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*Review Article*

# An Insight to Apoptosis

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**Abstract**

Apoptosis is considered as a tightly regulated active process signified by specific morphological and biochemical. On contrary to apoptosis, necrosis is a passive, energy independent pathologic process. The significance of understanding the apoptosis cascade mechanism is imperative as apoptosis being component of both physiological and pathological process. Apoptosis can be stimulated by both physiological and pathological conditions and hence play a role in maintenance of normal homeostasis and in pathogenesis of several diseases. Signaling for apoptosis occurs via caspase dependent and independent pathways that are initiated either from triggering events within the cell or from outside the cell by ligation of death receptors. Present review aims to provide an overview regarding apoptosis, its morphological and biochemical characterstics, its mechanism and its implication in health and diseases.

**Keywords:** Programmed cell death, Necrosis, Caspases, TUNEL, Phosphatidylserine.

## Introduction

The word "apoptosis" is derived from the ancient Greek word meaning the falling off of petals from a flower or of leaves from a tree in autumn. The term was first introduced by Kerr, Wyllie and Currie in 1972, to describe the morphological processes leading to controlled cellular self-destruction. As apoptosis was introduced as a term describing a specific morphology of cell death, it should not be used synonymously with the term "programmed cell death (PCD)", which usually occurs via apoptosis. (Lawson A 2003) The apoptotic mode of cell death plays an important role in the development, regulation and maintenance of the cell populations in both physiological and pathological conditions.

## How Apoptosis is Different from Necrosis?

In apoptosis, cell is an active participant in its own demise. This type of cell death is controlled, energy dependent and can affect individual or cluster of cells. In

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contrast, "Necrosis" is considered to be toxic process where the cell is a passive victim, follows an energy-independent mode of death and usually affects large field of cells. (Elmore S 2000) Although apoptosis and necrosis differ in their mechanism, there are evidences indicating that they both represent morphologic expression of shared biochemical network known as "Apoptosis-Necrosis Continum". (Zeiss CJ 2003).

**Table 1: Showing Difference between Apoptosis and Necrosis**
(Elmore S 2000, Roche] (Modified)

| **APOPTOSIS** | **NECROSIS** |
|---|---|
| **PHYSIOLOGICAL ASPECT** | |
| 1. Affect individual cell or cluster of cells. | Affect often contiguous cells. |
| 2. Evoked by physiologic as well as pathologic stimuli. | Induced by non-physiologic disturbances (hypoxia, toxins, viruses ischemia etc). |
| 3. Phagocytosis by macrophages or other cells (parenchymal or neoplastic). | Phagocytosis by macrophages only. |
| 4. Not associated with inflammation. | Associated with significant inflammatory response. |
| **MORPHOLOGICAL ASPECT** | |
| 5. Integrity preserved with blebbing of intact plasma membrane. | Loss of membrane integrity. |
| 6. Cell shrinkage and convolutions with intact organelles. | Cell swelling with disruption of organelles. |
| 7. Pyknosis and karyohexis. | Karyolysis, pyknosis and karyohexis. |
| 8. Formation of membrane bound vesicles (apoptotic bodies). | No vesicle formation, completelysis. |
| 9. Cytoplasm with organelles retained in apoptotic bodies. | Cytoplasm released. |
| **BIOCHEMICAL ASPECT** | |
| Energy dependent process (ATP required). | Energy independent process (ATP not required). |
| Transient loss of mitochondrial membrane potential. | Permanent loss of mitochondrial membrane potential. |
| Caspases dependent. | Caspases independent. |
| pH of the cell is acidic. | pH of the cell is unchanged. |
| Exteriorization of phosphatidylserine (PD) from inner to outer leaflet of plasma membrane. | Unchanged. |
| Non-random mono and oligonucleosomal length fragment of DNA(ladder pattern after agarose gel electrophoresis). | Random digestion of DNA (smear of DNA after agarose gel electrophoresis). |
| Prelytic DNA fragmentation. | Postlytic DNA fragmentation. |

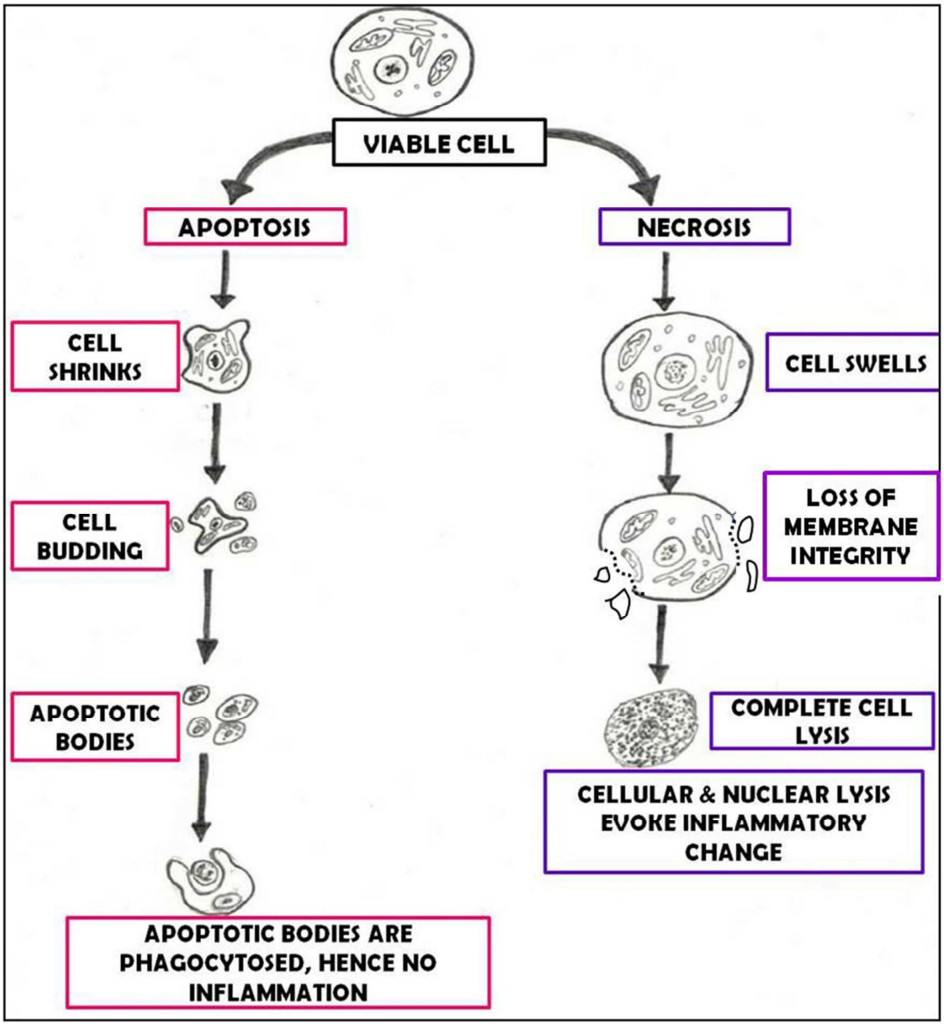


**Figure 1: Showing Difference between Apoptosis and Necrosis**

**Apoptosis in Biological Process-** Apoptosis plays dual role in biological process:

***I. Physiologic apoptosis*** is implicated in the following : (Singh N 2007, Rastogi R et al 2009).

1. Embryogenesis including implantation and organogenesis.

2. Development and involution of various organ system eg: Nervous system, Immune System and Regression of mullerian and wolfian duct.

3. Initiation of menstruation in adult female.

4. Autoregulatory mechanism of intestine eg: maintainence of iron absorption.

5. Normal placental development.

6. Production of erythrocytes via erthropoiesis.

7. Various infections eg: certain types of viral hepatitis, where apoptosis is needed to destroy pathogen invaded cells.

8. In wound healing, apoptosis is involved in removal of inflammatory cells and evolution of granulation tissue into scar tissue.

9. Remodelling of adult tissue eg: Follicular atresia of post-ovulatory follicle, post-weaning mammary gland involution.

10. Elimination of activated or autoregressive immune cells either due to maturation in central lymphoid organs (bone marrow or thymus) or in peripheral tissue.

*11. Age induced apoptosis:*

With the process of ageing, some of the cells die more rapidly as a result of apoptosis. This is because of generated oxidative stress as a consequence of accumulated free radical damage to mitochondrial DNA and hence known as age induced apoptosis. (Elmore S 2000, Harman D 1992).

*12. Apoptosis in oral tissues:*

During oral embryogenesis, regulation of delicate balance between cell death and cell survival and epithelial-mesenchymal interaction play an essential role in determining which cells to be shed and which one to survive. Epithelial cells require contact with each other for survival signals. Detachment of epithelial cells from neighbouring cells triggers apoptosis. This type of apoptosis induced by loss of adhesion of cells known as "Anoikis". (Loro LL et al 2005).

***II.*** *Pathologic apoptosis* involves: (Singh N 2007, Rastogi R et al 2009, Loro LL et al 2005).

1. *Inadequate apoptosis/Downregulated:* includes-

- Cancer (Oral, colorectal, Hepatic, Prostrate, Leukemia, Neuroblastoma).

- Autoimmune disorder (SLE, Myasthenia gravis, Autoimmune proliferative syndrome, Sjogren syndrome).

- Vesiculo-bullous lesion.

- Reactive oral lesions.

- Restinosis.

- Infections (viral infections).

2. *Extreme Apoptosis/Upregulateds:* includes-

- Neurodegenerative disorder: Alzheimers diseases, Parkinsonism, Hutingtons chorea, Stroke, Brain truma, Spinal cord injury, Amylotropic lateral sclerosis, Retinitis pigmentosa, Epilepsy etc.

- Cardiovascular disorders: Heart failure, Myocardial infarction, Stroke.

- Hematologic disorders: Aplastic anemia, Myelodysplatic syndrome, T CD4 + lymphocytopenia.

- Others: Inflammation, Sepsis, Diabetes, Alopecia, AIDS, Polycystic ovarian diseases.

**Stages of Apoptosis**

The process of apoptosis is divided into 3 process:

*I.* ***Phase of Initiation****:* In this phase, cell receives various intra and intercellular signals via intrinsic and extrinsic pathway resulting in apoptotic death sequence.

Apoptotic inductors can be physiological (hormones, cytokines etc), biological (bacteria, viruses, parasites etc), chemical (medication) or physical (radiations, toxins etc). In addition, one stimulus can generate different and even opposing effects in different cell types and in different development stages of same cell type. (Torres L et al 2003, Thompson CB, 1995).

*II.* ***Phase of Execution****:* Once the cell has received the signal to induce apoptosis, it loses contact with neighbouring cells and undergoes characteristic morphologic and biochemical alterations. (Torres L et al 2003).

*III.* ***Phase of Elimination****:* In this phase, remnants of cells undergoing apoptosis are quickly and efficiently eliminated by professional phagocytes or non-professional phagocytes such as dendritic

cells, epithelial cells and fibroblasts therefore not associated with inflammation. (Torres L et al 2003, Gregory CD 2000).

**Morphological Alterations in Apoptosis**

1) *Nuclear alterations:* Morphological hallmark of apoptosis in nucleus are chromatin condensation & nuclear fragmentation which can be seen with light microscopy, electron microscopy, fluorescence microscopy and also by using labelled dUTP by the enzyme terminal deoxynucleotide transferase nick end (TUNEL) method. Structural protein processed by caspases associated with apoptotic morphology includes actin, spectrin, gelsolin, β-catenin, Laminin A & B, Keratin 18 & 19 etc. (Chamond RR et al 1999, Saraste A et al 2000, Ziegler U et al 2004).

2) *Alteration in Cell membrane & Cytosol:* Initially during apoptosis, cell detaches from its substratum & adjacent cells. Membrane & organelles are well preserved. Subsequently cells start to show extensions or protrubrences of plasma membrane commonly referred to as blebs. Following cell shrinkage, these blebs separate forming apoptotic bodies, which are round, smooth membrane bound remnants densely packed with organelles & nuclear fragments. (Elmore S 2000, Chamond RR et al 1999, Saraste A et al 2000, Ziegler U et al 2004) Apoptotic bodies are rapidly phagocytosed by neighbouring cells including macrophages & parenchymal cells hence there is essentially no inflammatory reaction associated with process of apoptosis.

3) *Mitochondrial alterations:* Mitochondrial membrane permeabilisation has a central role during apoptosis degradation cascade. Proapoptotic members of Bcl-2 family are involved in initiation of mitochondrial membrane permeabilisation where as antiapoptotic factors of Bcl-2 family inhibits this process. Moreover there is loss of transmembrane potential. (Chamond RR et al 1999, Ziegler U et al 2004).

**Microscopy of Apoptotic Cells:** In light microscopy with hematoxylin and eosin stain, an apoptotic cell appears as a small round or oval mass, having dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments.

Ultrastructurally, an apoptotic cell will show characterstic peripheral aggregation of electron-dense nuclear material but there can also be uniformly dense nuclei. (Gobe G et al 2008).

**Biochemical Alterations in Apoptosis:**

1. ***Internucleosomal DNA Fragmentation****:* It is a characterstic form of DNA degradation which occurs by activation of endogenous DNAase in which genome is cleaved at nucleosomal sites, generating a "ladder" of DNA fragments when analysed by agarose gel electrophoresis and by TUNEL assay. (Saraste A et al 2000, Ziegler U et al 2004, Compton MM 1992, Nagata S. 2000).

2. ***Cytoplasmic Acidification****:* Acidification as a concomitant of cell death was first reported by Nedergard in case of neuronal injury and subsequently by Burry and Eastman who recognised acidification to be a feature of apoptosis. (Gottlieb RA. 1996) It has been suggested that a drop of cytoplasmic pH will have a profound effect on activity of enzyme like DNAase II, protease, transglutaminase and sphingomyelinase. (Barry MA et al 1993, Meisenholder GW et al 1996, Melino G et al 1994, Kanfer JN et al 1994).

3. ***Externalisation of Phosphatidylserine (PD)****:* In normal cells, PD is present on inner leaflet of plasma membrane but in apoptotic cell, this phospholipid "flips" out and is exposed on the outer layer membrane, where it is recognised by macrophages. (Ziegler U et al 2004, Savill J 2000).

4. ***Other Biochemical Feature Includes****:* Caspases interaction, Loss of mitochondrial membrane potential, Increase of free ionic calcium, Proteolysis of laminin B. (Chamond RR et al 1999, Saraste A et al 2000, Ziegler U et al 2004).

**Apoptosis Signalling Cascade**

***Major Players in Apoptosis Includes:***

*1)* ***CASPASES****:* The term caspases is derived from cysteine-dependent aspartate-specific proteases. The caspases are family of cysteine proteases homologous to C. elegans ced-3 and are of central importance in the apoptotic signalling network which are activated in most cases of apoptotic cell death. (Lawson A 2003).

*Classification of Caspases:* Depending upon the length of amino terminal prodomain. (Rastogi R et al 2009).

*Initiator (Apical):* Caspase-2, Caspase-8, Caspase-9, Caspase-10.

*Effector (Executioner):* Caspase-3, Caspase-6, Caspase-7.

*2)* ***Bcl-2 Family Proteins***: The member of Bcl-2 family are a group of crucial regulatory factors in apoptosis. Bcl-2 proteins are characterised by presence of conserved sequence motifs known as Bcl-2 homology (BH) domains. 4 domains have been described- BH1, BH2, BH3, BH4. [Singh N 2007, Strasser A et al 2000, Fan JJ et al 2005, Paula C et al 2003).

**Table 2: Showing Classification of Bcl-2 Family Proteins: Depending upon their Function Domain Present** (Fan JJ et al 2005, Paula C et al 2003)

| **GROUP I PROTEINS**- | **GROUP II PROTEINS** |
|---|---|
| Comprised of **antiapoptotic proteins** including A1/Bfl1, Bcl-2, Bcl-w, Bcl-xL, Boo/Diva, Mcl-1, NR-13 and Nrf3 in mammals, BHRF-1, E1B19K, Ks-Bcl-2, LMW5-HL and ORF16 in bacteria, and Ced-9 in *C. elegans* They all have **four Bcl-2 homology (BH) domains**: BH1, BH2, BH3 and BH4. | Comprised of **proapoptotic proteins**. Among these, 2 subfamilies have been identified.<br>**Bax subfamily:** Possesses BHI, BH2, BH3 domains but lack BH4 domain. consists of Bax, Bak, and Bok.<br>**BH3 only proteins:** Possesses BH3 domain only and lack other domains. Consist of Bid, Bim, Bik, Bad, Bmf, Hrk, Noxa, Puma, Blk, BNIP3, and Spike. |

*3)* ***Tumor Necrosis Factor Receptor Superfamily****:* Extrinsic apoptosis signalling is mediated by the activation of so called "death receptors" (DRs). These cell surface DRs belongs to Tumor necrosis factor receptor (TNFR) superfamily. They transmit their apoptotic signals following binding with specific ligands. (Lawson A 2003, Strasser A et al 2000) The best characterised family member in humans are- TNF-R1, Fas (Apo1 or CD95), DR-3 (Apo3, WSL-1, TRAMP or LARD), DR-4/ TRAIL-R1, DR-5 (TRAIL-R2, Apo-2, TRICK2 or KILLER), DR-6 and NGF-R. (Paula C et al 2003, French LE et al 2003).

*4)* ***Adaptor Molecules****:* Adaptor proteins are the link between cell death initiator i.e caspases and the cell death regulator i.e death receptors and Bcl-2 family members. These links take form physical association between TNFR family members on one side and caspases on other allowing caspases aggregation and activation. Examples of adaptor proteins includes: Fas-associating death domain protein/mediator of receptor-induced toxicity (FADD/MORT1), TNF-R1-associated death domain protein (TRADD), and receptor-interacting protein (RIP). (Strasser A et al 2000).

**Molecular Mechanism of Apoptosis-** Apoptosis is a tightly regulated and highly efficient cell death program which requires interplay of multitude of factors.

***Types of Apoptotic Mechanism:***

1. Via stimuli/signals arising outside or inside the cell-

- Extrinsic (Death receptor activated) or Intrinsic (mitochondria activated).

- Caspases dependent or Caspases independent.

- p53 dependent or p53 independent.

2. Via reactive oxygen species (ROS).

Apoptosis may be activated by variety of stimuli including Ionising radiations anticancer drug, Heat, ultraviolet light, oxygen free radicals, hydrogen peroxide etc. (Kam P et al 2000).

***Extrinsic/ Death Receptor Activated Pathway*** - Involves the initiation of apoptosis through ligation of plasma membrane death receptors therefore also known as Death receptor pathway resulting in the recruitment of adaptor proteins. Caspase 8, 10, 2 are the caspases involved in the death receptor pathway which needs to be activated. (Fan JJ et al 2005, Paula C et al 2003) Types of signalling involved in extrinsic apoptosis are Fas L Signalling, TNF Signalling, Apo 3L and Apo 6L Signalling & TRAIL Signalling. (Paula C et al 2003).

***Intrinsic/ Mitochondrial Cell Death Pathway*** – Also known as Mitochondrial cell death pathway since apoptosis occurs secondary to imbalance in intracellular homeostasis. (Fan JJ et al 2005, Paula C et al 2003). Following an apoptotic trigger, several apoptogenic proteins are released from mitochondria. (Hail N et al 2006) Chief molecule involved in execution of this pathway is cytochrome c which is is released into cytoplasm and interacts with apoptotic proteases activating factor-1 (Apaf-1), dATP/ATP and procaspase-9 resulting in formation of massive complex known as apotosome. Other mitochondrial proapoptotic factor are Second mitochondrial activator of caspases/ Direct IAP binding protein of low isoelectric point (SMAC/DIABLO) and apoptosis inducing factor (AIF). (Lawson A 2003, Paula C et al 2003).

Endoplasmic reticulum (ER) has also been implicated in process of apoptosis. The characterstic mediator of apoptosis under ER stress is caspase 12. ER stress is particularly caused by accumulation of unfolded and misfolded proteins in ER lumen and/or the perturbation of calcium ion homeostasis. (Fan JJ et al 2005, Morishima N et al 2002).

***Common Pathway****:* Once activated either via extrinsic or intrinsic pathway, apoptosis activator caspase such as caspase 2, 8, 9, 10 will activate other downstream apoptosis executioner caspases including caspase 3, 6, 7 which will further activate other proteins. (Fan JJ et al 2005).

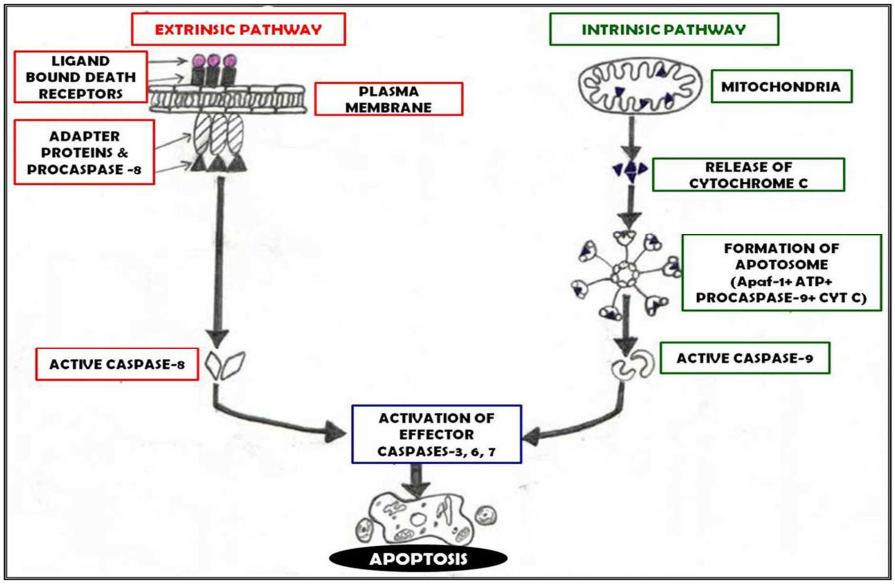


**Figure 2: Showing Pathways of Apoptosis Mechanism**

**Non-Caspase Dependent Mechanisms** (Hail N et al 2006).

1) *Via Non-caspase proteases* like cathepsins, calpain and granzyme and Omi/HtrA2**.**

2) *Via ex-mitochondrial proteins* like Apoptosis Inducing Factor (AIF), Mitochondrial Endonuclease G, WOX1/WWOX/FOR, AIF Homologous Mitochondrian Associated Inducer of Death (AMID) and Cytochrome c.

**Apoptosis Induction via Reactive Oxygen Species (ROS)**

ROS and mitochondria play an important role in apoptosis under both physiologic and pathologic conditions. ROS includes free radical species such as hydroxyl (OH), alkoxyl (RO) or peroxyl (ROO-) superoxide (O2-) or nitroxyl radical ( NO-) and non-radical hydrogen peroxide (H2O2), organic hydroperoxides (ROOH) and hypocholorous acid (HOCL). (Simon HU et al 2000) ROS are generated by inflammatory cells which accumulate in both allergic (French LE et al 2003, Simon HU 1997) and non allergic (Simon HU 2000, Dibbert B et al 1999) inflammation. ROS can induce apoptosis in many different cell system. For eg: H2O2 induced apoptosis in neutrophil which can be prevented by catalase. (Kasahara Y et al 1997) ROS can induce apoptosis either via Death receptor mediated apoptosis or through mitochondria, Endoplasmic reticulum and calcium. (Hail N et al 2006, Simon HU et al 2000).

**Regulation of Apoptosis**

Apoptosis is a controlled genetically programmed event which gets activated when a cell encounters a specific death inducing signal or death stimulus. All cells of a multicellular animal might undergo systematic self destruction unless cell death is constantly inhibited by survival signals as provided by other cells e.g. growth factors, hormones, nutrients. These survival signals control apoptosis by maintaining an equilibrium between pro-apoptotic and antiapoptotic regulatory molecules. Certain molecules, acting as modulators of apoptosis are- Bcl-2 Family, Voltage Dependent Anion Channel (VDAC)/Porins, Adenine Nucleotide Translocator (ANT), Inhibitor of Apoptotic Proteins (IAP), FLIP Proteins, Cytotoxic T-Cells, PI-3 Kinase Pathway. (Brunelle JK et al 2002, Chen M et al 2002, Mayer B et al 2003, Boise LH et al 1997).

**Clinical Implication of Apoptosis in Oral Health and Diseases:**

Proper function of the apoptotic machinery is of fundamental importance during the growth and development of the organism, because apoptosis in accord with cell division ensures the proper shaping and the structural and functional integrity of the various tissues and organs. (Nikitakis N G et al 2004, Matalova E et al 2004) During oral embryogenesis, regulation of the delicate balance between cell death and cell survival and epithelial-mesenchymal interaction play an essential role in determining which cell to be shed and which one to survive. Selected cells are destined to die by apoptosis termed as PCD (Programmed cell death) (Loro LL et al 2005).

Apoptosis plays an important role during tooth morphogenesis. Teeth are example of epithelial mesenchymal organs and are often used as model for studying the nature of such interactions and signalling controlling morphogenesis, histogenesis and cytodifferentiation. (Kim JY et al 2006, Lesot H et al 1996, Vaahtokari A et al 1996) During tooth development apoptosis found to occur in dental lamina, enamel organ, enamel knot, ameloblasts and odontoblasts and periodontal tissues. (Matalova E et al 2004, Lucas H et al 2010).

A dysfunctional apoptotic system can lead to either excessive removal or prolonged survival of cells. Therefore, dysregulation of apoptosis is involved in the pathogenesis of a variety of diseases like reactive oral lesions, (Jafarzadeh H et al 2006) recurrent apthous ulceration, (Honma T et al 1985) periodontal lesions, (Jarnbring F et al 2002) mucocutaneous lesions (erythema multiforme, lupus erythematosus, pemphigus vulgaris, epidermolysis bullosa, lichen planus,) (Chrysomali E et al 1997, Vaishnaw AK et al 1999, Puviani M et al 2003, Yoneda K 2001, Murrah VA et al 2006) viral infections, (Chamond RR et al 1999) candidal infection, (Villar CC et al 2010, Rouabhia M et al 2012) sjogren syndrome, (Manganelli P et al 2003) salivary gland tumors, (Jia L et al 2004) odontogenic cysts, (Artese L et al 2008) odontogenic tumors, (Kumamoto H et al 2005) leukoplakia (Tanda N et al 2000) and Oral squamous cell carcinoma. (Kaufmann SH et al 2000).

**Conclusion**

Apoptosis is regarded as a vigilantly regulated active process, being initiated by various physiologic and pathologic stimuli, characterized by specific morphological and biochemical alterations mediated by several molecules and regulated by balance between proapoptotic and antiapoptotic signals. The importance of understanding the mechanism of apoptosis is crucial because it has got a vital role in both health and disease. Moreover, the pervasive association of apoptosis in the pathobiology of disease lends itself to therapeutic intervention at many different checkpoints.

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