotherapy may work better is by partial synchronization of cells in the most radiosensitive phase of the cell cycle.
- We describe how cell cycle and DNA damage checkpoint control relates to exposure to ionizing radiation.
- studies have focused more specifically on how cell cycle checkpoints, including mutations in p53 and p21,
- as well as the cell cycle phase, determine radio responsive-

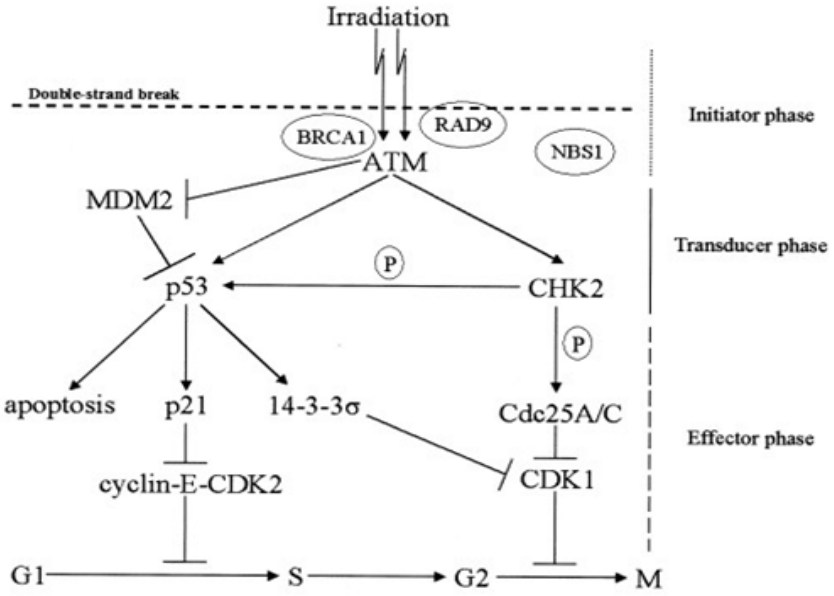
Radiation and Cell Cycle Signaling

- The phosphatidyl-inositol kinase-related protein ATM is the most proximal signal transducer initiating cell cycle changes after the DNA damage induced by ionizing radiation.

- Likewise, the rapid induction of ATM serine/threonine protein kinase activity after ionizing radiation has also suggested that ATM acts at an early stage of signal transduction in mammalian cells.
- Mammalian ATM is a member of a family of protein kinases that include ATM-Rad3-related (ATR), DNA-dependent protein kinase, and FRAP, which are related because they have a similar carboxy-terminal kinase domain.
- Until recently, the mechanism by which ATM kinase activity increases after radiation exposure was poorly understood.
- It was initially believed that double-strand breaks in the DNA induced by ionizing radiation either directly or indirectly signaled to ATM.
- However, the rapidity with which ATM is phosphorylated after ionizing radiation suggested that ATM was not activated by direct binding to the DNA strand breaks.
- Recently, Bakkenist and Kastan showed that, instead, ATM activation may result from changes in the structure of chromatin brought about by intermolecular autophosphorylation and ATM dimer dissociation.
- Once dissociated, ATM can then potentially phosphorylate numerous downstream targets, including p53, MDM2, CHK2, NBS1, RAD9, and BRCA1.
- ATM's essential role in DNA damage and repair is highlighted by the **extreme sensitivity to ionizing radiation** of cells with defective ATM and/or lacking ATM
- This is also the case in patients with ataxia telangiectasia who have a mutated ATM gene.
- These patients have a characteristic phenotype consisting of a heightened cancer predisposition, extreme sensitivity to radiation, and cell cycle abnormalities.
- In particular, cells from ataxia telangiectasia patients show defective G1, S, and G2 arrest after ionizing radiation.
- ATM has various targets.
- After cells are exposed to ionizing radiation, ATM phosphorylates p53, stabilizing the protein and prolonging its half-life.

- Ionizing radiation also leads to the phosphorylation of serines 15/20 on p53, which negatively influences the binding of p53 to the oncoprotein MDM2.
- MDM2 normally binds to p53, thereby targeting it for degradation in the ubiquitin-dependent proteosome pathway.
- By disrupting p53–MDM2 binding, ATM inhibits the degradation process, thus prolonging the half-life of p53.
- This prolongation of p53's otherwise short half-life after a DNA-damaging event has been extensively studied and has been found to correlate with cellular responses such as cell cycle arrest and apoptosis.
- ATM also activates human checkpoint kinase 2 (CHK2) in cells after exposure to ionizing radiation.
- CHK2 in turn phosphorylates p53, further stabilizing p53.
- CHK2 activity also is necessary for the phosphorylation of the dual-specificity phosphatases.
- Cdc25A/C, which inactivates the enzymes, blocking CDK1 activation and causing a G2 arrest.
- Other targets of ATM include BRCA1, NBS1, and RAD9
- The ATM-mediated phosphorylation of:
- **NBS1** is required for the proper execution of **the intra-S phase** checkpoint,
- that of BRCA**1** is associated with both the **S phase** checkpoint and the **G2/M transition**, and
- **RAD9** is linked to the **G1/S checkpoint** activation.
- Cells with mutations in the NBS1 gene share a variety of phenotypic similarities with ATM deficient cells such as chromosomal instability, increased radiation sensitivity, and defects in cell cycle checkpoints in response to ionizing radiation.

- Although NBS1 is not required for the activation of ATM and its downstream targets after ionizing radiation, cells mutated at the ATM phosphorylation site of NBS1 do display an abrogated S phase checkpoint after exposure to ionizing radiation.
- ATM appears to control S phase arrest after ionizing radiation by phosphorylating NBS1 on Ser 343;
- however, the mechanism by which phosphorylation of Ser 343 affects DNA replication after ionizing radiation remains unknown.



- Similar to NBS1,
- ATM is required for phosphorylation of BRCA1 in response to ionizing radiation.
- ATM resides in a complex with BRCA1 and phosphorylates BRCA1 in a region that contains clusters of serine-glutamine residues
- Phosphorylation of this domain appears to be functionally important because a mutated BRCA1 protein that lacks these key phosphorylation sites is unable to rescue the radiation hypersensitivity of BRCA1-deficient cell lines.
- Cells deficient in BRCA1 show genetic instability, defective G2/M checkpoint control, and reduced homologous recombination.
- Additionally, BRCA1 regulates expression of both the p21 and GADD45 proteins.

- RAD9 is a 1309 amino acid protein, with a C-terminal region that shows localized sequence identity with BRCA1
- RAD9 is required for DNA damage checkpoint in all phases of the cell cycle, and loss of RAD9 impairs checkpoint-induced cell cycle arrest and increases genomic instability.
- The RAD9 protein is constitutively phosphorylated in undamaged cells and undergoes hyperphosphorylation on exposure to ionizing radiation.
- Hyperphosphorylation of RAD9 is induced by ionizing radiation through ATM phosphorylation of Ser 272.
- Cells mutated at the RAD9 Ser 272 residue are defective in the G1/S checkpoint after exposure to ionizing radiation.
- DNA damaged-induced hyperphosphorylation of RAD9 appears to be normal in NBS1-deficient cells.
- This may be because RAD9 operates upstream of NBS1, or alternatively, that RAD9 functions separately from NBS1.
- p53 is a key DNA damage checkpoint protein that is indispensable for the mounting of a complete DNA damage response.
- However, whether p53 induces apoptosis or cell cycle arrest is a complex matter and depends in part on the abundance of the p53 protein (in general, low protein levels lead to cell cycle arrest and high protein levels lead to apoptosis), specific posttranslational modifications, and
- p53's interaction with such downstream activators as GADD45 as opposed to p21.
- Thus, although p53's upregulation of GADD45 may play a role in apoptosis by activating the JNK and/or p38 MAPK signaling pathways, p53's activation of p21 after exposure to ionizing radiation leads to cell cycle arrest.
- p21 belongs to the Cip/Kip family of CDK inhibitors, which also includes p27 and p57.

- Although members of the Cip/Kip family share broad specificity in their binding to, and inhibition of, most CDK/cyclin complexes, only p21 is directly involved in DNA damage-induced cell cycle arrest.
- Specifically, the p21 protein binds to, and inactivates, cyclin-E-CDK2 complexes, which results in hypophosphorylation and cell cycle arrest at the G1/S transition.
- p53 can also upregulate the transcription of 14-3-3 which inhibits G2 progression by sequestering CDK1 in the cytoplasm.
- In this way, ATM and p53 play important roles in both G1/S and G2 checkpoint regulation after exposure to ionizing radiation.

Irradiation and Cell Cycle Arrest

- Multiple pathways are involved in the maintenance of genetic integrity after exposure to ionizing radiation, most of which are related to the cell cycle
- Cells commonly respond to DNA-damaging agents by activating cell cycle checkpoints.
- These checkpoints provide for a controlled temporary arrest at a specific stage of the cell cycle to allow the cell to correct possible defects.
- Ionizing radiation induces arrests in the G1, S, and G2 phases of the cell cycle.
- The G1 checkpoint prevents the replication of damaged DNA before the cell's entry into S phase, and
- the G2 checkpoint prevents the segregation of aberrant chromosomes during M phase
- Two molecularly distinct G2/M checkpoints can be identified.

1. The first of these G2/M checkpoints occurs **early** after exposure to ionizing radiation,

- is very transient,
- is ATM dependent, and
- is dose independent.
- represents the failure of cells that had been in G2 at the time of irradiation to progress into mitosis.

- the "**late**" G2/M accumulation,
  - begins to be measurable only several hours after ionizing radiation,
  - is ATM independent,
  - is dose dependent, and
- represents the accumulation of cells that had been in earlier phases of the cell cycle at the time of radiation exposure
- G2/M accumulation after exposure to ionizing radiation is not affected by the early G2/M checkpoint and is enhanced in cells lacking the radiation-induced S phase checkpoint, such as those lacking NBS1 or BRCA1 function
- Most cells with wild-type p53 exhibit only a transient delay in the G1 and G2 phases of the cell cycle after RT.
- Although it is widely accepted that p53 mediates G1 arrest
- wild-type p53 cells do not always display G1 arrest after exposure to radiation
- It also appears that when irradiated cells undergo wild-type p53-dependent G1 arrest, they do not subsequently arrest in G2.
- However, if wild-type p53 cells are irradiated after the G1 checkpoint, the cells do arrest in G2 but do not show a delay in the subsequent G1 phase.
- p53's role in the G2/M checkpoint is not as clear.
- Numerous studies have shown that p53 and p21 mutant cells are capable of G2 arrest in response to DNA-damaging agents, including ionizing radiation
- In many of these studies, however, high doses of radiation were applied to cells that were growing asynchronously or synchronized in the S phase.
- Under these conditions, the data suggest that neither p53 nor p21 is involved in the G2/M checkpoint, because cells deficient in p53 or p21 were still able to arrest in G2 after exposure to ionizing radiation.

- Although p53 appears to be dispensable for the initiation of the delay at the G2/M checkpoint after exposure to ionizing radiation, p53- or p21-deficient cells do show a shorter G2/M delay.
- Thus, it appears that although p53 and p21 are not needed for the initiation of G2/M arrest, they are required for the sustaining of G2/M arrest after DNA damage.
- This has also been borne out by data showing that a given dose of radiation induces a longer G2/M delay in radiosensitive cell lines than in matched normal or resistant cells.
- A G2 delay in tumor cells may provide time for repair processes to operate that are critical for ensuring cell survival after sublethal DNA damage.
- In contrast, numerous studies have shown that the disruption or abrogation of the G2/M checkpoint leads to the radio sensitization of p53-mutated cells.
- Likewise, tumor cells treated with either caffeine or pentoxifylline, compounds that disrupt the G2 checkpoint, are sensitized to ionizing radiation.
- Although p53 is dispensable for the initiation of the delay at the G2 checkpoint, the ATM-CHK protein kinase pathway appears to be essential, because the inhibition of CHK1 in p53-deficient cells greatly sensitized them to radiation.
- This validates the use of CHK inhibitors as an anticancer strategy.
- The CHK inhibitor UCN-01 (7-hydroxystaurosporine) represents one such attempt.
- UCN-01, which has significant in vivo activity (unlike its parent compound staurosporine) was originally developed as a selective protein kinase C inhibitor.
- However, recent studies have suggested that UCN-01 has multiple divergent effects on cell cycle dynamics.
- In particular, UCN-01 functions not only as a CDK inhibitor causing G1arrest, it can also inhibit CHK1, and in so doing, abrogates the G2 checkpoint.
- Additionally, numerous DNA-damaging agents, including radiation, 5-fluorouracil, camptothecin and temozolomide, appear to act supra-additively with UCN-01 in terms of cytotoxicity.

- For example, the inhibition of CHK1 in p53-deficient cells greatly sensitized the cells to radiation.
- Although preclinical testing showed therapeutic efficacy for UCN-01, clinical trials of UCN-01 have yielded mixed results.
- In addition, in Phase I clinical trials, UCN-01 was found to bind avidly to human plasma proteins, resulting in a long half-life that required adjustment of the administration schedule.
- In many patients, the subsequent dose of UCN-01 therapy after the first course was reduced by 50%.
- The dose-limiting toxicities included hyperglycemia, acidosis, and adverse pulmonary events.
- One partial response occurred in a patient with melanoma, and a protracted (4 year) period of stabilization of minimal residual disease was observed in a patient with anaplastic large-cell lymphoma.

Cell Cycle: Effect of Cell Synchrony on Radiosensitivity

- The importance of the p53 and p21 status in determining radiosensitivity is somewhat complex.
- In general, loss of p53 is associated with a more radioresistant phenotype, but in some instances, loss of p53 either has no effect on radiation sensitivity or, conversely, is associated with a more sensitive phenotype.
- As an explanation for such discrepancies, it has been suggested that p53-mediated radio resistance is more important in cells that depend on apoptosis, instead of necrosis, for cell death.
- In the case of p21 mutation, when examined in vitro using a clonogenic assay that assessed cell survival, the loss of p21 appeared to affect more the mode of cell death (i.e., apoptosis vs. necrosis) than the overall level of cell killing.
- However, when using tumor regrowth delay or in vivo clonogenic assays to assess for differences, a p21 mutation did appear to sensitize tumors to radiation.

- Furthermore, the loss of p21 in ATM knockout mice caused increased radiosensitivity.
- Such contrasting results emphasize the importance of considering the cellular context when dissecting the role of p53 and p21 in radiosensitivity.
- Beginning in the late 1960s, researchers started to examine the dependence of the radiation response on the age or phase of the cell in the growth cycle.
- Initial studies in synchronized Chinese hamster cells showed a differential response of the cells to radiation depending on the phase of the cell cycle they were in at irradiation.
- In general, cell survival data showed that cells were most sensitive to irradiation during mitosis and in G2,
- Less sensitive in G1, and least sensitive during the latter part of the S phase.
- In the 1960s and 1970s, the effects of the cell cycle phase or age were examined in synchronization studies in a variety of cell lines (e.g., HeLacells, Yoshida sarcoma cells, mouse fibroblasts, and mouse L cells).
- In most of these early experiments, synchronization was achieved using excess thymidine (thymidine block), serum starvation, mitotic "shake-off," or hydroxyurea.
- More recently, lovastatin, centrifugal elutriation, and fluorescence activated cell sorting have been used as methods to isolate phase-specific populations of cells.
- The method of synchronization determines the phase of the cell cycle that cells are arrested in.
- For example, excess thymidine blocks cells in the S phase, and lovastatin arrests cells in the early G1 phase
- Regardless of the method of synchronization, however, maximal radiosensitivity has been universally found to occur during mitosis, with resistance rising during the S phase and reaching a maximum in the latter part of the S phase.

- Given these initial findings, the concept of synchronizing tumor cells in a phase of the cell cycle that is sensitive to radiation was recognized as a potentially important way to enhance the clinical efficacy of RT.
- ionizing radiation can retard the rate of progression of proliferating cell populations through various phases of the cell cycle, causing cells to accumulate in the G2 phase and keeping cells from undergoing mitotic division.
- In general, the effects of G2 blockade increase with radiation dose, but even low doses of radiation can result in cell cycle phase redistribution and, with time, may lead to partial synchronization.
- Given this, split or fractionated doses of radiation may be more efficacious, in part, by inducing a transient cell cycle arrest, after which a secondary RT fraction is administered exactly at the height of cell accumulation in the most radiosensitive cell cycle phase (G2/M).
- This suggests that the redistribution of cells in a particular phase would determine the response of an initially asynchronous population to fractionated high- and low-dose RT.
- Ngo etal. showed that the sequential exposure of Chinese hamster cells to low- and high-linear energy transfer gradually enriched the population of G2 cells, which showed increased radiosensitivity to sequential radiation exposure.
- Others have similarly shown that fractionated radiation can effectively synchronize cells in a more sensitive state for irradiation.
- Using human prostate cells, Geldof et al.showed that doses of 2 Gy or 4 Gy led to a shift toward a predominance of cells in the G2/M phase, causing the prostate cells to be more sensitive to radiation.
- Doses of 2 Gy have also been studied and been shown to increase considerably the G2/M phase fraction as well.
- Doses of 1 Gy have been shown to induce hyperradiosensitivity in a number of cell lines, including hamster fibroblasts and various human cancer cell lines.
- As the most sensitive and immediate indicator of cellular reactions to radiation, the hyper radiosensitivity effect on the cell should be reflected in the cell cycle.

- However, as Bartkowiak et al. noted, one would expect that, below a certain threshold of repair induction, cells would completely "ignore" damage and continue through the cell cycle unaltered.
- Nonetheless, Bartkowiak et al. showed that cells are extremely sensitive to the G2/M checkpoint and accumulate in a dose-dependent manner with doses as low as 0.2 Gy.
- Others have shown that the cell-cycle phase also has an important influence on the response to low-dose radiation of human tumor cell lines.
- Because the magnitude of hyper radiosensitivity appears to be greatest in the G2 phase, this also suggests that tumors with larger cell growth fractions and/or an aggressive proliferative response to treatment may be the best candidates for treatment using low-dose fractions.
- Despite the overwhelming evidence that the cell phase plays some role in radio sensitization, it cannot entirely account for the increased radiosensitivity observed for fractionated RT.
- Changes in repair fidelity or efficiency resulting from the induction of repair processes in a dose- or damage-dependent manner may also play a role.
- Another area that requires additional investigation is whether p53-deficient cells that have a foreshortened G2/M duration remain as sensitive to a fractionated RT regimen.
- To better understand the molecular events that govern sensitivity to radiation damage in different phases of the cell cycle, several investigators have examined cell cycle– dependent DNA damage and repair mechanisms after exposure to ionizing radiation.
- From work with synchronized populations of cells, it is clear that radiation-induced chromosomal damage and micronuclei formation depend on the cell cycle distribution.
- Ionizing radiation can produce both different types of, and quantitative differences in, chromosomal aberrations at various stages of the cell cycle.
- Illustrating the latter point, in Chinese hamster cells, the frequency of chromosomal aberrations after irradiation was about three times greater for G2 phase cells than for S and G1 phase cells.

- However, in mouse cells, the frequency of translocations was significantly greater in G1 and S phase cells, than in G2 phase cells.
- Furthermore, Tallon et al. (221) reported that primary human lymphocytes undergo a cell cycle– dependent induction of aneu-ploidy after irradiation.
- Cells exposed to radiation during the G1 phase exhibited a greater frequency of centromere positive micronuclei than cells in the G2 phase at exposure.
- In addition, G1 phase exposure induced a centromere-positive micronuclei dose–effect relationship that was not observed after G2 phase exposure.
- Paglin et al. examined breast cancer cells and noted that after the irradiation of G1 and S phase– enriched cell populations, S phase cells were more prone to micronuclei formation than G1 cells.
- Not only does the degree of radiation-induced damage depend on the cell cycle, the nature of the cell cycle repair varies with the phase of the cell.
- In particular, Iliakis and Okayasu (225), who studied double-strand break repair in CHO cells, observed faster kinetics in the G1/S and mid-S phases than in the G1 phase.
- Taken together, these data suggest that the degree of chromosomal damage and repair after irradiation also depends to some extent on the cell cycle phase.

However, this effect varies depending on the cell lines examined and the radiation dose used.

- Some of the variation in the molecular and cell cycle response to ionizing radiation is believed to be due to the intrinsic radiosensitivity of certain human tumor cells
- Some studies have shown that, although the low dose irradiation of human tumor cells induced substantial cell synchrony, the extent of cell blocking and cell killing increased together, and thus cell cycle arrest probably was not very important to the effectiveness of RT
- These studies point out that the use of cell synchronization as a therapeutic tool is limited and most likely clinically untenable.

Chapter 4: General Effects of Radiation

By: Dr. Abhishek Gupta

4.1: Oral mucous membrane:

- **Mucositis**: defined as the reactive inflammation of the oral and oropharyngeal mucous membrane during radiotherapy in the head and neck region.
- It is characterized by atrophy of squamous epithelial tissue, absence of vascular damage, and an inflammatory infiltrate concentrated at the basement region (Handschel et al., 1999).
- Although the **etiopathogenesis** of radiation mucositis still is not fully clear, it most likely can be considered as a **four-step inflammation** consisting:

1. Inflammatory /vascular phase,

2. an epithelial phase,

3. a bacterial phase, and

4. a healing phase.

Mechanisms of mucositis

- Historically, mucositis was thought to arise solely as a consequence of **epithelial injury**.
- It was hypothesized that radiation nonspecifically targeted the rapidly proliferating cells of the basal epithelium, causing the loss of the ability of the tissue to renew itself.
- Radiation-induced mucositis was typically recognized as an **'outside-in 'process**, in which DNA strand breaks occurred in oral basal-epithelial cells.
- Mucositis is a biologically complex process that involves a dynamic, interactive sequence of panmucosal events that ultimately targets epithelial stem cells.
- Morphological observations indicate that changes in the submucosal endothelium and connective tissue precede epithelial damage.

- Damage to endothelial walls was seen in electron micrographs within 1 week of an acute radiation challenge in animals and at least 5 days before epithelial breakdown.
- Paris et al. suggested that endothelial damage might even be the initiating event in triggering radiation-induced mucositis.
- A role for platelet aggregation as a component of endothelial radiation-induced mucosal injury was indicated by the detection of increased salivary levels of the cytokine platelet-activating factor (PAF) in patients with radiation-induced.
- Radiation induced apoptosis of submucosal fibroblasts precedes epithelial injury.
- Contrary to the assumption that damage that is induced by radiation focused within basal-layer stem cells,

- the mucosal distribution of **c-FOS** has been found to be predominantly localized to the nuclei of spinous-layer cells,

- whereas **c-JUN** is found in all nuclei of all epithelial cell layers, except for the basal layer.

- Furthermore, both transcription factors are present in other cell types in the mucosa, including fibroblasts, endothelial cells and macrophages.

Pathobiology

Initiation

- Radiation initiate both DNA and non-DNA damage.
- DNA strand breaks result in direct cellular injury that targets cells in the basal epithelium as well as cells within the submucosa.
- Simultaneously, reactive oxygen species (ROS), which are crucial mediators of downstream biological events, are generated.
- Although the mucosa seems to be absolutely normal at this stage, a cascade of events begins in the submucosa that ultimately results in mucosal destruction.

Primary Damage Response

- DNA strand breaks result in the activation of several transduction pathways that activate transcription factors such as **p53** and **nuclear factor-κB (NF-κB)**.
- Cell-membrane-bound molecules that are released during lipid peroxidation result in the upregulation of immediate-response genes, such as those encoding c-JUN and and c-JUN amino-terminal kinase (JNK).
- These upregulate other transcription factors, such as **NRF2** .
- NF-κB activation can have both **pro-apoptotic and anti-apoptotic consequences**.
- NF-κB upregulate a range of cell-adhesion molecules.
- The upregulation of genes due to radiation-induced transcription-factor activation results in the production of pro-inflammatory cytokines, including **TNF-α, IL-1β and IL-6**.
- It seems likely that their presence stimulates early damage to connective tissue and endothelium and initiates mesenchymal–epithelial signaling, reduces epithelial oxygenation and, ultimately, results in epithelial basal-cell death and injury.
- Other non-DNA events occur at the same time that accelerate mucosal damage.
- Radiation hydrolyse the cell-membrane lipid sphingomyelin through the activation of **sphingomyelinase or ceramide synthase**.

Signal Amplification

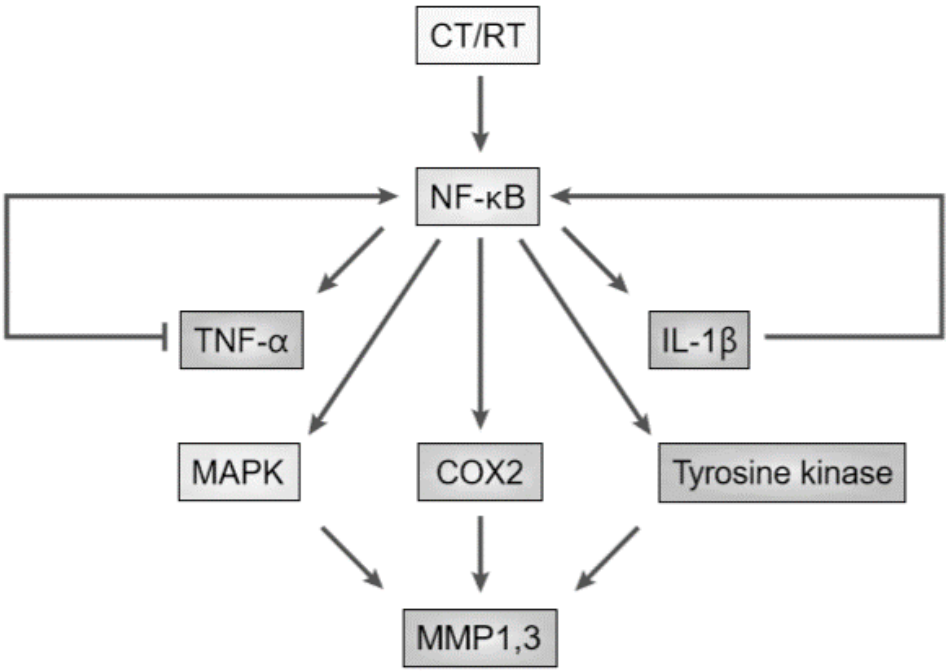
- Initial activation of transcription factors > gene upregulation - a broad range of biologically active proteins accumulate and target the tissues of the submucosa.
- pro-inflammatory cytokines

  - damage tissue,

  - also provide a positive-feedback loop to amplify the primary damage that is initiated by radiation.

- This pathway ultimately results in the activation of caspase 3 and in cell death.

- TNF-α also activates sphingomyelinase.
- So, its increased level in the tissue amplifies pro-apoptotic signals that are mediated by the ceramide pathway.
- both TNF-α and IL-1β induce MMP1 and MMP3 activation.
- **MMP1** (an interstitial collagenase) causes destruction of the collagenous subepithelial matrix,
- **MMP3** (also known as stromelysin 1), breaks down the epithelial basement membrane and potentially promotes the dissemination of other destructive signals.
- This changes as ulceration develops.



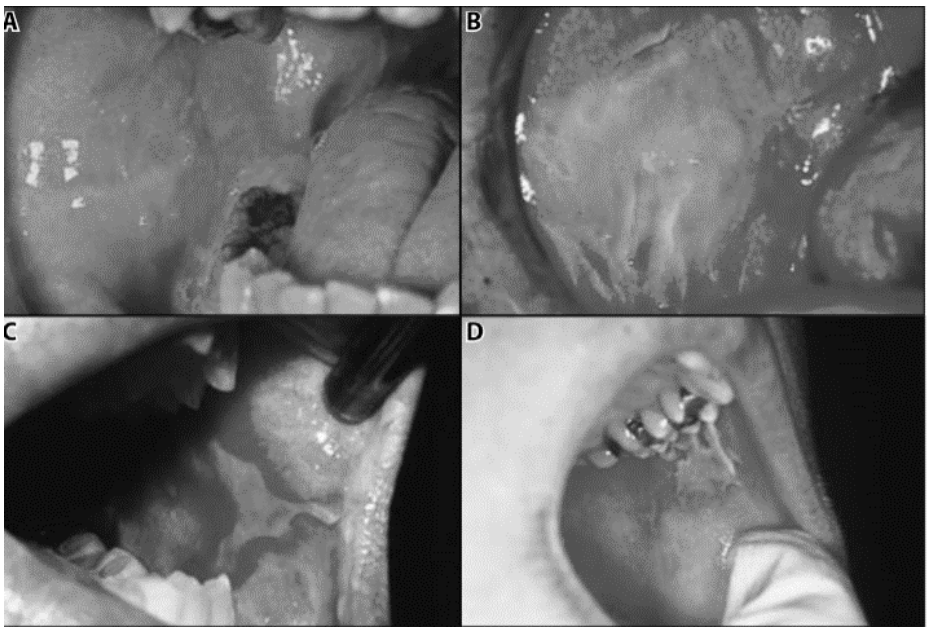
Ulceration

- The loss of mucosal integrity results in extremely painful lesions that are prone to superficial bacterial colonization.
- Cell-wall products from colonizing bacteria are likely to penetrate into the submucosa, where they activate infiltrating mononuclear cells to produce and release additional pro-inflammatory cytokines.

- This probably promotes the expression of pro-apoptotic genes and potentiates tissue injury.

Healing

- In most cases, mucositis is an acute phenomenon that is self-resolving once cancer therapy ends.
- Although there are parallels between the resolution of mucositis-induced ulcers and the healing of other types of mucosal injury, it is likely that the sequence of events that leads up to mucositis modulates the process.
- Signals from the submucosal extracellular matrix and mesenchyme govern the rate of epithelial-cell migration, the rate of proliferation and the differentiation of healing tissue.
- Clinical Stages of Oral Mucositis



Although the cells and tissues of the submucosa and epithelium respond immediately and robustly to radiation and chemotherapy, the clinical appearance of the mucosa is deceivingly normal during the primary damage phase (A).

Early superficial changes in the mucosa may be seen during the signaling and amplification phases, but the benign clinical appearance is in stark contrast to the biologic

havoc that is taking place beneath the epithelium, which ultimately results in basal cell injury, apoptosis, and death (B).

The toll of direct and indirect basal cell injury and death is most dramatically manifest by frank ulceration. This is the most symptomatic phase of mucositis and the one that is associated with the major significant adverse health and economic outcomes associated with the condition (C).

In the majority of cases, signaling from the submucosa to the epithelium results in spontaneous healing (D).

- First clinical signs: at the end of the **first week**
- no consensus regarding first sign.
- Some authors describe a **white discoloration** of the oral mucosa, which is an expression of hyperkeratinization as the first symptom, followed by or in combination with erythema Others consider **erythema** to be the first. After **20.0-30.0 Gy at 1.8-2.0 Gy per day**, the mucosa becomes erythematous.
- The early radiation reaction causes local discomfort as well as difficulties in drinking, eating, swallowing, and speech.
- About 20-30% of the patients will need artificial feeding
- Around **third week** of radiotherapy, more severe symptoms of mucositis, such as the formation of pseudomembranes and ulceration, may appear
- Some authors consider pseudomembranes to be ulcers covered by fibrinous exudate Others suggest that pseudomembranous mucositis is

    - related to yeast stomatitis or

    - to colonization of the oral cavity with Gram-negative bacilli

- After an additional 10.0-20.0 Gy, patches of mucositis (**pseudomembrane**) tend to begin to appear.
- Mucosa of the oral cavity does not react in the same manner at all locations.

- Mucositis is **most severe in the soft palate**, followed, in order, by the mucosa of the hypopharynx, floor of the mouth, cheek, base of the tongue, lips, and dorsum of the tongue.
- The most common infection in the oral cavity during or shortly after radiotherapy is candidiasis (Epstein, 1990; Ramirez-Amador et al., 1997).
- However, treatment of yeast and Gram-positive cocci with topical anti-fungals and disinfectants failed to relieve such complications.
- same holds for herpes simplex virus
- Clinically important late changes rarely occur until doses greater than 50.0 Gy (conventional fractionation) are imposed.
- Mucosal **ulceration** remains rare for doses < 65.0 Gy.
- Some mucosal atrophy and loss of mucosal mobility after conventionally fractionated doses of 60 to 70 Gy in **6 to 7 weeks** is common,
- Necrosis, chronic ulceration, and bone exposure seldom occur unless the delivery of dose is accelerated or the total dose exceeds 70 Gy in 7 weeks.
- Once acute effect has subcided and several months have elaspsed it is possible to see the **subacute** changes of mucosal atrophy, loss of mobility and pliability caused by submucosal scar ring.
- chronic ulcer characterizes the late effects & caused by ischemia that results from progressive scarring and thrombosis of small vessels in the submucosa.
- These effects are irreversible and may appear as early as 6 months or as late as l-5 years after irradiation.

Grade I: mucositis is generally asymptomatic or patient may show intolerance to spices or hot food (end of 1st and 2nd week)

Grade II: focal areas of desquamation, serosanguineous discharge (3rd week)

Grade III: Progresses to confluent mucositis with severe pain (4th to 5th week)

Grade IV: Ulceration, necrosis and sometimes bleeding.

Management of Mucositis:

Before Radiotherapy

- Detail clinical history
- Complete dental examination
- Complete dental examination
- Instruction for oral hygiene
- Treatment of Dental infections
- Application of flouride.
- **Chlorhexidine**: broad spectrum antibiotic rinse, not recommened by NCCN for prevention of OM in patients with solid tumors, undergoing RT
- A clinical benefit has **not** been demonstrated in large randomized, controlled trials.
- Also, CHX has been a/w an increase in oral mucosal inflammation and OM assessment scores, general mouth discomfort, taste alterations and staining of teeth.
- No difference in incidence and duration of OM was found
- **Magic mouthwash** (lidocaine, diphenhydramine and antacid): pain relieving & coating of mucosa
- Efficient as chx rinses
- **Fluoride supplementation-1.1% neutral sodium fluoride gel or a 0.4% stannous fluoride gel.**
- The gel should be used to brush gently on the teeth followed by expectoration an d rinsing the mouth gently.
- The fluoride should be applied at least once a day.
- Pharmacologic Management
- **Anti-Inflammatory Agents**

Benzydamine hydrochloride

- nonsteroidal antiinflammatory drug that inhibits proinflammatory cytokines including TNF-α.
- In a Phase III trial, Benzydamine hydrochloride mouthrinse reduced the severity of mucositis in patients with head and neck cancer undergoing radiation therapy of cumulative doses up to 50-Gy radiation therapy.
- Based on studies, it is **recommended** to use of this agent in patients receiving moderate-dose radiation therapy

Saforis

- It is a proprietary oral suspension of **L-glutamine** that enhances the uptake of this amino acid into epithelial cells.
- Glutamine may reduce mucosal injury by reducing the production of proinflammatory cytokines and cytokine-related apoptosis; and may promote healing by increasing fibroblast and collagen synthesis.
- In a Phase III study, this topical agent reduced the incidence of clinically significant chemotherapy-induced oral mucositis compared to placebo.

Amifostine

- Radiation protection agent
- is thought to act as a scavenger for harmful reactive oxygen species that are known to potentiate mucositis.
- However, because of insufficient evidence of benefit, various guidelines could **not** be established regarding the use of this agent in oral mucositis in radiation therapy patients.

RK- 0202

- It consists of the antioxidant, ***N-acetylcysteine***, in a proprietary matrix for topical application in the oral cavity.

- In a placebo-controlled phase II trial in patients with head and neck cancer, this agent significantly reduced the incidence of severe oral mucositis up to doses of 50-Gy radiation therapy.

Beta carotene

- a vitamin A derivative, is a scavenger of singlet oxygen.
- Based on the findings of different randomized controlled study, it is of the view that supplemental dietary beta-carotene led to a mild decrease in the severity of radiotherapy-induced oral mucositis.

Antimicrobial lozenges:

- No longer recommended
- A study comparing polymyxin, tobramycin and amphotericin B vs placebo found no difference in prevention of the development of severe OM in patients undergoing RT

Immunomodulatory Drugs

Pentoxifylline

- reduced the frequency and severity oral mucositis.
- Contradictory to this, some workers reported a significant aggravation of symptoms when they studied the effect of IV Pentoxiphylline in 92 patients.
- However, no difference in symptoms was noted in patients who undergone chemo radio therapy.

Indomethacin

- a nonsteroidal anti-inflammatory drug inhibiting prostaglandin synthesis is noted to delay the onset of mucositis.

Immunoglobulin

- Treatment with low-dose intra muscular immuno globulin is said to decrease the severity and duration of radio therapy-induced oral mucositis.

- Immunoglobulin has also been tried as a therapeutic agent in radiation- induced mucositis in various clinical trials and the observations were promising

Growth Factors

- granulocyte macrophage colony stimulating factor (**G- MCSF**),
- granulocyte colony stimulating factor **(G-CSF),**
- keratinocyte growth factor (**KGF**) and Interleukin 11.
- GMCSF or GCSF can reduce the severity of OM by accelerating neutrophil recovery.
- applied as topical and parenteral agents.

Palifermin:

- recombinant keratinocyte growth factor
- several mechanisms of action, such as inhibition of DNA damage andapoptosis i n the epithelial cell; downregulation of proinflammatorycytokines and also stim ulation of epithelial cell proliferation, growth, differentiation and migration.
- In a trial: 60 mcg/kg/day IV or placebo 3 days before and 3 days after, WHO grade ¾ OM was observed in 67 of the 106 patients (63 %) and 97 % in placebo group
- Among ¾ grade patients,3 median days for palifermin and 9 days for placebo
- These outcomes resulted in reduced use of opioids.
- Adverse events were minimal, most notably a transient skin rash, mucosal changes, altered taste sensation, and thickened tongue

Low Level Laser Therapy

- Although the exact mechanism of action is unclear, longstanding interest is focused on low level laser therapy (LLLT) as a preventive technique for OM

- It has been assumed that LLLT may reduce the levels of proinflammatory cytokines and/or reactive oxygen species (ROS) which contribute to the pathogenesis of OM.
- Antunes et al randomized 38 patients to low-power laser therapy group or a standard care control group.
- In the laser group, 31.5% had OM of grade 2 or lower, compared with 94.7% in the control group.
- In another larger randomized trial, Schubert et al. compared 2 different low-level lasers (650 and 780 nm) and placebo in 70 patients undergoing HCT.
- The 650-nm wavelength reduced the severity of OM and pain scores.
- LLLT was well-tolerated, and no adverse events were noted.
- However, the authors also noted that further study is needed to truly establish the efficacy of LLLT and to define the optimal laser parameters, including optimal wavelength, energy density, and schedule.

Ozonated Water

- anti-inflammatory, antimicrobial, biosynthetic (activator of lipid, protein and carbohydrate metabolism), antihypoxic, bioenergetic, hemostatic and analgesic properties.
- Ozone is very successful in lysing bacteria, fungi, yeast and mould.
- Ozone directly attacks inflamed cells, with loss of enzyme layer, therefore ensuring that the attack is solely targeted on these cells.

Role of Safe Radiotherapy

- Computed tomography (**CT**)-based target delineation, Intensity-Modulated Radiation Therapy (**IMRT**), and simple, custom-made, intraoral devices that are designed to exclude uninvolved tissues from the treatment portals or to provide shielding of tissues within the treatment area.
- **Stents** can be useful in excluding the palate mucosa during treatment of the tongue or floor of the mouth.

- More frequent use of electron-beam and/or sophisticated three-dimensional conformal, multibeam, wedged-pair, or oblique treatment plans.
- Packing gauze between metallic dental restorations and mucosa of the lateral tongue and buccal area - minimize the dose from scattered radiation.

After Radiotherapy

- The most popular mucositis scales are radiation therapy oncology group (**RTOG**) for radiotherapy,
- World Health Organization (**WHO**) for chemotherapy,
- common toxicity criteria of **NCI** for chemotherapy and radiotherapy.

Radiation Therapy Oncology Group (Rtog)

Grade	Description
0 (none)	No change over baseline
I (mild)	Irritation, may experience slight pain, not requiring analgesic
II (moderate)	Patchy mucositis that may produce inflammatory serosanguinitis discharge; may experience moderate pain requiring analgesia
III (severe)	Confluent, fibrinous mucositis, may include severe pain requiring narcotic
IV (life-threatening)	Ulceration, hemorrhage, or necrosis

	WHO Scale	NCI–CTC Clinical	NCI–CTC Functional
Grade 1	Oral soreness, erythema	Erythema	Minimal symptoms, normal diet; minimal respiratory symptoms but not interfering with function
Grade 2	Ulcers but able to eat solids	Patchy ulcerations or pseudomembranes	Symptomatic but can eat and swallow modified diet; respiratory symptoms interfering with function but not interfering with ADL
Grade 3	Oral ulcers and able to take liquids only	Confluent ulcerations or pseudomembranes; bleeding with minor trauma	Symptomatic and unable to adequately aliment or hydrate orally; respiratory symptoms interfering with ADL
Grade 4	Oral alimentation impossible	Tissue necrosis; significant spontaneous bleeding; life-threatening consequences	Symptoms associated with life-threatening consequences
Grade 5	N/A	Death	Death

Management Strategies

- Normal saline (0.9%) rinses can often provide temporary relief of mild to moderate OM pain
- Lidocaine products (viscous, gel, or solutions) can provide good topical anesthesia for OM discomfort and pain.

Anti-Inflammatory

- **Dinoprostone** (prostaglandin E2), **misoprostol** (Prostaglandin E1) and **prednisolone** have been used; however the results are not encouraging.
- **Benzydamine**, a non-steroidal anti-inflammatory agent that inhibit TNF-a, shown to be effective to control oral mucositis and pain due to radiotherapy
- infectious disease protocols will usually recommend the use of prophylactic antiviral and antifungal agents.
- herpes simplex virus: **acyclovir** or valacyclovir would be indicated.

- **Fluconazole** and **clotrimazole** prophylaxis have been shown to reduce candidiasis in cancer patients
- Additionally, hematopoietic growth factors are indicated in patients with neutropenia along with prophylactic antibiotics to prevent infection.

Therapeutic Interventions

- **Sucralfate**
- a cytoprotective agent used for gastro intestinal ulcerations, is a basic aluminum salt of sucrose octasulfate, and may be useful in palliation of established mucositis by its coating and protective actions.
- This was tried in radio therapy cases by different authors in different combinations with varied results.
- **Kaolin pectin**
- combined with diphenhydramine, which is a H1-histamine antagonist and local anesthetic,
- found to reduce oral pain without reducing the degree of mucositis in a double blind randomized and controlled study
- **Honey**
- antioxidant properties can increase cytokine release and also has antimicrobial effects.
- Furthermore, it can prevent tissue cells from oxidative damage that leads to agei ng, disease susceptibility and death.
- can reduce inflammation and oedema, stimulate epithelialisation and tissue rege neration and therefore mayimprove granulation and debridement which acceler ate tissue repair and wound healing results
- Noronha et al. reportedthat honey produced faster wound healing in patients wi th Grade 2 and 3
- **Caffeine**

- hypoalgesic, antioxidant and anti-inflammatory properties.
- Coffee or coffee specific compounds which contain antioxidant properties also have some protective effects against tissue damage, oxidative DNA damage
- **Propolis**
- contains**flavonoids** which have been reported to have a wide range of biological properties:

  - antibacterial,

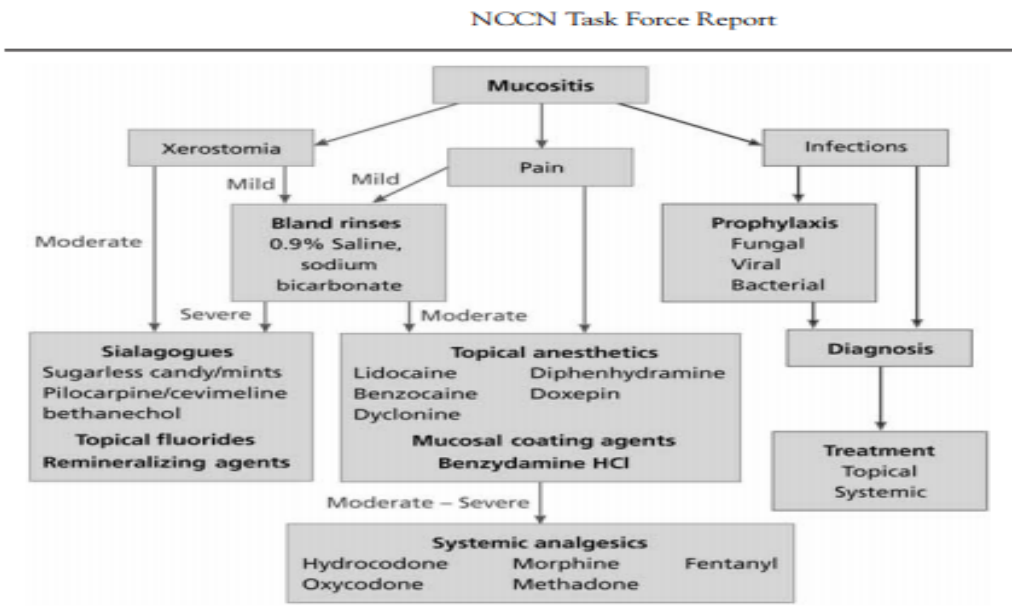
  - antiviral,

  - antiallergic,

  - anti-inflammatory and

  - vasodilatory actions.

- a comparative Phase III clinical trial study with larger number of cases should be done to confirm the efficacy of the product.



Guidelines from the Multinational Association of Supportive Cancer Care for Management of Patients with OM

Basic Oral Care and Clinical Practices

1. Use of a soft toothbrush on a regular basis.

- Elements of good clinical practice should include the use of validated tools to regularly assess oral pain and oral cavity health.

2. The panel recommends patient-controlled analgesia with **morphine** as the treatment of choice for OM.

Radiotherapy: Prevention

3. The panel recommends the use of **midline radiation blocks** and 3-dimensional radiation treatment to reduce mucosal injury.

4. The panel recommends **benzydamine** to prevent radiation-induced mucositis in patients with head and neck cancer receiving moderate- dose radiation therapy.

5. The panel recommended that **chlorhexidine not** be used to prevent OM in patients with solid tumors of the head or neck who are undergoing radiotherapy.

6. The panel recommends that **antimicrobial** lozenges **not** be used to prevent radiation-induced OM.

Radiotherapy: Treatment

7. The panel recommends that **sucralfate not** be used to treat radiation-induced OM.

4.2: Taste Buds

By: Dr. Kumari Sonam Jha

- Irradiation of the taste buds typically imparts **hypogeusia** or **ageusia**.
- caused by **direct** damage of the cells in the taste buds OR
- Irradiation may damage **nerve fibers** that innervate taste buds, causing taste cell death indirectly (Conger and Wells, 1969; Nelson, 1998), as maintenance of mature taste cells requires nerve contact (Sollars et al., 2002; Miura et al., 2004; Oakley and Witt, 2004).

- At a dose of **20.0 Gy**, it can be estimated from animal models that approximately 20-30% of the taste cells are destroyed in each taste bud.
- When 60 Gy of irradiation was given, more than 90% of patients lost their sensitivity to taste.
- The timing for loss of taste cells is broadly congruent with the onset of functional taste loss in patients, which is first observed after **1 week** of radiotherapy (Mossman and Henkin, 1978),
- with more broad taste dysfunction in patients by the **3rd – 4th weeks** (Ruo Redda and Allis, 2006; Yamashita et al., 2006; Yamashita et al., 2009; Epstein and Barasch, 2010):
- Posterior two-third: effects the bitter and acid flavors.
- Anterior third: effects sweet and salty flavors.
- **Bitterness** is the basic taste most influenced (some decrease in the threshold value for bitterness whereas other studies have reported an increase)
- Changes in **umami** taste thresholds differ: increases significantly after radiation therapy and does not return to baseline, whereas recognition of other basic tastes is slightly and only temporarily impaired
- The differential tissue exposures during radiation treatment clearly affect the development of taste alterations in cancer patients.
- Taste thresholds for all basic tastes increase significantly when radiation therapy is directed to the whole tongue.
- However, taste thresholds do not increase when only the tongue tip is irradiated
- About 85% of patients receiving irradiation of the head and neck experienced **unpleasant taste changes**
- The most prevailing taste alteration reported is perception of a metallic or bitter taste or aftertaste.
- A metallic and/or bitter taste has been associated with low levels of irradiation

- These changes in the taste perception may also be attributed to the **salivary changes** that occur due to radiation.
- It is only the **relative improvement** in taste that patients report before they notice return of salivary function that clinically suggests independent effects.

4.3: Teeth

By: Dr. Abhishek Gupta

- Prior to calcification: tooth buds get destroyed
- After initiation of calcification: inhibition of cellular differentiation causing **malformation** or **arrest of growth.**
- Developing stage: development may be retarded
- Adult teeth: The pulp shows decreased vascularity, reduced cellularity and the tooth becomes more prone to pulpitis.
- According to Carpenter and Dury, 10 Gy is sufficient to cause permanent damage to mature ameloblasts and 30 Gy is sufficient to arrest dental development.
- However, Fromm et al. and Goho identified alterations in dental development after 40 Gy dose
- Radiation damage occurs simultaneously to the bone, periodontal ligament, and pulp.
- Radiation effects on teeth are limited to the irradiated area
- may cause:

- enamel defects (discolorations and hypoplasia)

- root development, premature apex closure,

- dental development delay or retained teeth.

- Dental anomalies shape (microdontia, macrodontia and taurodontia) and number (hypodontia), and root formation disturbances (blunted root, tapered root and root development delay)

- Other anomalies, such as supernumerary teeth, have also been detected

- Children's teeth exposed to RT, particularly with a dose over **20 grays** can cause root shortening (dwarfism) or abnormal curvature (hypocalcification).

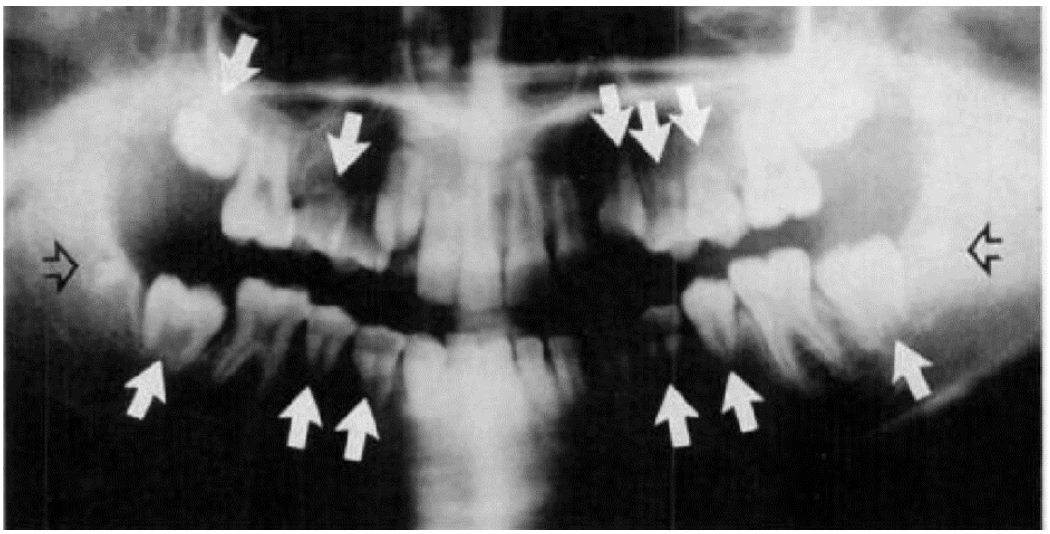
- More than 85% of head and neck rhabdomyosarcoma survivors, (doses >40 gray), had significant dental abnormalities:

  - Mandibular or maxillary hypoplasia,

  - **increased caries,**

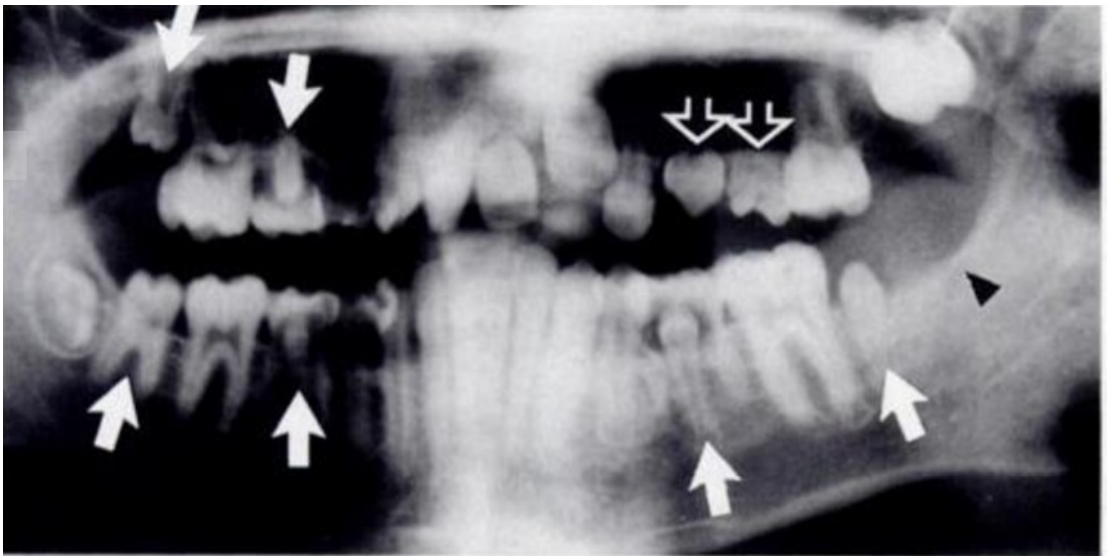
  - **hypodontia, microdontia, root stunting**

  - and xerostomia



effects of chemotherapy and radiation therapy in a patient who had rhabdomyosarcoma of the nose diagnosed at age 7 years.

Panoramic radiograph shows root stunting of bicuspids and second molars (solid arrows) and microdontia of third molars (open arrows).



effects of combination chemotherapy and radiation therapy in a 13-year-old who had rhabdomyosarcoma of the left orbit diagnosed at age 3 months.

Panoramic radiograph shows absence of third molar (arrowhead), mlcrodontla of second molars and bicuspids (solid arrows), and rootless left maxillary teeth (open arrows).

Histologically,

- **irradiated presecretory odontoblasts** change from columnar to cuboidal shape.
- Mitotic activity ceases, although the cells do not die."
- **Osteodentin**" forms between the arrested odontoblasts and the pulp.
- The osteodentin is secreted by osteoblast-like cells originating from undifferentiated pulp mesenchyme.
- The pulp mesenchyme forms these cells either due to direct radiation damage, or due to induction by the damaged odontoblasts.
- The osteodentin is visible microscopically as a " niche" in the dentin, or as a wavy, irregular dentinoenamel junction.

- It is delineated from normal dentin both apically and incisally, indicating that only **presecretory odontoblasts** are damaged by low-dose radiation.
- Osteodentin also differs chemically from normal dentin.
- In normal dentin, **phosphorylated phosphoprotein (PP-H**) is the predominant noncollagenous protein.
- Low-dose radiation effects noted in enamel appear to be due to damage to the underlying dentin and not to direct ameloblast injury.
- Nucleation of enamel crystals requires a properly mineralized dentin substrate.
- Enamel crystals theoretically grow from existing dentin crystals at the dentinoenamel interface. Or, dentin crystals actually may grow into the enamel matrix to induce enamel crystal formation.
- Abnormal osteodentin alters dentinogenesis, which alters the mineralization of enamel**.**
- **Enamel hypoplasias** over the defective dentin are the result.

➢ **Radiation Caries**:

- This is a rampant form of caries.
- These lesions occur secondary to changes in the salivary glands and saliva
- Clinically **three types** of radiation caries are seen:

i. Primarily involving cementum and dentin in the cervical areas. This lesion progresses around the tooth circumference and ultimately results in the amputation of the crown.

ii. Generalized superficial lesions attacking the buccal, occlusal, incisal and palatal surfaces of the teeth.

iii. Dark pigmentation of the crown.

- Radiation-induced dental effects in adults essentially are indirectly produced by salivary changes that occur when the glands are included in the treatment portals, **not by direct irradiation of the teeth themselves**.

- Whether direct irradiation of teeth alters their organic or inorganic components, making them more susceptible to decalcification, has **not been shown clearly**
- The literature reports that there is destruction of prismatic structures in irradiated **enamel** resulting in a demineralization pattern that differs from that of nonirradiated enamel.
- A study in rats has indicated the loss of organization in enamel prisms after doses higher than **0.5 Gy**.
- Reports have indicated that the interaction of ionizing radiation with the enamel structure reduces its mechanical properties.
- enamel demineralization is a controversial topic in the current literature.
- Some studies have indicated no difference between the patterns of in vitro demineralization and in situ remineralization, concluding that the enamel interaction with ionizing radiation is not the main cause of the initial enamel demineralization.
- With regard to microhardness in **dentin**, it has been stated that this property decreases after only **10 Gy,**and with doses **higher than 60 Gy** dentin is severely weakened, losing its capacity to support enamel.
- Kielbassa et al evaluated the effects of radiation on dentin microhardness and found that within the limits of an in vitro study, dentin is severely affected by radiation

Pulp: significantly increased amounts of collagen fragments by direct radiogenic destruction.

- may contribute to secondary fibrosis and decreased vascularity, thereby impairing the odontoblastic metabolism.
- The obliteration of the dentine tubules, preceded by a degeneration of the odontoblast processes, was found to be the result of direct radiogenic cell damage with hampered vascularization and metabolism particularly in the area of the terminations of the odontoblast processes.
- Grotz and coworkers suggested that a deficit in metabolism combined with a latent damage of the parenchyma ultimately resulted in functional symptoms such as subsurface caries.

- Subsurface caries is a main factor contributing to the atypical and comparatively rapid progress of irradiation caries which may not be explained by hyposalivation alone.
- Irradiation did not measurably affect the extent of collagen destruction of mineralized dental tissue, which may be related to the relatively low concentration of this protein in dentin and enamel.
- In a study, after 60 Gy of radiation, none of the 40 teeth tested showed a positive response to the **Pulp Sensitivity Test** and after 4 to 5 months. after the beginning of RT these teeth were still unresponsive to PST but without any sign or symptom of pulp mortification, thus excluding the possibility of tissue necrosis caused by RT.
- In the recent report on **pulse oximetry** testing of teeth in patients undergoing radiotherapy, it was shown that the oxygen saturation in the pulp is the lowest immediately after completion of the RT and regains higher values again after 4 to 5 months after the initiation of treatment.
- The most common pattern (Type 1) affects the cervical aspect of the teeth and extends along the cementoenamel junction.
- A circumferential injury develops and crown amputation
- The third and least common pattern (Type 3) presents as color changes in the dentin.
- The crown becomes dark brown/black and occlusal and incisal wear may be seen

Fluoride supplementation -

- 1.1% neutral sodium fluoride gel or a 0.4% stannous fluoride gel.

- The gel should be used to brush gently on the teeth followed by expectoration an d rinsing the mouth gently.
- The fluoride should be applied at least once a day.
- **Caesin derivative coupled with calcium phosphate (CD –CP)**
- Remineralizing toothpaste – delivers soluble calcium & phosphate ions
- **Fluoride rinses -** not adequate to prevent tooth demineralization.

- Instead, a high-potency fluoride gel, delivered via **custom gel-applicator trays**, is recommended.
- Several days before radiation therapy begins, patients should start a daily 10-minute application of a 1.1% neutral pH sodium fluoride gel or a 0.4% stannous fluoride (unflavored) gel.
- For patients reluctant to use a tray, a high-potency fluoride gel should be **brushed** on the teeth following daily brushing and flossing.
- Either 1.1% neutral pH sodium or 0.4% stannous fluoride gel is recommended.
- Patients with radiation-induced salivary gland dysfunction must continue **lifelong daily** fluoride applications.
- The incorporation of sodium carboxymethyl cellulose (water-soluble polymer) into aqueous acidulated phosphate fluoride (APF) gels produces a viscous solution that improves the ease of application using custom-made trays.
- In custom-made trays, viscous gels flow under pressure and which facilitates penetration between the teeth.
- A neutral pH gel (e.g., 2% w/v neutral F ion releasing gel, **9000 ppm F**) can be applied for treatment of conditions such as exposed or carious dentine, hypomineralized porous enamel surfaces, and dental erosions.
- Sodium fluoride is chemically very stable, has an acceptable taste and is non-irritating to the gingivae.
- Additionally, it does not cause discoloration of tooth tissues or dental restorations.
- In contrast, **APF** or **stannous fluoride gels** may cause discoloration and etching of restorations.
- APF gels therefore should not be used in patients with composite resin metal–ceramic or ceramic restorations

Instructions for Patients Using Supplemental Fluoride:

IF USING A TRAY	IF USING A BRUSH-ON METHOD
• Place a thin ribbon of fluoride gel in each tray.	• After brushing with toothpaste, rinse as usual.
• Place the trays on the teeth and leave in place for 10 minutes. If gel oozes out of the tray, you are using too much.	• Place a thin ribbon of gel on the toothbrush.
• After 10 minutes, remove the trays and spit out any excess gel.	• Brush for 2 to 3 minutes.
• Do not rinse.	• Spit out any excess gel.
• Rinse the applicator trays with water.	• Do not rinse.
• Do not eat or drink for 30 minutes.	• Do not eat or drink for 30 minutes.

Possible acute toxicity in dental clinic: APF gel

Product	Concentration of			Amount containing PTD for 10 kg child (1-2 year old)	Typical amount used
	Salt	Fluoride			
	%	%	ppm		
APF gel	2.72	1.23	12,300	**4 ml**	**5 ml**

- A shift to a cariogenic flora has been documented in patients following head and neck radiation therapy.
- Topical fluorides and chlorhexidine rinses may reduce levels of *Streptococcus mutans*.
- A **2% chlorhexidine gel** applied in mouth guards demonstrated an enhanced ability to control cariogenic flora in cancer patients with xerostomia.

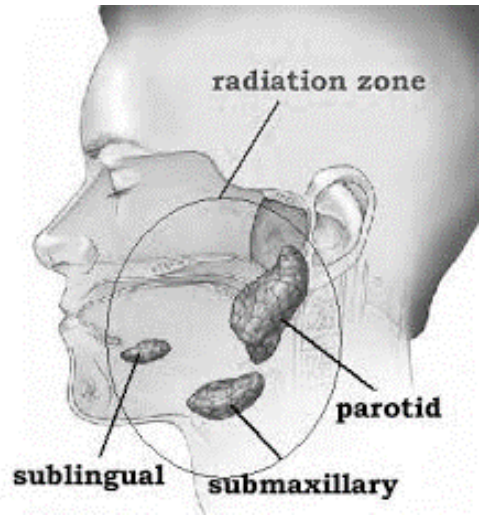
Casein phosphopeptide-amorphous calcium phosphate

- **Reynolds and colleagues** reported that CPP-ACP (Casein phosphopeptide-amorphous calcium phosphate) binds readily to the surface of the tooth, as well as to the bacteria in the plaque surrounding the tooth.
- In this way, CPP-ACP deposits a high concentration of ACP in close proximity to the tooth surface.
- The authors proposed that under acidic conditions, this localized CPP-ACP buffers the free calcium and phosphate ions, substantially increasing the level of calcium phosphate in plaque and, therefore, maintaining a state of **supersaturation** that inhibits enamel demineralization and enhances remineralization.
- The addition of CPP-ACP to **chewing gums, lozenges, mouth rinses, toothpaste,** and even some foods is a promising tool for preventing dental caries.

4.4: Salivary Glands and Radiation

By: Dr. Abhishek Gupta,

Dr. Kumari Sonam Jha



- At rest (basal or **unstimulated** function), it is estimated that the minor glands may produce up to half of the saliva in the oral cavity.
- With **stimulation**, however, the major glands predominate and minor gland secretions account for less than 10% of the saliva.
- Unstimulated salivary function is very important for both the comfort and protection of the oral cavity as this is the functional state the great majority of the time.
- It is important to check unstimulated function when evaluating a symptomatic dry mouth patient for salivary gland dysfunction.
- Tumour cells are actively dividing cells with a high mitotic index, and their DNA is the cellular target in radiation therapy because critically damaged DNA leads to cell death.
- In contrast, salivary glands are highly specialised organs in which cells are **well differentiated** with a **slow cell division cycle** and therefore have a relatively low mitotic index.

- The **differentiated salivary acinar cells** have a median life-span of over a month, and **serous cells** are characterised as noncycling, functionally mature cells in interphase in an extended G1 position in the division cycle.
- Nonetheless, they are surprisingly highly sensitive to ionising radiation, as demonstrated by the functional and morphological glandular changes occurring shortly after irradiation.
- In both human and animal studies, serous acinar cells seem to be more radiosensitive than the mucous types, but the specific mechanism behind this apparent difference in sensitivity is still **unknown**.
- Since the serous cells normally are not growing and dividing, it is unlikely that cell death occurring shortly after radiation should be associated with cell division.
- Thus, it has been suggested that acute radiation-induced salivary gland hypofunction is attributable to early serous cell death as a result of membrane disruption and interphase death caused by apoptosis.
- It is still an open question whether the nuclear changes occurring in cell death are caused directly by radiation or indirectly by other mechanisms, including alteration of cell membranes and/or release of lysosomal enzymes.
- The difference between serous and mucous cells in radiosensitivity may be attributed to the presence of **heavy metals in the serous secretory granules**, which upon absorption of radiation energy promote the release and action of free radicals.
- The apparent selective destruction of serous acini may explain the **increase in saliva viscosity and decrease in pH** as well as the earlier reduction in parotid flow than in resting whole saliva flow during treatment.
- On the other hand, a recent study found no difference between the functional response of the parotid and the submandibular/sublingual glands on exposure to radiation therapy.
- In contrast, Tsujii performed 99MTc-Per- technetate-sialography in 145 patients who received radiotherapy for head and neck cancer.
- The parotids appeared more radiosensitive than the submandibular glands at O-3 months following 20-70 Gy,

- But after 3 months both glands were similarly impaired.
- In both glands, the stimulatory response remained impaired up to 6 months.
- Serous acinar cells are believed to develop and replenish via the replication of stem cells in ductal segments
- When the stem cells of a functional subunit of the gland (secretory acini and connecting duct branch system) are inactivated by radiation it is unlikely that function can subsequently be reestablished and normalized.
- Thus, the severity of glandular damage is dependent on the *irradiated gland volume, the radiation dose, and the ability of surviving stem cells to repopulate*.
- Acinar cells surviving irradiation in vitro are functionally similar to nonirradiated cells.
- In humans it is not known to what extent effects secondary to the damage to vascular structures, (which include increased capillary permeability, interstitial oedema and inflammatory reactions,) contribute to the radiation-induced salivary gland tissue damage.
- However, the nerve function seems not to be significantly affected after radiation.
- Liu et al. determined the unstimulated and stimulated salivary flow rates in 47 patients at 0.5-25 years following mantle field radiotherapy for Hodgkin's disease or unilateral or bilateral facial fields for head and neck cancer.
- Compared to a group of unirradiated healthy controls, the irradiated patients had significantly smaller salivary flow rates.
- The magnitude of salivary flow rate reduction increased with **dose** and the **volume** of the salivary glands included in the radiation fields.

Acute effects on SG function and tissue

- A profound decrease in salivary flow occurs during the first week of radiation therapy.
- The decrease in salivary flow continues throughout the course of therapy, until flow rates are barely measurable after about 6 weeks.

- An 80% reduction in both parotid and submandibular /sublingual flow rates has been shown after the first 2 weeks of fractionated radiation therapy.
- These results suggest that the tolerance dose to the submandibular/ sublingual glands is comparable to that to the parotid glands.
- Affected individuals display a 50-60% loss of salivary flow within the first week of radiotherapy.
- Loss of acinar cells and glandular shrinkage also occurs during the acute phase

Chronic Effects

- Many studies have suggested that chronic effects of radiation may be the consequence of acute damage to salivary glands.
- Chronically, affected individuals continue to display significant decreases in unstimulated and stimulated salivary flow for several months or years following radiotherapy. In a subset of persons whose salivary glands received lower doses of radiation (< 25 Gy), there is recovery of salivary function within 12-24 months.
- However, many individuals have permanent salivary gland hypofunction, which has been attributed to attrition of acinar cells followed by replacement with fibrotic tissue
- Generally, fully irradiated parotid glands exposed to doses exceeding 60 Gy sustain permanent damage resulting in hypofunction, and there is no recovery of gland function over time.
- Some studies have documented partial recovery of salivary gland function when radiation doses of less than 52 Gy.
- A threshold effect has been suggested at a mean dose of about 26 Gy

Different therapeutic radiation doses and their acute and late effects on human salivary gland function

Dose	Acute effects on salivary flow rate	Long-term effects on salivary flow rate
2.25 Gy	Salivary flow ↓ 24 hours a single dose of 2.25 Gy [103]	
10–25 Gy	UWS ↓ to 40% [148] and SPS ↓ to 50% after 1 week of fractionated therapy and progressive reduction of flow during therapy to almost immeasurable flow after 6–8 weeks [44, 52, 57, 95, 106, 149]	Permanent salivary hypofunction [30, 104] Recovery in UPS and SPS (<24–26 Gy), permanent reduction in UPS and SPS (>24–26 Gy) after 1 year [49]
30–50 Gy	SWS and SPS ↓ [58, 83, 95]	Permanent salivary hypofunction [104] UWS↓ (slightly), SWS ↓ to 20% after 9 years [134] SWS and SPS ↓ to 47–54% [57] Partial recovery of SPS after 18–24 months [58, 141]
50–70 Gy	SWS↓ [44]; UPS and SPS↓ to 20% after 2 weeks (fully irradiated glands) and UPS and SPS↓ to 50% after 2 weeks in spared glands [47]; UPS, SPS, SM/SL ↓ [26, 172]	UPS and SPS immeasurable in fully irradiated glands and ↓ to 50% in spared glands after 3 months [47] UPS and SPS unchanged in spared contralateral glands after 2 years [49] SWS ↓ 5 years after therapy [91] SPS ↓ (total dose >52 Gy); recovery after 2–18 months (total dose <52 Gy) [57] Less reduction in SPS after 3 months using CHART [81, 83]

Changes in composition of saliva during and after radiotherapy of the head and neck region

Salivary constituents	During radiation therapy	Long-term perspective
Immunoglobulins	s-IgA, IgG ↑	Normal after 18 months
Lysozyme	↑	↑ after 6 months, normal after 18 months
Total protein	↑, EGF ↓	
Lactoferrin	↑	
Amylases	↓ or →	↓ or →
Albumin	↑	
Electrolytes	Na^+ ↑ or →, Cl^- ↑, K^+ → or ↑, Mg^{2+} ↑, total calcium ↑, HCO_3^- ↓, total phosphate ↓	Normal after 18 months

- **Preventive Therapies**

Improvements in Radiation Physics

- One of these technologies, intensity modulated radiotherapy (**IMRT**), allows for maximal treatment of a tumor while sparing normal tissues and reducing side-effects (Braam *et al.*, 2006).

- However, one study evaluated tumor recurrence in three persons who underwent salivary gland sparing close to the treatment site and concluded that caution should be used when delineating treatment parameters (Cannon and Lee, 2008).

- The major limitations of these technologies include availability of equipment, distance to experienced treatment centers, tumor location in relation to other tissues, and anatomic changes that occur during treatment (St John *et al.*, 2006; Robar *et al.*, 2007; Seiwert *et al.*, 2007).

- Radiation Dose Delivered to Salivary Glands

- Numerous studies have defined maximal dose calculations for salivary gland exposure to minimize side-effects.

- Clinically, radiation exposure of parotid salivary glands is kept below 2 Gy/day and a cumulative dose of 24-26 Gy, to allow for recovery of salivary function (Eisbruch *et al.*, 1999; Li *et al.*, 2007).

- Using planar salivary gland scintigraphy combined with single-photon-emission computed tomography (SPECT), Bussels *et al.* have determined the amount of salivary excretion fraction (SEF) lost in different anatomical slices within the parotid gland following conformal radiotherapy (Bussels *et al.*, 2004).

Amifostine

- It is dephosphorylated by alkaline phosphatase, yielding an active free thiol that can scavenge free radicals and limit indirect damage by ionizing radiation.

- Studies have indicated that accumulation of the active metabolite of amifostine, **WR-1065**, is selective to normal tissues including the salivary glands, which may be due to lower alkaline phosphatase activity in tumor vasculature than in normal vasculature. In 1999, a phase III clinical trial examining the radioprotective effects

of amifostine on salivary glands led the FDA to approve it as an agent for preventing radiation-induced xerostomia

- The study reported that amifostine administered intravenously 15 to 30 min prior to doses of fractionated radiation (~ 2 Gy/day;cumulative dose of 50-70 Gy) reduced the occurrence of acute xerostomia (grade ≥ 2) from 78% to 51%, and the occurrence of chronic xerostomia (grade ≥ 2), 1 yr after treatment, from 57%to 38%.

- A randomized controlled trial of standard fractionated radiation (1.8 Gy - 2.0 Gy/day for 5 days/week for 5-7 weeks) with or without Amifostine for Injection, administered at **200 mg/m2 as a 3-minute i.v. infusion 15-30 minutes prior to each fraction of radiation**, was conducted in 315 patients with head and neck cancer.

- Patients were required to have at least 75% of both parotid glands in the radiation field.

- The incidence of Grade 2 or higher acute (90 days or less from start of radiation) and late xerostomia (9-12 months following radiation) as assessed by RTOG Acute and Late Morbidity Scoring Criteria, was significantly reduced in patients receiving Amifostine.

- Persons receiving amifostine also have a reduced caries incidence.

- Importantly, overall survival after 2 yrs was not significantly affected by amifostine—a concern for any radioprotective therapy.

- A later phase III trial ended after 41% of individuals discontinue amifostine due to severe side-effects, including **hypotension vomiting**, and **allergic reaction.**

- After a review of several studies using a range of doses, the researchers concluded that roughly 25% of persons receiving intravenous injections of amifostine discontinue treatment.

- It has been suggested, however, that subcutaneous injection of amifostine may reduce toxicity.

- Due to high toxicity and claims that it may protect tumors, others have begun looking at

alternatives to amifostine-

- One alternative is the **nitroxide tempol**.
- In a study with fractionated radiation (6 Gy/day for 5 days), mice were administered tempol (i.p. or topical) 10 min prior to each dose (Cotrim *et al.*, 2007a).
- After 8 wks, these mice had significantly higher levels of stimulated salivary flow than mice treated with radiation alone.
- Preliminary results are promising, and tempol may soon be ready for clinical trials.

Growth Factors

- Studies have indicated growth factors' potential use as radioprotectants.
- These endocrine proteins activate cellular signaling pathways promoting *cell survival, DNA repair, and growth*.
- One study indicated that insulin like growth factor (IGF1) is a potent activator of Akt in salivary acinar cells cultured from rat parotid glands (Limes and *et al.*,2003a).
- One growth factor that is currently undergoing clinical trials for the prevention of radiation-induced xerostomia is **keratinocyte growth factor (KGF).**
- Unfortunately, a recent phase II trial of recombinant human KGF (**palifermin**) had mixed results (Brizel *et al.*, 2008).
- In persons receiving standard fractionated radiotherapy (2 Gy/day; cumulative dose of 70 Gy), palifermin provided no protection against xerostomia (grade ≥ 2) up to 12 wks post-treatment.
- In those receiving hyper-fractionated doses of radiation (1.25 Gy twice *per* day to a cumulative dose of 72 Gy), however, palifermin seemed to offer some protection, although the results were not significant.

- Recently, two novel methods have been proposed for the delivery of growth factors to salivary glands prior to irradiation.
- One study showed that rat cells treated with **basic fibroblast growth factor** (bFGF) 4 hrs prior to a single dose of radiation have a 44% reduction in apoptosis (Thula *et al.*, 2005).
- Importantly, the study demonstrated that polymer spheres loaded with **bFGF** can be used for the delayed release of growth factor over 28 days—roughly the length of a radiotherapy regimen.
- Another proposed mechanism for the delivery of growth factors is by gene transfer with adenoviral vectors (Cotrim *et al.*, 2007b).
- Adenoviruses expressing bFGF (**AdbFGF**) or vascular endothelial growth factor (**AdVEGF**) was administered *via* cannulation to the submandibular glands of mice 48 hrs prior to irradiation (15 Gy).
- Microvascular density of the gland assessed 4 hrs post-treatment was reduced by 50% in control mice, but by only 20% in mice treated with either AdbFGF or AdVEGF.
- These results corresponded with similar improvements in salivary flow rates measured after 8 wks.

Supportive Therapy

- Generally, palliative treatments for radiation-induced xerostomia are **muscarinic-cholinergic agonists** intended to stimulate secretion from remaining salivary cells or the use of artificial saliva and mouth moisturizers.
- One such drug, **pilocarpine**, had been approved by the FDA for this purpose.
- Another, **cevimeline**, which is already approved for Sjogren's syndrome, has undergone open-label studies for use in affected individuals following radiotherapy (Chambers *et al.*, 2007a, b).
- Both drugs improve salivary flow, are fairly short-lived and due to a non-specific mechanism of action, can cause a variety of side-effects, including nausea, diarrhea, and excessive sweating.

- Overall, these treatments are not well-suited for long-term treatment; thus, an emphasis has been placed on restorative therapies.
- Both should represent the first line of treatment in RT induced xerostomia.
- On the basis of the best available evidence, the results of the meta-analysis provide evidence that pilocarpine offers statistically significant clinical benefits for the symptomatic treatment of radiation-induced xerostomia in patients with head and neck cancer.
- However, the authors of this systematic review found the best available evidence in the meta-analysis in 3 studies, 1 of which showed no effect.
- The authors of this systematic review suggest that these patients take **5 milligrams of pilocarpine 3 times daily for approx. 12 weeks,** and that there is need for further study.
- In a study on 255 subjects, Overall, 175 i.e 68.6% experienced expected treatment-related AEs, most mild to moderate.
- The global efficacy evaluation at the last study visit showed that cevimeline improved dry mouth in most subjects (59.2%).
- Cevimeline **45 mg t.i.d**. was generally well tolerated over a period of 52 weeks in subjects with xerostomia secondary to radiotherapy for cancer in the head-and-neck region.

Pharmacologic Stimuli[10]

Parasympathomimetic		
Agent	Activity	Remarks
Pilocarpine	Nonselective muscarinic agonist	Sweating, nausea; half-life of 50 minutes
Bethanechol	M3 muscarinic agonist	Unhydrolysable
Carbachol	M3 muscarinic agonist	Unhydrolysable
Cevimeline	M1 and M3 selectivity	Half life of 3-4 hours
Physostigmine	Cholinesterase inhibitor	Toxic
Anethole trithione	Choleretic	–
Bromhexine	Mucolytic agent	–

Mechanical and Gustatory Stimuli[10,11]

Stimulant	Effect	Advantage	Disadvantage
Chewing Gum	Thin, watery saliva	More volume, mild taste	Only dentate, Too strong taste
Sucking Ointment	Longer time	Mild taste	Foamy saliva
Taste:			
Menthol	Mucous saliva	All glands stimulated	Too strong taste
Sweet	Mucous saliva	Inexpensive	Cariogenic
Acid	Watery saliva	Large volume	Erosive
Vitamin C	Chemical reduction	Reduces viscosity	Erosive

Palliative Therapy

- When the function of the salivary glands is completely destroyed, stimulatory measures have no effect.
- In these cases, some palliation can be obtained by wetting the oral tissues with homemade or commercially available products, including special toothpastes, oral gels, mouthwashes, and saliva substitutes.

- A number of dentifrices are well tolerated by patients with dry mouth, including those of **Biotène Oralbalance gel** ®, and **Zendium Saliva**®.

Homemade Mouthwashes	
Saline	
Emser salt	
Bicarbonate	
Glycerol	
Lemon	
Tea	
Commercial Mouthwashes	**Substantial Base**
Biotène®	Enzymes
Oralbalance®	Enzymes
Zendium Saliva®	Enzymes
Saliva Substitutes	
Glandosane®	Carboxymethylcellulose
Saliva Orthana®	Porcine gastric mucin
Xialine®	Xanthan gum

- Mucins or xanthan gum are added to simulate not only the moisturizing properties, but also the viscoelastic properties of natural saliva.
- Currently, ongoing research is aimed at developing saliva substitutes that provide protection against microorganisms in addition to providing moisture and lubrication.
- Potential additive agents are **histatin** derivatives.
- Klestov et al, Visch et al, and Vissink et al have determined that the most useful indices of the effectiveness of artificial saliva are the degree of nocturnal discomfort and difficulty in talking.
- Furthermore, the success of artificial saliva usage is strictly dependent on adequate instructions.
- In addition, there is also a great variation in tolerance to artificial saliva among patients.
- Because of this variability it is worthwhile to use different types of saliva substitutes in a particular patient in order to select the most effective substitute in that patient.
- A comparison of effects of saliva substitutes and saliva stimulants indicates that the effect of a treatment also depends on the remaining secretory potential of the salivary glands.

SEVERE HYPOSALIVATIN	MODERATE HYPOSALIVATION.	SLIGHT HYPOSALIVATION
A saliva substitute with gel-like properties should be used during the night and when daily activitiesare at a low level. - During the day, a saliva substitute with propertiesresembling the viscoelasticity of natural saliva, such as substitutes that have xanthan gum and mucin (particularly bovine submandibular mucin) as a base should be applied.	- If gustatory or pharmacologicstimulation of the residual salivarysecretion does not provide sufficient amelioration, saliva substitutes with a rather low viscoelasticity, such as substitutes that have carboxymethyl cellulose, hydroxypropyl-methylcellulose, mucin (porcinegastric mucin), or low concentrations of xanthan gum as a base are indicated. - During the night or other periods of severe oral dryness, the application of a gel is helpful.	- Gustatory or pharmacologic stimulation of the residual secretion is the treatment of choice. - Little amelioration is to be expected from the use of saliva substitutes.

- Restorative Therapies

Gene Transfer

- A recent review describes a clinical trial for the use of adenoviral mediated gene transfer in treating persons with chronic radiation induced xerostomia (Baum *et al.*, 2006).
- The authors suggest that water is the crucial component protecting the upper GI tract.

- Therefore, they propose that increasing the water permeability of ductal cells that remain following radiotherapy may alleviate the symptoms of chronic xerostomia.
- To achieve this, they plan to deliver an adenoviral vector expressing the water channel protein human aquaporin-1 (AdhAQP1) to salivary glands *via ductal* cannulation.
- This approach has been tested extensively *in vivo*.
- In one study, ductal cannulation of AdhAQP1 to the submandibular glands of rats resulted in a roughly five-fold increase in AQP1 present in membranes throughout the gland (Delporte *et al.*,1997).
- Rats treated with AdhAQP1 had salivary flow rates 2 to 3 times higher than those of rats treated with the control vector (Delporte *et al.*, 1997).
- A similar study in miniature pigs, with a single dose (20 Gy) of radiation targeted to 1 parotid gland, reported that delivery of AdhAQP1 after 17 wks resulted in recovery of parotid flow to roughly 80% of pre-irradiation values, *vs*. 20% in animals receiving a control vector (Shan *et al.*, 2005).
- Unfortunately, another study showed post-irradiation salivary flow improvements in only 2 of 3 rhesus monkeys treated (O'Connell *et al.*, 1999a).
- While adenoviral gene therapy has had several experimental set-backs and may still have issues with the host immune response, there is still some optimism that it is a viable therapeutic option (Cotrim and Baum, 2008).

Artificial Salivary Gland

- Tran *et al.*, 2006 - design consists of a biodegradable polymer tube covered with an extracellular matrix protein, such as collagen, on which a monolayer of polarized epithelial cells can be grown.
- It has been demonstrated that primary cells from rhesus monkey parotid glands can proliferate on a poly-L-lactic acid membrane coated with collagen (Tran *et al.*, 2006).
- These cells, which appear to be ductal, are correctly polarized and can limit fluid movement from the basal to the apical surface.

- When transduced with an adeno associated virus expressing aquaporin-1 (AAV2-hAQP1), roughly 9% of the cells became positive for AQP1 within 72 hrs, allowing for a six-fold increase in fluid movement.
- The percentage of transduced cells was low, and there are questions about whether these channels alone will be enough to establish proper osmotic gradients for secretion.
- However, the work is promising.

Stem Cell Transplantation

- It has been proposed that the loss of salivary function post-irradiation is due to attrition of the salivary stem cells necessary for maintaining a healthy gland (Konings *et al.*, 2005b).
- Based on this hypothesis, a 2008 study revealed that salivary stem cell transplantation post-irradiation can rescue glandular function (Lombaert *et al.*, 2008).
- To determine whether cultured stem cells could re-populate a damaged gland, a group of investigators grew salivary stem cells from male mice in culture for 3 days and injected them into the submandibular glands of female mice 30 days post-irradiation (15 Gy) (Lombaert *et al.*, 2008).
- Remarkably, after 90 days, the glands of these mice were repopulated by donor-derived proliferatingacinar cells, as determined by the presence of the Y chromosome.
- These mice also exhibited a marked recovery of salivary flow at the same time-point, demonstrating the first use of transferring salivary specific cells to restore glandular function.
- It was proposed to use autologous salivary stem cells to re-populate the glands of affected individuals after radiotherapy.
- Unfortunately, it was found that these cells lost expression of stem cell markers after 3 days in culture, which would diminish their use after the ~ 30-day radiotherapy regimen.
- It is clear that the next step involves the development of methods for maintaining the pluripotency of these cells in culture.

- Future Directions
- In the past few years, a wealth of research has been conducted that has improved our understanding of radiation-induced salivary gland dysfunction and affected the development of new treatment strategies.
- One major obstacle in the field has been identification of the salivary stem cell.
- Rachidi *et al.* evaluated the radiosensitivity of keratinocyte stem cells and their direct progeny progenitor cells (Rachidi *et al.*, 2007).
- Interestingly, the stem cells were radioresistent, and the progenitor cells were radiosensitive.
- This dichotomy of radiosensitivity within a particular tissue may have applicability to the salivary glands as well.
- Identification of stem cells or progenitor cells within the salivary gland and their fate following radiotherapy directly influences the type of therapy that could be beneficial.
- This path to improved care for the secondary side-effects of radiotherapy on salivary glands will no doubt be challenging, and several different approaches to success could be envisioned.

Salivary ph

- Baseline pre-RT - 6.50+-1.07
- 3 weeks during RT - 6.23+-1.5
- **6weeks during RT - 5.59 +- 1.62**
- 3months post RT - 6.21+-1.09
- 6 months post RT - 6.63+-.89
- Wetting properties of **mucin-containing** and **carboxymethylcellulose-containing** substitutes on poly (methyl methacrylate) were significantly better than those of human saliva.

- Mucin-containing artificial salivas had the best wetting properties on the acrylic resin for the materials tested.
- Patients with insufficient saliva benefit from wetting their dentures before placing them in the mouth.
- Salivary substitutes, artificial saliva and salivary stimulants therefore can be beneficial for the denture-wearing patient in terms of helping with adhesion and cohesion and, subsequently, prosthesis retention.
- Patients can be advised to spray their prostheses with artificial saliva before denture insertion and before meals.
- Although the use of adhesives in patients with xerostomia and hyposalivation requires additional care, it often is necessary to stabilize a removable prosthesis.
- Patients should be instructed to wet their prostheses before applying adhesive, and a combined use of artificial saliva and denture adhesive appears to be beneficial.

Skin:

- Early erythema may appear from a single dose of about 450 rads.
- With lower doses no erythema occurs.
- The skin lags the mucosa by l-2 weeks, but similarly exhibits erythema. (usually 10 to 16 weeks)
- In the skin, the analog of mucositis is desquamation; initially "dry desquamation" and later "moist desquamation."
- The acute radiation reaction of skin is characterized by vasodilatation and increased permeability seen after single doses exceeding 20 Gy.

ii. Late or chronic signs:

- Loosening of hair and epilation.
- Dryness and atrophy of skin, due to destruction of thesweat glands.
- Progressive pigmentation, telangiectasis and keratosis.
- Indolent type of ulcerations.

- **Fibrosis**: 4 to 12 weeks after a single dose of 15 to 25 Gy, 36 to 48 weeks after lower doses of 5 to 10 Gy.A dose-dependant increase in collagen content is seen as early as 1 week after irradiation. This effect declines between weeks 12 and 24 and increases again between weeks 36 and 48.

- There is an early progressive and sustained deposition of collagen in skin after irradiation rather than a "lag" between exposure and the late development of fibrosis.

- The early increase in collagen is possibly caused by an inflammatory response that results from changes in vascular permeability, extravasation of plasma protein, and fibrin deposition, possibly stimulated by complement kinin generating systems, prostaglandins, lysosomal enzymes, or other compounds that are liberated from irradiated lymphocytes, macrophages, and polymorphonuclear leukocytes.

4.5: Osteoradionecrosis

- Ewing was the first to identify the osseous changes associated with RT in 1926, and called it "**radiation osteitis**".

- Watson and Scarborough in 1938, postulated the causes of "radiation osteitis" to be radiation, trauma, and infection.

- Meyer 1970 Classic sequence in the pathogenesis of osteoradionecrosis of the jaws has been accepted as radiation – trauma -- infection.

- The role of trauma as the absolute initiating factor in osteoradionecrosis has has been challenged.

- Daly and Drane have reported a 39% incidence of osteoradionecrosis unassociated with any specific trauma.

- Bedwinek et al have also identified "spontaneous osteoradionecrosis" and related it to higher radiation doses.

- Wong *et al*. defined ORN as "a slow-healing radiation induced ischemic necrosis of bone with associated soft tissue necrosis of variable extent occurring in the absence of local primary tumour necrosis, recurrence or metastatic disease"

- Chranovic et al. suggested to add to this definition a minimum period of bone exposure of **three months**

- Store and Boyson defined ORN as "radiological evidence of bone necrosis within the radiation field, where tumour recurrence has been excluded"
- The **National Cancer Institute** defined ORN as "a disorder characterized by a necrotic process occurring in the bone of the mandible".
- According to the most recent literature, ORN of the jaws is defined as exposed irradiated bone that fails to heal over a period of 3 months without any evidence of persisting or recurrent tumor
- Although the pathogenesis mechanism is still under investigation the most frequently reported reason is radiation arteritis, which leads to the development of a hypocellular, hypovascular, and hypoxic environment (Marx, 1983b, Fenner et al., 2010).

sequence suggested by MARX's 1983 study is as follows:

(1) radiation,

(2) hypoxic-hypocellular-hypovascular tissue,

(3) tissue breakdown (collagen lysis and cellular death exceeding synthesis and cellular replication), and

(4) chronic non-healing wound (in which energy, oxygen, and structural precursor demand exceed supply).

- Another recently proposed theory suggests that osteoclastic injury due to radiation, leads to hampered osteoclast-mediated bone turnover. This in turn leads to ORN.
- Delanian et al., published a new theory, the fibro atrophic theory in 2004.
- It states that the radiation induced fibro-atrophic mechanism leads to ORN.
- This constitutes three phases: the profibrotic phase, the constitutive organized phase, and the late fibro-atrophic phase.

The pre-fibrotic phase - changes in endothelial cells predominate with an acute inflammatory response.

The second is a constitutive, **organized phase** in which abnormal fibroblastic activity predominates, and the extracellular matrix becomes disorganized.

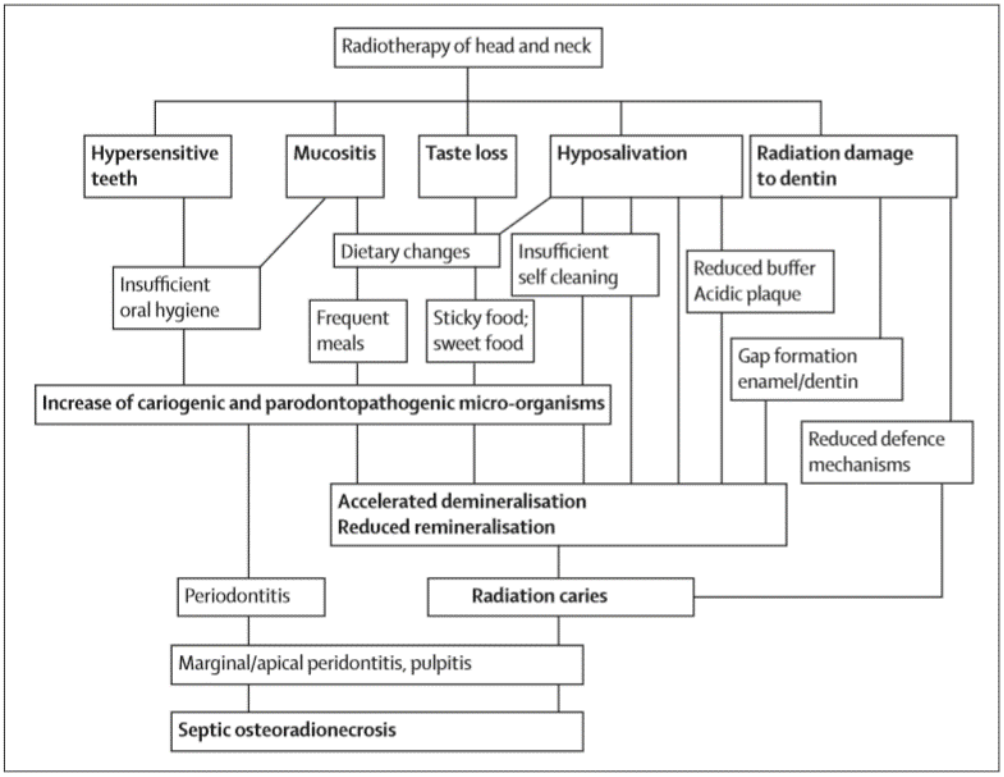
Finally, in the **late fibro atrophic phase**, attempted tissue remodeling forms fragile healed tissues, which have a serious inherent risk of late reactivated inflammation in the event of local injury, and in bone may result in necrosis.

- The average age of patients with ORN is over 55 years Mandibular ORN predominates when compared with maxillary ORN (ratio mandible: maxilla is approximately 24:1.
- Previous studies, the incidence of ORN in a population that has received head and neck irradiation was estimated to be 4.74 to 37.5%.
- Recent studies have shown an incidence decreased to lower than 5% and have attributed the phenomenon to improved dental preventive care and improved radiation techniques, such as 3-dimensional conformal RT (3DCRT) and intensity-modulated RT (IMRT)
- Peterson et al. reviewed 18-years of literature regarding the impact of cancer therapies on the prevalence of ORN, and reported a weighted ORN prevalence of **7.4% for conventional RT, 6.8% for chemoradiotherapy**, **5.3% for brachytherapy**, and **5.1% for IMRT**
- Ben-David et al. reported no case of mandibular ORN after IMRT for head and neck cancer, using a strict prophylactic dental care policy.

Risk Factors

- Numerous factors have been associated with the risk of ORN development
- They can be divided into three main groups

1. **Tumor-related factors**
2. **Treatment-related factors**
3. **Patient-related factors**



The **incidence** of osteonecrosis appeared to be directly related to the **radiation dose** to the bone.

- In a study, Osteonecrosis developed in 85% of the dentulous patients and 50% of the edentulous patients who received more than 75.0 Gy to the bone.
- None of the patients who received less than **65.0 Gy** developed osteonecrosis.

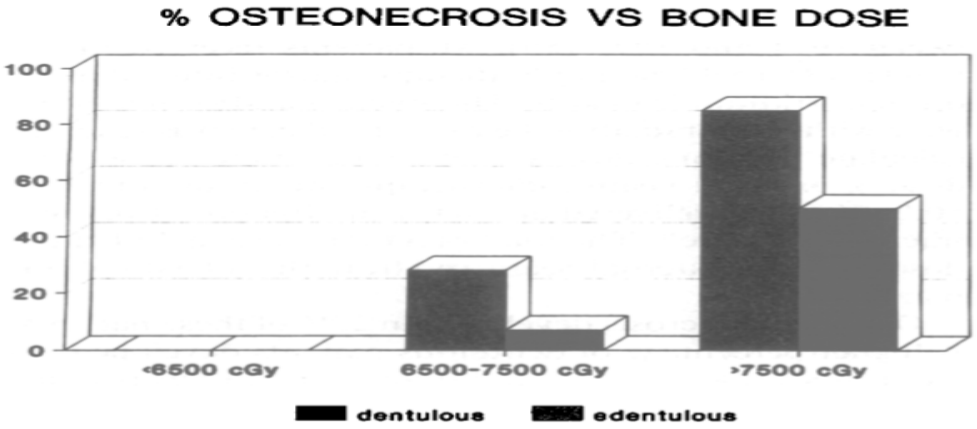


Fig. 13. Likelihood of osteonecrosis vs. dose delivered to bone in dentulous and edentulous patients. Data from (59).

Table 4. Association of dental extractions and osteonecrosis in 78 irradiated dentulous patients*

	Radiation dose (cGy)		Osteonecrosis	
Time of extraction	Range	Mean	Incidence	Mean time of onset after treatment (months)
No extractions	6940–9280	(7871)	5/41 (12%)	29
Before radiotherapy	7580–9610	(8500)	3/19 (16%)	41
After radiotherapy	6700–8100	(7346)	11/18 (61%)	20
Total	6700–9610	(7666)	19/78 (24%)	22

* Radiation for dentulous patients without osteonecrosis ranged between 4950 and 9700 cGy (mean 6450 cGy).

- Thus, it seems reasonable to conclude that while spontaneous osteoradionecrosis can occur in any patient, it is most likely in dentulous patients who require tooth extractions after radiation therapy.
- Patients who are edentulous prior to treatment have a relatively low risk of osteonecrosis, and dentulous patients who have only pretreatment extractions or no extractions appear to have risks similar to those of edentulous patients.
- **Early-onset ORN** is defined as clinical features noted within 2 years of RT.
- It is predominantly caused due to high radiation doses that are >70 Gy.
- **Late-onset ORN** is postulated due to trauma in a chronically hypoxic environment
- With the increasing use of pentoxifylline but not HBO, and in the **absence of a classification that includes the extent of ORN and its symptoms,** *A. Lyons et al.* have developed a new classification and have used it in a series of patients with the condition.

Classification of osteoradionecrosis.

Stage	Description
1	<2.5 cm length of bone affected (damaged or exposed); asymptomatic Medical treatment only
2	>2.5 cm length of bone; asymptomatic, including pathological fracture or involvement of inferior dental nerve, or both Medical treatment only unless there is dental sepsis or obviously loose, necrotic bone
3	>2.5 cm length of bone; symptomatic, but with no other features despite medical treatment Consider debridement of loose or necrotic bone, and local pedicled flap
4	2.5 cm length of bone; pathological fracture, involvement of inferior dental nerve, or orocutaneous fistula, or a combination Reconstruction with free flap if patient's overall condition allows

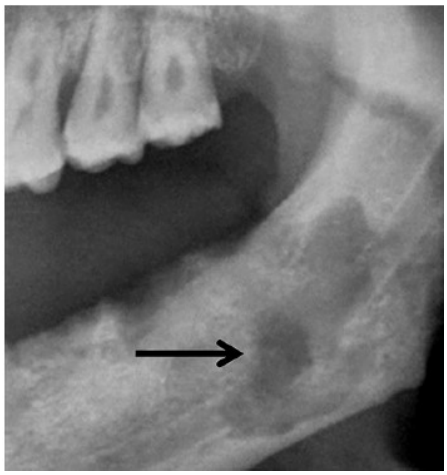
Radiological considerations

- Radiological investigations are required in ORN to detect the presence, severity, and extent of ORN, and to monitor the progress of conservative treatment, if instituted.
- Major diagnostic concern in a suspected case of ORN is to exclude tumour recurrence.
- Various morphological imaging techniques that contribute to the evaluation of ORN are conventional radiographic techniques [**mainly panoramic radiography (PR), multidetector CT (MDCT), and MRI**].

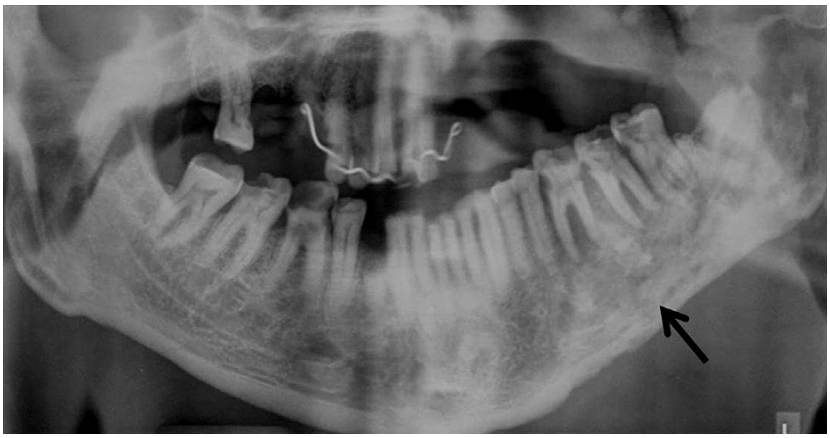
Panoramic radiography (PR)

- Conventional radiography, most commonly PR, has been widely used for evaluation of suspected ORN.

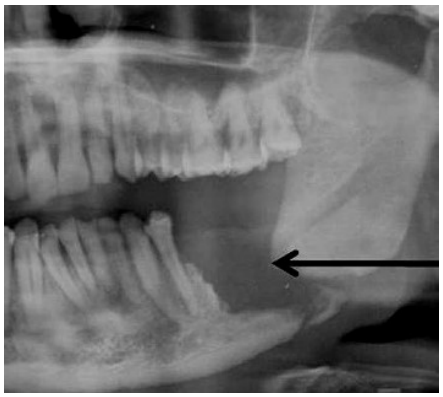
- PR depicts osseous changes of ORN, however, with lesser sensitivity than cross-sectional imaging techniques.
- Early osseous changes are not easily detected.
- PR is also not able to depict accurately the soft-tissue changes associated with ORN.
- As a two-dimensional (2D) projection- several limitations, such as magnification, superimposition, misrepresentation, and distortion of structures.
- However, PR is a readily available, fast, and convenient technique, which involves reduced radiation exposure.
- Hence, **PR is recommended for follow-up and monitoring patients who are at risk of ORN; but is not very accurate for evaluation of extent**.
- Radiation damage to the mandible can lead to loss of bone mass with resorption of the osseous trabeculae.
- On OPG, it is seen initially as rarefaction of the affected bone, or later, as lytic areas within the mandible.



- Disorganization and thickening of trabeculae can also be one of the features of radiation damage.

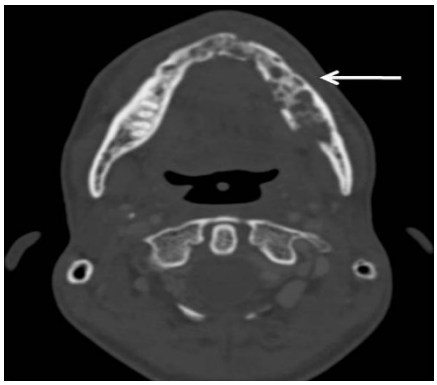


- Sequestrum, which is defined as "dead bone", may be seen as a radiodense area amidst the affected rarefied portion of the mandible.
- Progression of the disease can lead to pathological fracture in severe cases, which is seen as a cortical break.

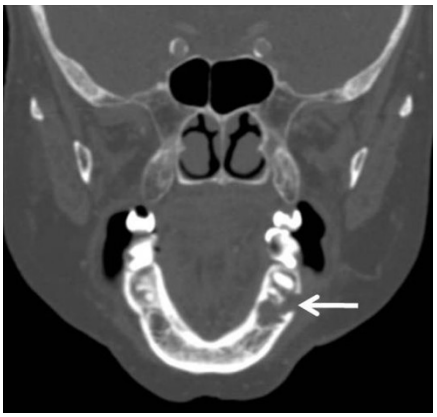


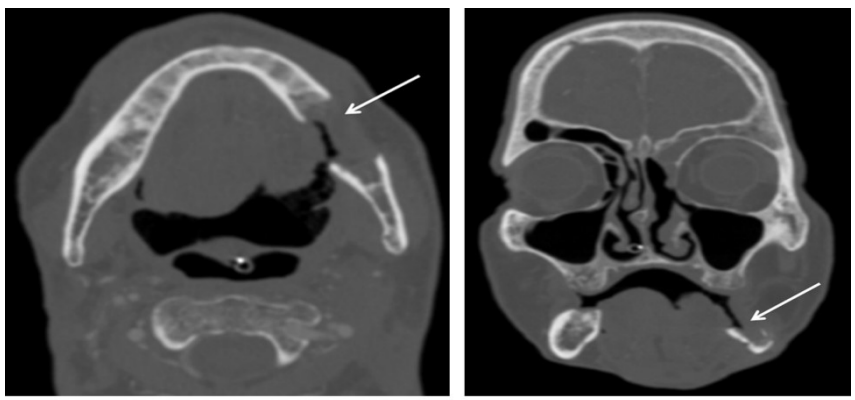
- **Multidetector Ct (Mdct)**
- MDCT can accurately evaluate the extent and severity of the osseous changes, along with the associated soft-tissue changes, if any.
- Store and Larheim compared the efficacy of CT and PR in the diagnosis and pre-surgical evaluation of mandibular ORN, by evaluating 31 cases.
- They concluded that CT is superior to PR in visualizing the radiological features of ORN and the anterior posterior extent of the lesion.
- They recommended CT in a diagnostic dilemma or when surgical intervention is contemplated.

- At CT, ORN may present as loss of osseous trabeculae in the spongiosa.
- It can manifest as - osteolytic mandibular lesions.



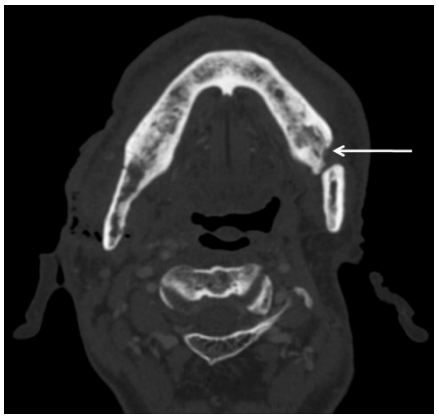
- or cortical erosions, involving the buccal or lingual surface Bicortical involvement can occur.



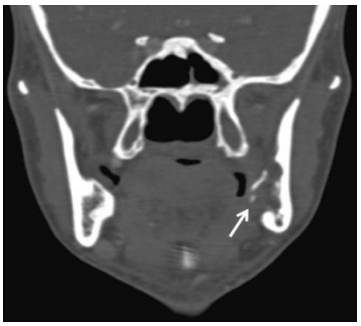


(a) (b)

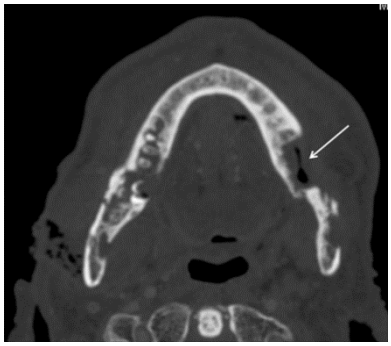
- In severe cases, leading to pathological fractures



- Bone sequestrum may be seen as sclerotic fragments in the involved region of the mandible that are separated from the adjacent cortex.



- Bone fragmentation and gas bubbles may also be encountered in areas of ORN.



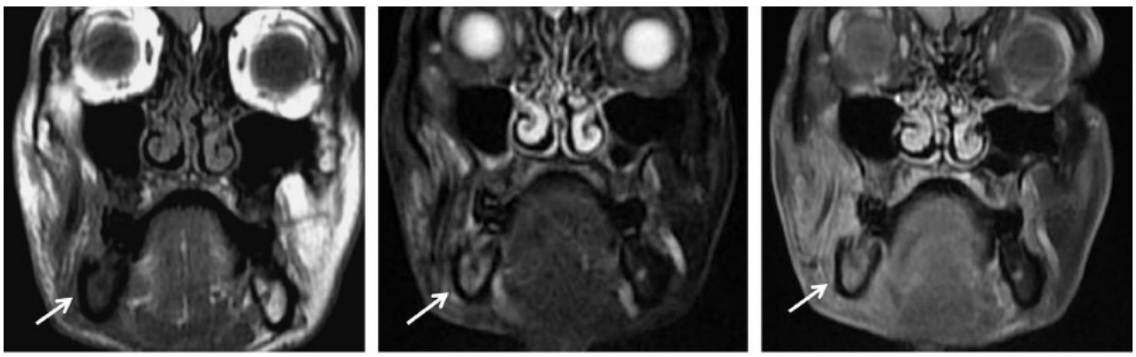
Cone-Beam Ct (Cbct)

- CBCT uses a divergent cone-shaped beam, obtaining multiple planar projections in a single rotation.

- CBCT provides accurate images in formats that allow three-dimensional (3D) visualization of the maxillofacial region, thus achieving a transition of dental imaging from 2D to 3D images.
- However, it has limited soft-tissue contrast resolution compared to MDCT.
- So, where evaluation of soft tissues is required, such as in suspected mandibular ORN, the appropriate imaging technique is **MDCT** or **MRI**, rather than CBCT.

MRI

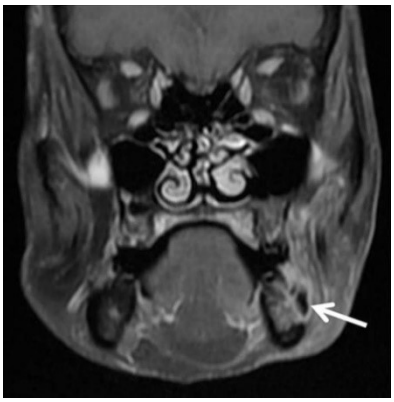
- MRI meticulously depicts marrow alterations, cortical erosions, soft-tissue changes, and complications of ORN.
- MRI of patients with ORN reveals altered marrow signal intensity in the involved part of the mandible,
- usually appearing hypointense on T1-weighted images (Fig a),
- hyperintense on T2-weighted (Fig b).
- and short-tau inversion recovery (STIR) images, these areas show intense post-contrast enhancement (Fig c)

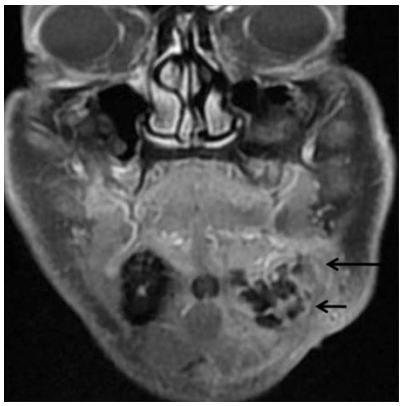


(a) (b) (c)

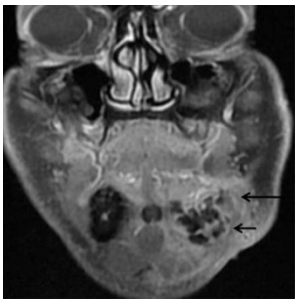
- Chong et al. and Bachmann et al. found similar marrow signal intensity changes.
- However, Fujita et al. studied 13 patients with mandibular ORN and classified their MRI findings into three groups.
- They found the **commonest** MRI presentation to be homogeneous low signal intensity on both T1-weighted and T2-weighted images in the involved portion of the mandible.

- They postulated this to be suggestive of **fibrosis** of bone marrow due to long-standing ORN, inflammatory changes having settled down by the time of the MRI investigation.
- The **second** group showed low signal intensity on T1-weighted images and inhomogeneous high signal intensity in a diffuse surrounding area of low signal intensity on T2-weighted images.
- This was suggested to be due to **acute inflammatory** changes in the irradiated fibrous bone marrow.
- The **third pattern** was of homogeneous low signal intensity on T1-weighted images and high signal intensity on T2-weighted images; which they postulated to be due to either inflammatory changes or loose fibrosis associated with marked cellularity.
- Further osseous changes associated with ORN include cortical erosions, which can be appreciated as loss of the hypointense cortical definition.
- Bone sequestrum, fragmentation, and pathological fractures can be appreciated at MRI;





- **however, these osseous changes are better evaluated at CT.**
- The osseous lesions may be associated with soft-tissue abnormalities, which appear hypointense on T1-weighted images, hyperintense on T2-weighted and STIR images, and show intense post-contrast enhancement.
- Post-contrast enhancement may also be seen in the masticator muscles, giving a "pseudo-mass appearance", as in CT.



Radiological differentiation of mandibular ORN and tumour recurrence

- The major concern in patients presenting with radiological features of ORN is to exclude tumour recurrence.
- Tumour recurrence also commonly presents as osteolytic lesions with associated soft-tissue mass.
- Tumour recurrence is usually encountered within 2 years of treatment of the primary tumour; whereas the time to presentation of ORN can be variable (early or late ORN).
- The introduction of functional imaging techniques such as **diffusion-weighted imaging (DWI), PET, and SPECT**, however, has added a new dimension to the radiological evaluation of ORN.

Management / preventive

- Frequent follow-up should be done for patients after completion of radiotherapy.
- Scaling and root planning should be done under proper antibiotic coverage if proper oral hygiene is not maintained by the patient.
- Carious lesions should be restored immediately.
- Dental extractions after irradiation should be avoided and postponed if possible.
- Consequently, endodontic therapy should be the treatment of choice in many cases.
- Endodontic therapy has been shown to be a viable alternative to exodontia, since traumatic injury will be kept to a minimum thus reducing the risk of osteoradionecrosis.

- The initial infection-based hypothesis resulted in treatment based on antibiotic therapy and surgical debridement.
- Later work by Marx described bacteria as a superinfection rather than as being involved in pathogenesis,
- But nonetheless **antibiotic therapy** in acute episodes of pyogenic infection in ORN, guided by microbiologic samples and culture and sensitivity assays, remains a cornerstone of treatment.
- Conservative management has traditionally involved minimal surgical debridement and hyperbaric oxygen therapy (HBOT)
- Patients with aggressive ORN often require radical resection and free flap reconstruction to achieve a satisfactory outcome.
- HBOT is well known to have a positive influence the out-comes of operation by promoting angiogenesis in irradiated tissues, but some authors have reported good out comes without its use.
- Contemporary understanding of the pathophysiology of ORN based on the concept of radiation-induced fibrosis has allowed the introduction of new therapeutic regimens comprising **pentoxifylline**, **tocopherol**, and **clodronate**

1. **The pre-fibrotic phase** - changes in endothelial cells predominate with an acute inflammatory response.
2. **The second is a constitutive**, **organized phase** in which abnormal fibroblastic activity predominates, and the extracellular matrix becomes disorganized.

3. Finally, in the **late fibroatrophic phase**, attempted tissue remodeling forms fragile healed tissues, which have a serious inherent risk of late reactivated inflammation in the event of local injury, and in bone may result in necrosis.

- **Fibroatrophic Theory**
- Endothelial cell injury occurs directly from radiation and indirectly from the free radical or reactive oxygen species (ROS) generation.

- Subsequent cytokine production triggers an acute inflammatory response and generates further production of ROS from inflammatory cells.
- Endothelial injury with small vessel thrombosis leads to necrosis, ischaemia and tissue injury with further cytokine production.
- Ultimately these cytokines stimulate the **trans differentiation** of fibroblasts into myofibroblasts and include fibroblast growth factor b, TGFb1, tumour necrosis factor a (TNFa) and interleukins.
- This activation results in a fibrotic pattern of tissue turnover with greater **proliferation** and production of **abnormal extracellular matrix.**
- There is a reduced ability to degrade these components, especially in the absence of bone-forming cells that have been depleted by the effects of radiation.
- The replacement of bony tissue with a fibrous matrix is exacerbated in bone that has an end arterial blood supply, like the mandible.
- Maxilla
- The mandible was involved in 100% of the cases; no case was diagnosed in the maxilla. (Morrish et al.,1981, Kluth et al.,1988, Store and Boysen, 2000, Vanderpuye and Goldson, 2000, Notani et al., 2003, Chopra et al., 2011, Gevorgyan et al., 2013, Lambade et al., 2013).
- This could be attributed to the restricted localized blood supply and the higher bone density in the mandible, the inclusion of the mandible in the radiation field and the higher amount of radiation being absorbed by the mandible during RT (Morrish et al., 1981, Vanderpuye and Goldson, 2000, Lambade et al., 2013) as well as the high number of anastomoses in the maxilla and its frequent restriction from the irradiation field (Beumer et al., 1984, Thorn et al., 2000, Reuther et al., 2003).
- The posterior region of the mandible was more frequently affected than the anterior one. (Thorn et al., 2000, Reuther et al., 2003) and could be attributed to the fact that posterior areas are almost always included in the radiation field during RT of both oropharynx and regional lymph nodes (Epstein et al., 1987b, Thorn et al., 2000)

- They also undergo maximum load during mastication and are often subject to dental extractions which are implicated in the occurrence of ORN (Jereczek-Fossa and Orecchia, 2002)

- **Bone Exposure (E).**

Grade 0 : no bone exposure

Grade 1: largest dimension of exposure **less than 1 cm**

Grade 2: largest dimension of exposure **1 to 3 cm**

Grade 3: largest dimension of exposure **greater than 3 cm** or bone destruction extending to the **sinus floor**, evidenced by periapical or panoramic radiography or CT scan

- **Infection (I).**

Grade 0: no symptoms and signs of infection

Grade 1: erythema and swelling of the mucosa **less than 5 mm** in width measured from the bone exposure margin, with or without serous discharge

Grade 2: erythema and swelling of the mucosa extending **5 mm or more** beyond the bone exposure margin, with or without serous discharge

Grade 3: purulent discharge or facial swelling and erythema indicative of **cellulitis** or the presence of **acute sinusitis**

- **Bleeding (B).**

Grade 0: **no** clinical signs or history of bleeding

Grade 1: **intermittent** bleeding that stopped spontaneously

Grade 2: one or more episodes of active bleeding with need to

apply pressure for **less than 30 minutes**

Grade 3: one or more episodes of active bleeding with need to apply pressure for **30 minutes or more**

- Pentoxifylline

- Methylxanthine derivative
- Has multiple effects including vascular dilatation and increased erythrocyte flexibility effects, both of which enhance blood flow.
- Furthermore, pentoxifylline has anti–TNF-a activity and is thought to reduce the cytokine cascade driving the ORN process.
- It has also been shown to reduce proliferation of dermal fibroblasts and limit ECM production by these cells.
- In vitro experiments have also shown promotion of collagenase activity in these cells.
- Tocopherol
- A fat-soluble vitamin (vitamin E) and is a weak antioxidant agent.
- Scavenging reactive oxygen species involved in the pathogenesis of ORN, wherein they induce cell membrane peroxidation among other deleterious effects.
- Tocopherol also shows partial inhibition of TGF-b1 and an antifibrotic effect mediated by procollagen genes.
- Clodronate
- This agent is a first-generation, non-nitrogenous bisphosphonate approved for use in osteoporosis, hyperparathyroidism, hypercalcemia of malignancy, and multiple myeloma.
- Clodronate reduces bone resorption through reducing osteoclast numbers and activity.
- It is also known to reduce inflammatory cytokines IL-1b, IL-6, and TNF-a.
- Clodronate also has been shown to act on osteoblasts to increase bone formation and reduce fibroblast proliferation.
- Pentoxifylline and Tocopherol Combined Therapy with or Without Clodronate
- Delanian and colleagues have described 2 phase II trials of combined therapy for ORN of the mandible.

- In the first, 18 consecutive patients were treated with pentoxifylline and tocopherol.
- Each had at least 13.4 mm of exposed mandibular bone and all had been prescribed pentoxifylline, **400 mg twice daily** and tocopherol, **1000 IU orally** for 6 to 24 months.
- The worst affected cases (n = 8) were also given clodronate, 1600 mg daily for 5 days per week.
- The second trial, published in 2011, reported on 54 patients who received radiation for head and neck cancer a mean of 5 years before the onset of ORN.
- This treatment regimen had evolved to combined pentoxifylline and tocopherol as described earlier, with **clodronate, 1600 mg given 5 days per week**, and prednisone, 20 mg with ciprofloxacin given on the other 2 days.
- **study showed that prolonged treatment (16 +-9 months) was safe and well tolerated.**
- **All patients in the study experienced improvement, with anexponential progressive and significant reduction in exposed bone**
- Based on the current understanding on ORN pathophysiology, new protocols have been suggested for its prevention.
- Patients who required multiple dental extractions or extensive surgical extractions, or both, can be given **eight weeks of pentoxifylline 400 mg twice daily with tocopherol 1000 IU, starting a week before the procedure.**

C. Madrid et al. Osteoradionecrosis: An update. Oral Oncology 46 (2010) 471–474

- **Hbo Therapy**

- Hyperbaric oxygen therapy is still widely used for ORN prevention and management, although this practice has had recent challenges.
- A randomized/prospective clinical trial using HBO and penicillin was carried out by Marx et al.
- This trial demonstrated that HBO **reduced the development of osteoradionecrosis after tooth removal** and this reduction was statistically significant.

- HBO stimulates a neo-angiogenesis, improves the tissue perfusion and stimulates the formation of collagen; therefore, HBO aids the healing process of the damaged tissues.

Management of osteonecrosis of the jaws induced by radiotherapy in oncological patients.2015

- **Patients planned for more radical surgery, or patients facing preventative measures such as dental extractions, are advised to have 20 dives preoperatively followed by 10 dives postoperatively**.

- In Marx's HBO protocol study, only 15% of patients responded to HBO alone; most had operations if they did not respond to HBO, 14% had sequestrectomy, and 70% major reconstruction.

- These results suggested that HBO without aggressive surgical management was inadequate, and this was confirmed in subsequent studies of HBO in ORN

Kiki C. A. L. Cheriex. Osteoradionecrosis of the Jaws: A Review of Conservative and Surgical Treatment Options. Journal of Reconstructive Microsurgery Vol. 29 No. 2/2013

- Hbo Therapy

- It consists of inhaling 100% oxygen at an elevated pressure (1.5-3.4 atmospheric pressure)

- Mechanism of action

- HBO treatments bring oxygen to the hypoxic tissue by increasing the blood–tissue oxygen gradient; this favours the wound healing process by facilitating the reconstruction of irradiated tissues and preventing necrosis.

- In addition, HBO is bacteriostatic and bactericidal for many microorganisms. (enhance the phagocytic ability of leucocytes) Chouinard AF, Giasson L, Fortin M. Hyperbaric Oxygen Therapy for Head and Neck Irradiated Patients with Special Attention to Oral and Maxillofacial Treatments. J Can Dent Assoc. 2016;82(g24):1488-2159.

- **Short-term effects:** vasoconstriction, reduction of edema, phagocytosis activation and an anti-inflammatory effect.

- **Long-term effects:** stimulation of collagen production by fibroblasts, osteoneogenesis and, most important, neovascularization.
- The induced angiogenesis becomes detectable after 8 sessions.
- At 20 sessions, it reaches a plateau at 80–85% of non-irradiated tissue vascularity.
- The changes induced by HBO therapy on the tissue's oxygen pressure appear to be largely permanent, as, 3 years after completion of HBO treatment, oxygen pressure in the tissue has been observed to be 90% of what it was at the end of the treatment.
- Indications
- Burns
- Diabetic arteriopathic ulcers
- Air embolism,
- Carbon monoxide poisoning and
- Compartment syndrome,
- **Used as an adjuvant to both conservative and surgical treatment of ORN**
- The Marx's protocol for **ORN treatment**:

- 90 - minute session at 2.4 atmospheres, once a day for 30 days before the surgery and 10 days after the surgery OR

- If HBO therapy is used as a **preventive** method, the protocol is daily sessions for 20 days before surgery and 10 after
- **Contraindications**
- **Relative contraindications:** claustrophobia, seizure disorder, upper respiratory tract infection, chronic sinusitis and history of spontaneous pneumothorax.
- **Absolute contraindications:** are optic neuritis, history of bullous pulmonary disease, congenital pulmonary blebs,untreated pneumothorax and poorly controlled chronicheart failure.

- The presence of an active tumour was once a contraindication, but Feldmeier and colleagues, after reviewing the available clinical data, concluded that there is no evidence that HBO therapy induces tumour cell growth.
- Complications
- Transient myopia,
- Middle-ear barotrauma,
- Pneumothorax,
- Arterial air embolism,
- Oxygen toxicity seizure,
- Exacerbation of acute viral infection,
- Pulmonary oxygen toxicity and acute pulmonary edema.
- **Disadvantages**
- High cost,
- The limited treatment locations available,
- Time-consuming (thus difficulty in getting patients'compliance) and
- May delay the definitive treatment
- **Efficacy of HBO Therapy**
- A randomized placebo-controlled double-blind study was conducted to evaluate the effect of HBO therapy on ORN.
- The treatment group received 30 sessions of HBO before and 10 after surgery when such a treatment was needed. The controlled group was treated in the same manner but with a gas similar in composition to normal room air.
- The study was stopped after enrolling 68 patients when an interim analysis revealed a lower recovery rate in the HBO group (19.3%) compared with the placebo group (32.4%).

- **Controversy in the Literature**
- Today, the widespread use of HBO therapy for ORN treatment appears to be based on personal beliefs and experience rather than convincing scientific evidence.
- No consensus on its efficacy exists in the literature, which consists mainly of poorly controlled trials and cohort studies.
- The only available randomized controlled study (without a placebo group), conducted by Marx, demonstrates the benefit of HBO therapy over antibiotic therapy in the prevention of ORN following dental extraction.
- These results contrast with those of Annane and colleagues, which showed a negative effect of HBO therapy in the treatment of ORN.
- However, the patients enrolled in this study received HBO or placebo twice a day, which differs from the usual 1 session a day protocol.
- Overall, both studies dealt with relatively small cohorts (about 30 patients) and neither took into consideration the previous dental condition of the patient or the severity of the ORN, resulting in a low level of evidence.
- With these conflicting studies, it is, thus, not possible to draw conclusions on the efficacy of HBO therapy in the prevention and treatment of ORN.
- In recent years, various substances have been tested as alternative treatments for ORN, namely pentoxifylline (a peripheral vasodilator), vitamin E and clodronate (a bisphosphonate).
- These treatments are based on different pathophysiological theories of ORN: osteoclast suppression or fibro-atrophic process.
- The fact that these approaches are producing positive results raises doubts about the veracity of the theory behind HBO treatment and, thus, the efficacy of HBO treatment itself.

4.6: Muscles of Mastication

By: Dr. Rohit

- Trismus can be a significant side effect of RT, especially if the lateral pterygoid muscles are in the field.
- In a study, patients in whom the pterygoid muscles were irradiated and not the temporomandibular joint, 31 percent experienced trismus.
- In addition, radiation to the TMJ also was associated with a decrease in maximum vertical opening.
- The mechanisms by which mandibular hypomobility due to the radiotherapy devel ops, and the factors which determine speed of onset, severity and extent, are **poorly understood**.
- Its development is thought to progress in **three phases**:

1. An initial nonspecific inflammatory phase,

2. A fibrotic cellular phase, and

3. A matrix densification and remodeling phase.

- It is generally viewed to be the result of fibrosis leading to a loss of flexibility and ex tension.
- Limited mouth opening can interfere with proper oral hygiene and dental treatment.
- Therefore, before RT starts, patients who are at risk for developing trismus should receive **instruction in jaw exercises** that will help them maintain maximum mouth opening and jaw mobility.
- The dentist should measure the patient's maximum mouth opening and lateral movements before RT, and reevaluate mandibular opening and function at follow-up dental visits.
- For patients who experience reduced mouth opening, the intensity and frequency of the exercises should increase, and a physical therapy regimen prescribed.
- Tongue blades can be used to gradually increase the mandibular opening.
- Dynamic bite opening appliances have also been used.

4.7: Hematopoietic Injury

Dr. Kumari Sonam Jha

- leukopenia,
- leukemia,
- anemia,
- lymphopenia and loss of specific immune response.

4.8: Eyes

Dr. Kumari Sonam Jha

- Epilation of eyelashes
- Inflammation, fibrosis and decreased flexibility of the eyelid
- Damage to the lacrimal glands, leading to dryness
- Ulceration of the cornea
- Initiation of cataract formation from the periphery towards the center

4.9: Ears

By: Dr. Abhishek Gupta

- Columnar epithelium of the middle ear may be desquamated.
- Edema of the mucosa and collection of sterile fluid in the middle ear, which leads to obstruction of the eustachian tube – Radiation Otitis Media
- Deafness due to rupture of the eardrums

4.10: Testicles & Ovary

Dr. Rohit

- Suppression of germinal activity
- Alteration in fertility
- Functional changes in the offspring may be seen.

4.11: Radiation Effect on Embryos and Fetuses:

By: Dr. Abhishek Gupta

- considerably more radio-sensitive
- less than 0.25 mGy from a full mouth examination.
- most sensitive period: period of organogenesis (18-45 days)
- effects are deterministic in nature.
- > 50 days after conception does not cause gross malformations.
- general retardation of growth may persist through life.
- increased risk for childhood cancer, (leukemia and solid tumors), after irradiation in utero

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