International Journal of Dentistry and Oral Science (IJDOS)
ISSN: 2377-8075

Role Of Apoptotic Marker Caspase 3 In Periodontal Disease

Review Article

Arya. M. Loka[1*], Deepa Ponnaiyan[2], Harinath. P[3]

[1] Post graduate Student, Department of Periodontics, SRM Dental College, Ramapuram,Chennai, India.
[2] Reader, Department of Periodontics, SRM Dental College, Ramapuram, Chennai, India.
[3] Professor, Department of Periodontics, SRM Dental College, Ramapuram, Chennai, India.

Abstract

Periodontal diseases are characterized by the inflammation and destruction of supporting tissues of the teeth. Periodontitis is initiated by bacterial accumulation between the gingiva and teeth, which triggers an inflammatory-immune response within the host. In susceptible individuals, the initial acute inflammatory response fails to resolve, and a dysregulated chronic inflammation ensues, which destroys the supporting connective tissues surrounding the teeth. Caspases are cysteine protease enzymes that are commonly involved in apoptosis, pyroptosis, inflammation, systemic diseases, and as well as in periodontal diseases. Caspase 3 is a cysteine protease enzyme that plays a major role in periodontal disease progression. Emerging evidence now suggests a strong relationship between the extent and severity of periodontal disease and caspase 3 activation. In this review, the mechanisms of caspase activation and influence of caspase 3 and its corresponding CASP 3 gene in periodontal disease has been described in detail.CASP3 gene polymorphism in periodontitis has also been highlighted.

Keywords: Apoptosis; Caspase-3; Chronicperiodontitis; Gene Expression; CASP 3 Gene.

Introduction

Caspases are cysteine protease enzymes they play a vital role in many processes like inflammation, programmed cell death (apoptosis), necroptosis, pyroptosis [1]. Depending on function caspases are subcategorized into initiator caspases and executioner caspases and inflammatory caspases [2, 3]. Initiator caspases, (caspase-2,8 &-9) initiates the apoptosis signal while the executioner caspases (caspases 3,6&7) degrade into cellular components in order to induce the morphological changes [4] and inflammatory caspases (caspases 1, 4, 5, 11, 12, 13, and 14) are involved in inflammatory cytokine signalling [5]. In inflammatory conditions like gingivitis and periodontitis, oral micro-organisms induce apoptosis via activation of caspases. Evidence suggests that toxins released by keystone pathogens as well as other bacterial factors directly activates caspases leading to apoptosis [6-8]. Galluzi L et al concluded that till date 14 caspases were discovered, out of which 12 caspases were found in humans [9].

However, caspase 12 is found only in few humans. Caspases are activated either by homoactivation (requires recruitment of the zymogens to the adapter proteins) or by heteroactivation (requires action of another protease upon the caspase zymogen) [10]. These activated caspases are involved in apoptosis in two pathways commonly known as intrinsic and extrinsic pathways.

Caspase 3 is activated by both intrinsic and extrinsic pathway. In periodontitis, binding of Fas with FasL allows recruitment of Fas-associated death domain (FADD) and it leads to activation of the extrinsic pathway. Since caspase 3 is activated by both intrinsic and extrinsic pathway, caspase 3 is most commonly involved in periodontal disease progression than other caspases. Apart from inflammatory conditions like periodontitis and gingivitis, literature evidence also reveals that caspase 3 plays a role in orthodontic movements [11, 12] and as well as in premalignant conditions like oral submucous fibrosis and squamous cell carcinoma [13].

The levels of caspases have been studied in GCF and tissue sam-

***Corresponding Author**:
Dr. Arya Mitra Loka,
Post graduate Student, Department of Periodontics, SRM Dental College, Ramapuram, Chennai, India.
Tel: +918349602936
Email ID: aryamds369@gmail.com

Received: March 02, 2021
Accepted: April 03, 2021
Published: April 06, 2021

Citation: Arya. M. Loka, Deepa Ponnaiyan, Harinath. P. Role Of Apoptotic Marker Caspase 3 In Periodontal Disease. *Int J Dentistry Oral Sci.* 2021;08(04):2220-2223.
doi: http://dx.doi.org/10.19070/2377-8075-21000439

ples and observed that caspases levels are increased in periodontitis subjects compared to healthy subjects. Numerous studies have also concluded that hyperglycaemic status elevates caspases activity, levels and as well as caspases gene levels in periodontitis [14, 15]. Thus, the role of Caspase 3 as a biomarker in periodontal diseases is established.

This literature review explains about caspase 3 and its role in periodontal diseases like gingivitis, periodontitis, apical periodontitis etc, as well as the influence of oral microbial organisms on caspase 3 and gene expression in periodontal disease conditions.

Caspase 3

Galluzi L et al concluded that till date 14 caspases were discovered, out of which 12 caspases were found in humans 41 (as shown in Fig1).

Caspase 3 protein is encoded by the CASP3 gene. Caspase3 is active even under normal cellular conditions due to its basic nature. Since, caspase 3 is activated by both intrinsic and extrinsic pathway of apoptosis it is considered an executor enzyme (Fig 2). Caspase 3 is inhibited through the IAP (inhibitor of apoptosis) with protiens like c-IAP1, c-IAP2, XIAP, and ML-IAP [16].

Caspase 3 in periodontitis

Extrinsic pathway is most relevant in periodontitis.Fas receptor and Fas ligand (FasL) play an important role in activation of caspases in periodontitis. Fas is a protein encoded by Fas gene and it is also called as CD95. Fas receptors are present of gingival fibroblasts [17, 18]. Binding of Fas with Fas ligand (FasL) allows the recruitment of Fas-associated death domain (FADD) and leads to activation of death-inducing signalling complex (DISC) and this DISC activates caspases like 3, 6 and 7 leading to apoptosis of gingival cells.

Several oral microorganisms activate caspases either directly or indirectly resulting in periodontitis. For example: - A.actinomytemcomitans induces apoptosis in epithelial cells through leukotoxin [19, 20], P.gingivalis induces apoptosis in T-cells and fibroblasts through gingipains [21, 23]. Messina et al [24] observed that upregulated Fas and FasL levels in A.actinomycetemcomitans induced periodontitis rats.

Based on above literature evidence it can be concluded that oral keystone pathogens alter immune response and enhances caspase activity in periodontitis.

Caspase 3 protein in GCF, saliva and serum

Caspase 3 expression increases with progression of gingival inflammation, probing depth and clinical attachment level in chronic periodontitis. Pradeep A R et al 2016 observed serum and GCF levels of caspase 3 in chronic periodontitis subjects and healthy subjects and observed that caspase 3 levels were higher in patients with periodontitis and correlated with clinical periodontal parameters compared to healthy individuals [25]. Aral K et al in 2018 studied GCF levels of p53, caspase3, TNFα, TRAIL, IL1β, and IL10 in subjects with GAgP before and after non-surgical peri-

Figure 1. Shows different types of caspases.

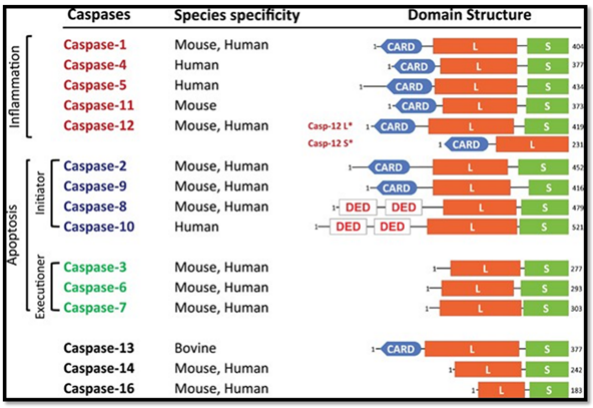
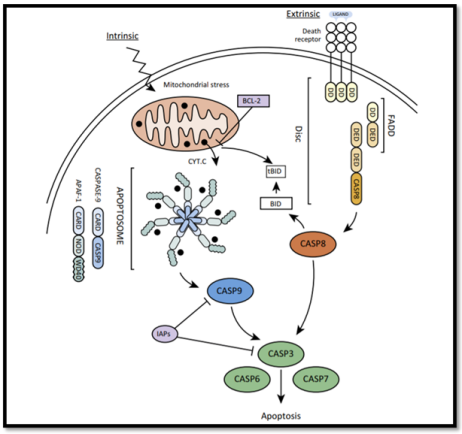


Figure 2. Shows caspase 3 is activated by both intrinsic and extrinsic pathways.



odontal treatment, ELISA analysis results showed that TRAIL, TNFα, and IL10 were similar at baseline and 3 and 6 months but caspase3 and IL1β levels were decreased at 3 months [26].

Caspase 3 in human gingival tissue

Immunohistochemistry studies have revealed that increased expression of caspase 3 could be involved in the inflammatory process associated with gingival tissue destruction in patients with periodontitis.

In an immunohistochemistry analysis, Das et al 2009 [27] observed intrinsic and extrinsic pro-apoptotic markers in inflamed human gingival samples and also observed that, increased cytochrome c and caspase-3 levels were detected in periodontal disease groups when compared to healthy group.

In a human gingival tissue immunohistochemistry analysis done by Bantel H et al., 2005 [28] observed that expression of apoptosis associated genes increased when exposed to pathogenic periodontal bacteria and expression of caspase 3 is increased in subjects with periodontitis than healthy subjects. In an immunohistochemistry study published by Lucas et al, 2010 [29] concludedthatapoptosis in periodontitis may be inhibited by elevated expression of TRAIL decoy receptors and cleaved caspase-3 inhibitors. Gomonal et al 2001 [30] studied apoptotic events in the gingival tissue of chronic adult periodontitis patients. After electron microscopic and immunohistochemistry analysis they observed positive staining for active caspase-3, Fas, Fas L, BCL2 and p53 is more in chronic periodontitis group when compared to control group.

Based on the above studies it can be concluded that caspase 3 is involved in periodontitis.

Influence of periodontitis on CASP3 gene

Caspase 3 gene is significant proapoptotic gene which has been observed consistently upregulated in gingival tissue sites.

Gonzalez et al 2011 studied 88 genes related to apoptotic pathway in gingival biopsies of healthy and periodontitis sites from non-human primates and comparison between healthy and periodontitis gingival tissues showed that the up or down regulated apoptotic genes in diseased gingival tissue are different in adults compared with aged animals. Furthermore, they observed that caspase 3 gene is 1.5 fold highly expressed in periodontitis compared to healthy, suggesting a potential role of caspase 3 in pathogenesis of periodontal disease [31].

Gene polymorphism i.e. variation at a single nitrogen base pair in gene, also plays a role in periodontitis disease progression by upregulatingcaspase levels even in systematically healthy subjects. Kang et al 2015 [32] recently, in a Korean population observed polymorphism in caspase 3 gene by assessing 201 SNP's of CASP3, out of which only 3 genotypes that is rs12108497, rs4647602 and rs113420705 were significantly associated with chronic periodontitis.

Considering the above data, it has been established that association of caspase 3 in chronic periodontitis however the link between periodontitis and CASP 3 gene polymorphism needs further research in larger samples and different populations. Several periodontopathogenic microorganisms and their products act on the cellular constituents of the gingival tissues and activates cellular processes that induce the destruction of the connective tissue as well as bone, for example A. actinomycetemcomitans expresses virulent factors which helps in formation of inflammasomes and these inflammasomes activates caspases like caspase 1 which leads to formation of inflammatory cytokines mainly interleukins like IL-1 and IL-18 [33].

Influence of Diabetes mellitus on caspases and its influence on periodontitis

In systemic diseases like diabetes mellitus, caspases are activated in other pathways as well like TNF pathway, polyol pathway, reactive oxygen species (ROS) etc. Graves et al 2006evaluated periodontal disease pathogenesis in diabetic patients and concluded that diabetes activates several pathways and activates caspases leading to apoptosis stimulation [34, 35]. Alikhani et al concluded that, diabetes mellitus activates polyol pathway and leads to elevation of Advanced Glycation End Products (AGEs). In periodontitis, AGEs induce fibroblast apoptosis by activating caspase 8,9 as well as caspase 3 [36].Joussen et al concluded that diabetes mellitus enhances TNF and Fas/FasL as well as caspase levels and activates extrinsic pathway of apoptosis [37] and similarly. Hesham et al in 2006 observed expression of Fas mRNA levels in diabetic periodontitis mice and also observed that inhibiting apoptosis improved healing and increased mRNA levels of collagen I and III in diabetic mice [38]. Yousef M et al 2016 evaluated caspase 3 activity in type II DM controls with and without periodontitis, after ELISA analysis concluded that caspase-3 play a role as a biomarker in periodontitis [39]. Manosudprasit et al 2017evaluated caspase-3, -8, and -9 levels of PMN in patients with chronic periodontitis and type 2 Diabetes mellitus (T2DM). After TUNEL, Annexin V assay and colorimetric assays they observed caspase-3 was significantly higher in T2DM patients with and without CP compared to healthy controls [40].

Discussion

Caspase-3 is activated by several other caspases so it is considered as executioner enzyme.

Above literature studies concluded that caspase3 levels were increased in GCF, saliva, serum and tissue samples are positively correlated with clinical parameters like probing depth, clinical attachment loss and caspases concentrations were increased proportionally with the severity of periodontal disease. CASP genes levels were upregulated in periodontal diseases. Caspases such as caspase 3 can be activated in several aspects, in addition to inflammatory conditions such as keystone pathogens, premalignant conditions, systemic diseases and age. Systemic diseases like diabetes mellitus also influences caspase3 levels, studies published by Graves DT et al [34, 35] concluded that diabetes mellitus influences caspases like caspase 3 and involves in periodontal disease progression. Yousef MK et al [39] concluded that caspase 3 can play as role of biomarker in chronic periodontitis. Apart from gingivitis and periodontitis, caspase 3 also plays a role in premalignant conditions.

However further studies in relation to caspase3 with other factors

such as smoking, stress etc in periodontal diseases are less. Further, longitudinal studies involving larger population, meta-analysis are needed and also genetic studies like gene polymorphisms of CASP3 gene are much required for better understanding confirming its function and role in pathogenesis of periodontal diseases.

Acknowledgements

We sincerely thank SRM Dental College, Ramapuram, Chennai for providing the study participants.

References

[1]. Lozano GM, Bejarano I, Espino J, González D, Ortiz A, García JF, et al. Relationship between caspase activity and apoptotic markers in human sperm in response to hydrogen peroxide and progesterone. J Reprod Dev. 2009 Dec;55(6):615-21.Pubmed PMID: 19734695.

[2]. Singh N. Apoptosis in health and disease and modulation of apoptosis for therapy: An overview. Indian J ClinBiochem. 2007 Sep;22(2):6-16.Pubmed PMID: 23105676.

[3]. Fan TJ, Han LH, Cong RS, Liang J. Caspase family proteases and apoptosis. ActaBiochimBiophys Sin. 2005 Nov 1;37(11):719-27.

[4]. Riedl SJ, Shi Y. Molecular mechanisms of caspase regulation during apoptosis. Nat Rev Mol Cell Biol. 2004 Nov;5(11):897-907.

[5]. Jiménez Fernández D, Lamkanfi M. Inflammatory caspases: key regulators of inflammation and cell death. J Biol Chem. 2015 Mar;396(3):193-203. Pubmed PMID: 25389992.

[6]. Arakawa S, Nakajima T, Ishikura H, Ichinose S, Ishikawa I, Tsuchida N. Novel apoptosis-inducing activity in Bacteroidesforsythus: a comparative study with three serotypes of Actinobacillusactinomycetemcomitans. Infect Immun. 2000 Aug;68(8):4611-5.Pubmed PMID: 10899863; PMCID: PMC98390.

[7]. Wang PL, Shirasu S, Shinohara M, Daito M, Oido M, Kowashi Y, et al. Induction of apoptosis in human gingival fibroblasts by a Porphyromonasgingivalis protease preparation. Arch Oral Biol. 1999 Apr;44(4):337-42. Pubmed PMID: 10348360.

[8]. Yang HH, Jun HK, Jung YJ, Choi BK. Enterococcus faecalis activates caspase-1 leading to increased interleukin-1 beta secretion in macrophages. J. Endod. 2014 Oct 1;40(10):1587-92.

[9]. Galluzzi L, López-Soto A, Kumar S, Kroemer G. Caspases connect cell-death signaling to organismal homeostasis. Immunity. 2016 Feb 16;44(2):221-31.

[10]. Stennicke HR, Salvesen GS.Properties of the caspases. BiochimicaetBiophysicaActa (BBA)-Protein Structure and Molecular Enzymology. 1998;1387(1-2):17-31.

[11]. Goga Y, Chiba M, Shimizu Y, Mitani H. Compressive force induces osteoblast apoptosis via caspase-8. J Dent Res. 2006 Mar;85(3):240-4.Pubmed PMID: 16498071.

[12]. Yan X, Chen J, Hao Y, Wang Y, Zhu L. Changes of caspase-1 after the application of orthodontic forces in the periodontal tissues of rats. Angle Orthod. 2009 Nov;79(6):1126-32.Pubmed PMID: 19852604.

[13]. Veeravarmal V, Austin RD, Siddavaram N, Thiruneelakandan S, Nassar MH. Caspase-3 expression in normal oral epithelium, oral submucous fibrosis and oral squamous cell carcinoma. J Oral MaxillofacPathol. 2016 Sep-Dec;20(3):445-452.Pubmed PMID: 27721610.

[14]. Pacios S, Andriankaja O, Kang J, Alnammary M, Bae J, de BritoBezerra B, et al. Bacterial infection increases periodontal bone loss in diabetic rats through enhanced apoptosis. Am. J. Pathol. 2013 Dec 1;183(6):1928-35.

[15]. Liu J, Jiang Y, Mao J, Gu B, Liu H, Fang B. High levels of glucose induces a dose-dependent apoptosis in human periodontal ligament fibroblasts by activating caspase-3 signaling pathway. ApplBiochemBiotechnol. 2013 Jul;170(6):1458-71.Pubmed PMID: 23686473.

[16]. Scott FL, Denault JB, Riedl SJ, Shin H, Renatus M, Salvesen GS. XIAP inhibits caspase-3 and -7 using two binding sites: evolutionarily conserved mechanism of IAPs. EMBO J. 2005 Feb 9;24(3):645-55.Pubmed PMID: 15650747.

[17]. Arnold R, Seifert M, Asadullah K, Volk HD. Crosstalk between keratinocytes and T lymphocytes via Fas/Fas ligand interaction: modulation by cytokines. J Immunol. 1999 Jun 15;162(12):7140-7.Pubmed PMID: 10358159.

[18]. Griffith TS, Brunner T, Fletcher SM, Green DR, Ferguson TA. Fas ligand-induced apoptosis as a mechanism of immune privilege. Science. 1995 Nov 17;270(5239):1189-92.

[19]. Kato S, Muro M, Akifusa S, Hanada N, Semba I, Fujii T, et al. Evidence for apoptosis of murine macrophages by Actinobacillusactinomycetemcomitans infection. Infect Immun. 1995 Oct 1;63(10):3914-9.

[20]. Arakawa S, Nakajima T, Ishikura H, Ichinose S, Ishikawa I, Tsuchida N. Novel apoptosis-inducing activity in Bacteroidesforsythus: a comparative study with three serotypes of Actinobacillusactinomycetemcomitans. Infect Immun. 2000 Aug;68(8):4611-5.Pubmed PMID: 10899863.

[21]. Wang PL, Shirasu S, Shinohara M, Daito M, Oido M, Kowashi Y, et al. Induction of apoptosis in human gingival fibroblasts by a Porphyromonasgingivalis protease preparation. Arch Oral Biol. 1999 Apr;44(4):337-42. Pubmed PMID: 10348360.

[22]. Baba A, Abe N, Kadowaki T, Nakanishi H, Ohishi M, Asao T, et al. Arggingipain is responsible for the degradation of cell adhesion molecules of human gingival fibroblasts and their death induced by Porphyromonasgingivalis. Biol Chem. 2001 May;382(5):817-24.Pubmed PMID: 11517936.

[23]. Sheets SM, Potempa J, Travis J, Casiano CA, Fletcher HM. Gingipains from Porphyromonasgingivalis W83 induce cell adhesion molecule cleavage and apoptosis in endothelial cells. Infect Immun. 2005 Mar;73(3):1543-52.Pubmed PMID: 15731052.

[24]. Li Y, Messina C, Bendaoud M, Fine DH, Schreiner H, Tsiagbe VK. Adaptive immune response in osteoclastic bone resorption induced by orally administered Aggregatibacteractinomycetemcomitans in a rat model of periodontal disease. Mol Oral Microbiol. 2010 Aug;25(4):275-92.

[25]. Pradeep AR, Suke DK, Prasad MR, Singh SP, Martande SS, Nagpal K, et al. Expression of key executioner of apoptosis caspase-3 in periodontal health and disease. J InvestigClin Dent. 2016 May;7(2):174-9.

[26]. Aral K, Aral CA, Kapila Y. The role of caspase-8, caspase-9, and apoptosis inducing factor in periodontal disease. J Periodontol. 2019 Mar;90(3):288-94.

[27]. Das P, Chopra M, Sun Y, Kerns DG, Vastardis S, Sharma AC. Age-dependent differential expression of apoptosis markers in the gingival tissue. Arch Oral Biol. 2009 Apr 1;54(4):329-36.

[28]. Bantel H, Beikler T, Flemmig TF, Schulze-Osthoff K. Caspase activation is involved in chronic periodontitis. FEBS J. 2005 Oct 24;579(25):5559-64.

[29]. Lucas H, Bartold PM, Dharmapatni AA, Holding CA, Haynes DR. Inhibition of apoptosis in periodontitis. . J Dent Res. 2010 Jan;89(1):29-33.

[30]. Gamonal J, Bascones A, Acevedo A, Blanco E, Silva A. Apoptosis in chronic adult periodontitis analyzed by in situ DNA breaks, electron microscopy, and immunohistochemistry. J Periodontol. 2001 Apr;72(4):517-25.Pubmed PMID: 11338305.

[31]. González OA, Stromberg AJ, Huggins PM, Gonzalez-Martinez J, Novak MJ, Ebersole JL. Apoptotic genes are differentially expressed in aged gingival tissue. J Dent Res. 2011 Jul;90(7):880-6.Pubmed PMID: 21471327.

[32]. Kang SW, Kim SK, Chung JH, Ban JY. Assessment of CASP gene polymorphisms in periodontal disease. Genet Mol Res. 2015 Dec 22;14:18069-77.

[33]. Zhao P, Liu J, Pan C, Pan Y. NLRP3 inflammasome is required for apoptosis of Aggregatibacteractinomycetemcomitans-infected human osteoblastic MG63 cells. ActaHistochem. 2014 Sep;116(7):1119-24.Pubmed PMID: 25001390.

[34]. Graves DT, Liu R, Oates TW. Diabetes-enhanced inflammation and apoptosis: impact on periodontal pathosis. Periodontol 2000. 2007;45:128-37. Pubmed PMID: 17850453.

[35]. Graves DT, Liu R, Alikhani M, Al-Mashat H, Trackman PC. Diabetes-enhanced inflammation and apoptosis--impact on periodontal pathology. J Dent Res. 2006 Jan;85(1):15-21.Pubmed PMID: 16373675.

[36]. Alikhani Z, Alikhani M, Boyd CM, Nagao K, Trackman PC, Graves DT. Advanced glycation end products enhance expression of pro-apoptotic genes and stimulate fibroblast apoptosis through cytoplasmic and mitochondrial pathways. J Biol Chem. 2005 Apr 1;280(13):12087-95.Pubmed PMID: 15590648.

[37]. Joussen AM, Poulaki V, Mitsiades N, Cai WY, Suzuma I, Pak J, et al. Suppression of Fas-FasL-induced endothelial cell apoptosis prevents diabetic blood-retinal barrier breakdown in a model of streptozotocin-induced diabetes. FASEB J. 2003 Jan;17(1):76-8.Pubmed PMID: 12475915.

[38]. Al-Mashat HA, Kandru S, Liu R, Behl Y, Desta T, Graves DT. Diabetes enhances mRNA levels of proapoptotic genes and caspase activity, which contribute to impaired healing. Diabetes. 2006 Feb;55(2):487-95.Pubmed PMID: 16443785.

[39]. Shoukheba, Malak.CASPASE-3 ACTIVITY AS A NOVEL BIOMARKER IN TYPE 2 DIABETES MELLITUS PATIENTS WITH CHRONIC PERIODONTITIS. Egypt Dent J. 2016; 62:32811-2821.

[40]. Manosudprasit A, Kantarci A, Hasturk H, Stephens D, Van Dyke TE. Spontaneous PMN apoptosis in type 2 diabetes and the impact of periodontitis. J Leukoc Biol. 2017 Dec;102(6):1431-1440.Pubmed PMID: 29021368.