THE EFFECT OF OXYGENATION ON APOPTOSIS OF THE FIBROBLAST CELL IN PERIODONTAL TISSUE RATS

Dian Mulawarmanti*, Syamsulina Reviangi*, Fanny M Laihad**
*Laboratory of Oral Biology (Biochemistry), ** Laboratory Oral Surgery
Faculty of Dentistry, Hang Tuah University

ABSTRACT

Background: Depriving tissues of oxygen is one of the more common mechanisms for cellular injury. Hypoxia can result from interrupted blood supply (ischemia), inadequate oxygenation of blood included aggressive periodontitis. Apoptosis is one of the main types of programmed cell death and involves a series of biochemical events leading to a characteristic cell morphology and death. Tissue oxygenation use Hyperbaric Oxygen Therapy (HBOT) is involved administration 100% oxygen under pressure. It has been used as an adjuvant therapy to improve wound healing.

Purpose: To investigated effects oxygenation on apoptosis of the fibroblast cell in periodontal tissue rats.

Methods: A total of 54 male Wistar rats were equally divided in healthy controls (group 1), diabetes (group 2), normal+HBOT (group 3), bacteria (group 4), diabetes+HBOT (group 5), bacteria+HBO (group 6). Experimental gingiva infection was induced by once daily administration of periodontopathogen bacteria is diluted in BHI media for 4 days. Ligature-induced periodontitis was created by tying silk ligatures on the necks of mandibular incisive until 30 days, treatment HBOT 2,4 ATA 3x30 minutes for 10 days and the animals were decapitated. The measurement fibroblast cell apoptosis in gingiva tissue after 50 days with Tunnel Assay method.

Results: It was found apoptosis fibroblast cell decreased significantly $p=0.000$ (18.17±1,500) after 10 sessions HBOT than without HBOT (28.1667±2.62202) and fibroblast cell bacteria-induced (22.6667 ±2.25154) decreased significantly $p=0.000$ than without HBOT (32.7778 ±2.15058).

Conclusions: Tissue oxygenation (HBOT) can reduces apoptosis in fibroblast cell in periodontal tissue rats

Key words: apoptosis, hyperbaric oxygen, periodontitis
INTRODUCTION

The number of people with diabetes worldwide was estimated at in 131 million 2000; it is projected to increase to 366 million by 2030 (Mealey, 2003). Periodontal disease was more prevalent and severe in people with diabetes than in people without diabetes, researchers sought specific biological mechanisms. A number of pathological mechanisms related to elevated levels of glucose in the blood have been defined, including the activation of the sorbitol pathway, the formation of advanced glycation endproducts (AGEs), the damaging effect of oxidative stress and altered lipid metabolism. The main problem that always found were discovered in patients with diabetes mellitus was not realized that he had been suffering from diabetes, most of them were found in the clinic and discovered in an advanced stage that has been accompanied by complications diabetes type 1. Chronic diabetes manifestations such as retinopathy, neuropathy, nephropaty, angiopati, atherosclerosis, periodontitis, and other complications such as disturbance of healing process (Graves et al, 2006).

Taylor and Borgnakke, 2008 identified periodontal disease as a possible risk factor for poor metabolic control in people with diabetes mellitus. Graves and colleagues the pathogenesis of periodontal disease in patients with diabetes and concluded that, in addition to inflammatory response, enhanced apoptosis (the sequence of programmed events leading to cell death) may contribute to periodontitis as a complication of diabetes. If apoptosis is enhanced, the effects, including delayed wound healing, can be detrimental. Therefore, enhanced inflammation leading to tissue destruction and diminished repair of damaged tissue may contribute to the periodontal tissue destruction seen in patients with diabetes (Alikhani Z, 2005; Liu, R, 2006). The damage periodontitis is begun in gingival epithelial cells, and then continued at the periodontal ligament fibroblast and osteoblast cells in alveolar bone. The function of fibroblast cell synthesis collagen fiber and as a immunoregulator cell that may affect the immune response by secreting chemokin, cytokines which able to trigger the immune response. Fibroblasts are able to express few regulators molecules multiple, such as cytokines, growth factors, chemokin, cell surface antigens, adhesion molecules, and substances with low molecular weight. The increasing apoptosis in periodontal fibroblast cells would disrupt the function of remodeling on the cell that has a function in tissue regeneration like tissues resorption that forms bind such as bone, cementum and periodontal ligament (Alikhani Z, 2005; Graves et al, 2006).
Hiperbaric Oxygen Therapy (HBOT) is a therapy with pure oxygen with more pressure than 1 atmosphere absolute (ATA). Mechanisms of action for HBOT are based on elevation of both the partial pressure of oxygen and hydrostatic pressure. The result of HBOT on inflammatory mediators in the diabetes is reported to have suppressive effect on pro-inflammatory cytokines such as IL-1, TNF-α, and IFN-γ. In the wound healing process it will occur vasoconstriction of blood vessels, thus reducing oedema, increased growth factor (GF) (Yuan, 2009). Based on the idea of some research results which are still unclear, especially the influence of HBOT. In this study it will examine the effects of HBOT to the number of fibroblast cells which have periodontal tissue apoptosis in rats (rattus novergicus) under conditions of hyperglycemia, which is used in HBOT. This research used HBOT 2.4 ATA is the most optimal therapy which is done in minimum side effects for 3x30 minutes with 5 minutes intervals for 10 days (Neumeister, M, 2005).

**Periodontitis in Diabetes**

Periodontitis in diabetes is the largest diabetes complications in the oral cavity and severity is associated with chronicity diabetes. High risk were mainly found in people who unregulated diabetes. In the unregulated diabetes were found thickening of blood vessels and becoming less elastic which will reduce the oxygen capacity and nutrients throughout the body, elevated levels of ROS (Reactive Oxygen Species) and NOS (Nitrogen Oxygen Species), the decrease of antioxidants, enzymes changes involving endothelial cells, blood vessels, and dysfunction neurovascular. This will increase oxidative stress that causes more complications of diabetes, which would damage the DNA, fat, protein, disruption of hemostasis and accumulation of molecules damage (Sadzevicien,R, 2005). Diabetes-causing microbes in patients with non-diabetes and diabetes are the same, the difference lies in the body's response to factors that increase the periodontitis incidence. The first factor is the change in microvascular and the second is a response to infection. In diabetes, there is a damage to the cellular immunity system, is hemotaksis neutrophil and macrophage function, the third is the increase in salivary glucose levels which can result plaque formation and microbial food sources oral cavity (Liu, 2006). AGEs accumulation affect the migration and phagocytic activity of polymorphonuclear cells, macrophages that influence pathogenic subgingival flora. Maturation and gradual changes in the subgingival microflora in pockets ulcer showed the changes of chronic
systemic disease, characterized by secretion of IL-1 and TNF-α, insulin resistance, influence on glucose metabolism. The interaction between phagocytic cells, macrophages with AGEs induce the expression of cytokines and oxidative stress (Sadzevicien, R 2005). Simultaneously, periodontal infection can induce persistent insulin resistance, followed by hyperglycemia, glycoxylation is irreversible nonenzymatic, accumulation of AGEs-protein bond, which is then accompanied by the degradation and destruction proliferation connective tissue (Graves, D.T. 2006).

In periodontitis diabetes are found the hemoglobin A1c levels which increase due to increased synthesis of cytokines and bacteria, which then caused the formation of AGEs in diabetes. This causes greater periodontal tissue destruction. Diabetes influence the process of healing and increase susceptibility to bacterial infections, including periodontitis, it was found decreased vascularization of new blood vessels and granulation tissue in diabetic rats. Delays in the healing process associated with a decrease in leukocyte infiltration and elevated levels of IL-6 during the final phase of healing process. Disruption of the healing process is also associated with hyperglycemia (Lamster, 2008).

**Diabetes, Periodontitis and Apoptosis**

Apoptosis is one program of death cell which can be triggered by many kinds signals with specific morphology changes. Apoptosis is important critical mechanism that can eliminate unfuction cell during the development phase, prevent immune system, and host response to the infection or tumor synthesis. Apoptosis occurs very quickly within an hour of effector caspase becomes active. Although apoptotic cell persentage is low. In apoptotic death cell give signals which is mediated by several genes that encode proteins to digestive enzymes called caspase. Caspase genes is part of cystein protease which will be active in the cells development also an active signal to cells destruction. Apoptosis can occur in the damage cells that can not be repaired, that can cause stress to the cell. DNA damage due to chemicals or radiation can trigger apoptosis through a tumor suppressor gene p53 (Liu, 2007).

The adequate healing process requires number of cells which is used for repairing process, apoptosis causes decreasing tissue formation and resulting wound healing process destruction. It has been proved in humans that apoptosis plays an important role in diabetes.
complications. There are some aspects that can trigger apoptosis, including ROS, cytokines and AGEs (Liu, 2007).

![Diagram showing apoptosis pathways in diabetes.](image)

Fig 1. Apoptosis Pathways in Diabetes (Graves, D.T. 2006)

Diabetes associated with activation of polyol pathway that can synthesize the formation of AGEs and phospholipase C activation, the increased levels of TNF-α, activation of protein kinase C, and oxidative stress formation (Al-Mashat, 2006). Synthesis ROS, TNF-α and AGEs have a potential effect on the healing process in the oral cavity or a direct effect on the response induced by bacteria in osteoblast cells or fibroblasts, such as decreased expression of collagen, or by indirect triggers inflammation and apoptosis of matrix producing cells. The production of ROS, TNF-α, and AGEs which increase in periodontitis resulted in impaired healing process and progression of periodontal disease (Nishikawa, T, 2007). Diabetes trigger cell apoptosis fibroblasts and osteoblasts due to bacteria, infection. In the rabbit bacteria induce inflammation and cell damage. The velocity of fibroblast cell apoptosis caused by activation of caspase-3 which increase, apoptotic cells fibroblast increase and the number of cells that function decrease. Barriers against caspase-3 can decrease fibroblast cells apoptosis. There are several mechanisms that have a response to apoptosis in diabetes. One of mechanism through activation of receptor cytokine 'death domains', such as TNF-α receptor-1 (TNFR1) or fas. Diabetes is associated with activation of TNF-α and fas / fas-ligand (Alikhani, 2005).
Apoptosis in diabetes and periodontal tissue inflammation occurs due to expression of IL-1 or IFN-γ triggers apoptosis, whereas there is damage to the receptor death domains, by altering the expression of pro-apoptotic gene or trigger the production of oxygen radicals. Advanced glycation end-products can also result in matrix-producing cells have apoptosis. There are mechanisms of AGEs can trigger apoptosis: the directory that is directly activate caspase, and indirect increases oxidative stress, or increase the expression of pro-apoptotic genes that regulate apoptosis (Liu T, 2007).

Hyperbaric Oxygen (HBO)

Hyperbaric Oxygen Therapy (HBOT) is a therapy in patients by administering 100% pure oxygen by inhalation of air pressure more than 1 Atmosphere Absolute (ATA) which is inserted in a chamber / high pressure air chamber (RUBT). In normal circumstances humans breathe 20% oxygen and 80% nitrogen at a pressure of 1 ATA or 760 mm Hg and oxygen partial pressure of arterial blood vessels around 100 mmHg. At a pressure of 1 ATA almost entirely of oxygen in the blood are transported in liquid form dissolved in plasma (3%) and in a form that binds to hemoglobin (± 97%) (Neumeister, M, 2005). Basic HBOT of pure oxygen (100%) continuously in a certain time. HBOT the amount of solved oxygen so it condition in air greater air pressure than normal atmospheric pressure, there is between 2-3 ATA. Changes in 1 ATA (atmospheres absolute) is equivalent to 14.7 psi (pounds per square inch) or 760 mm Hg or 33 feet below the sea water surface. In HBOT, the form of dissolved oxygen is very important compared with the form of oxygen bound by Hb, it is more easily consumed by the network through direct diffusion. HBOT can increase the amount of oxygen
dissolved in such a way as to achieve a practical situation where the oxygen demand of dissolved oxygen can be met without the use of oxygen bound. Function HBOT increases the concentration of oxygen in the blood, required for various biochemical enzymatic reactions, the function of cells respiration and normal tissue. HBOT doses used for clinical cases is between 2-3 ATA for giving more than 3 ATA cause neurological symptoms such as convulsions and loss of consciousness due to oxygen toxicity (Neumeister, M, 2005)

MATERIALS AND METHODS

The study was an experimental laboratory, the design used was Control Group Post Test Only Design. This study used types of Wistar rats of 7-8 weeks with body weight between ± 100-125 grams, which was developed at the Laboratory of Biochemistry Faculty of Medicine Airlangga University. Large replication used in this study, calculated by a formula determining the sample size (Steel and Torrie, 1991). STZ induced can cause hyperglycemia until it achieve blood glucose levels ≥ 300 mg / dl. Used 6 groups of Wistar rat species. Negative control group / K0 rats (normal) was not induced STZ, bacteria and HBOT. Positive control group / K1 STZ rats for 4 days induced a dose of 40 mg / kg until glucose levels > 300 mg / dl (Sigma), was not induced bacteria and HBOT. Treatment group 1 / P1 STZ rats induced a dose of 40 mg / kg (Sigma) until glucose levels > 300 mg / dl. Bacteria induction and hyperbaric oxygen therapy treatment group HBOT 2.4 ATA 2 / P2 STZ-induced rats for 4 days, a dose of 40 mg / kg (Sigma) until glucose levels > 300 mg / dl, without induction therapy given HBOT 2.4 ATA. Treatment group 3 / P3 STZ-induced rats for 4 days with a dose of 40 mg / kg (Sigma) until glucose levels > 300 mg / dl, induced bacteria and treated with HBOT.

Induction of STZ for 4 days with a dose of 40 mg / kg BW (Lamster, 2008), conducted the examination of blood glucose levels on day 4 of the initial induction of STZ to achieve glucose levels > 300 mg/dl (Lalla E, 2000). If on day 4 of blood glucose levels <300 mg / dl, STZ induction again with a dose of 40 mg / kg until blood glucose levels > 300 mg / dl, repeated again the following day. If on day 7 blood glucose levels <300 mg / dl rats were sacrificed. HBOTT is given for 3x30 minutes with 5 minute intervals to avoid toxicity, for 10 days continually.

At the end of treatment entirely sacrificed, tissues taken for examination of periodontal fibroblast cells which have apoptosis. Apoptotic cells detected using the terminal deoxynucleotidyl
transferase (TdT)-mediated deoxy-UTP nick end labeling (TUNEL) assay with the kit Trevigen (Gaithersburg, MD, USA). The number of fibroblast cells that had apoptotic calculated with hematoxylin-eosin staining. Apoptotic fibroblasts is calculated on the third ligament periodontal (ParagonBioservices. 2009). The study was conducted in Laboratory of Biochemistry, Airlangga University School of Medicine, Culturing bacteria carried in the Microbiology Laboratory Hang Tuah University Surabaya. Preparation and examination of apoptosis is carried out in Biomedicine Lab Universitas Brawijaya School of Medicine

RESULTS:

The effects of streptozotocin (STZ) on hyperglycemia to compare blood glucose levels before and after the administration of STZ in the fifth day (data obtained from control K1/ groups STZ and STZ groups + bacteria / P1 and P3) (the results of table 1). There was significant difference $p < 0.05$ ($p = 0.000$) between groups before and after STZ induction.

The analysis showed STZ administration may cause hyperglycemia in subjects between before and after the administration of STZ. Expression of fibroblast cell apoptosis is obtained from observation of periodontal ligament fibroblasts.

Fig. 3. Apoptosis in fibroblast cells periodontal tissues Rat (Rattus norvegicus)
A. Control; B. STZ; C. STZ+bacteria; D. STZ+HBOT; E. STZ+bacteria+HBOT; F. HBOT. Apoptosis is marked by a brown color in the cell nucleus (black arrow)
Table 1. Mean and standard deviation periodontal ligament fibroblast cell apoptosis in each experimental group, calculated according to Pizem et al (2003)

<table>
<thead>
<tr>
<th>Group</th>
<th>X</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>One way Anova</th>
</tr>
</thead>
<tbody>
<tr>
<td>K0/normal</td>
<td>9.67</td>
<td>0.577</td>
<td>9</td>
<td>10</td>
<td>F=23,435</td>
</tr>
<tr>
<td>K1/stz</td>
<td>22.33</td>
<td>3.055</td>
<td>19</td>
<td>25</td>
<td>P=0.000</td>
</tr>
<tr>
<td>K2/h+HBOT</td>
<td>32.67</td>
<td>3.215</td>
<td>9</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>P1/bacteria</td>
<td>19.67*</td>
<td>2.517</td>
<td>30</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>P2/stz+HBOT</td>
<td>20.33*</td>
<td>4.163</td>
<td>15</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>P3/bacteria +HBOT</td>
<td>12.67</td>
<td>2.517</td>
<td>18</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

There is increased expression of fibroblast cell apoptosis between the control group K1/STZ (22.33 ± 3.055) compared with normal K0/kontrol group (9.67 ± 0.577). Concluded that the induction of STZ increased apoptosis of periodontal fibroblast cells. HBOT therapies against apoptosis of fibroblast cells STZ rat group showed a significant difference p <0.05 between which no treatment was given 2.4 ATA HBOT (22.03 ± 3.055) with the given HBOT (20.33 ± 4.16). HBOT concluded that therapy can reduce periodontal fibroblast cell apoptosis hyperglycemia rats. HBOT Therapy 2.4 ATA did not affect the changes in expression of apoptosis K0/normal groups of rats (9.67 ± 0.577) and rats treated K2/normal HBOT (32.67 ± 3.215). It is concluded that the therapeutic HBOT effect changes in expression apoptosis periodontal fibroblast cells of normal rats at the end of induction. The influence periodontopathogen bacteria on the expression of periodontal fibroblast cell apoptosis between K1/STZ group (22.33 ± 3.055) with P1/STZ + bacteria group (19.67 ± 2.517) showed significant differences p <0.05.

It is concluded that the suspected bacterial induction of apoptosis resulted in increased expression of periodontal fibroblast cells at the end of treatment. The effect of HBOT 2.4 ATA to the fibroblast cell apoptosis which is bacteria-induced in STZ rat group between groups P1/STZ + bacteria (19.67 ± 2.517), hyperglycemia is not induced bacteria with bacteria-induced in STZ rats group that were given therapy P3/STZ HBOT + bacteria + HBOT (12.67 ± 2.517) showed a significant decrease p <0.05 (table). Expression of apoptosis fibroblast cell periodontal tissues differ between groups of normal rats (9.67 ± 0.577), STZ group (22.33 ± 3.055) and STZ + bacteria
group (19.67 ± 2.517). Induction of STZ increased the expression of apoptotic fibroblast cells rat periodontal, and additional induction bacteria that will increase the of apoptosis rat periodontal fibroblast cells were significantly p <0.05. ATA 2.4 HBOT therapy can decrease the expression of periodontal fibroblast cell apoptosis rats STZ-induced rats groups of bacteria were significantly p <0.05 (table).

Fig. Scheme apoptosis and each controle and treatment groups

DISCUSSION

Hyperglicemia condition caused by STZ induced can cause destruction β cell of pancreas and influence insulin secretion (Lalla, 2000). Induction of STZ, which is a potential ingredient to DNA methylation, which is an N-nitroso-containing compound which is a nitric oxide donor in pancreatic islet, can cause damage to cells that secrete insulin (Bennet, 1981). STZ enters β cells through glucose transporter (GLUT-2) that cause DNA alkylation. DNA damage induces activation of poly ADP ribocytlation of DNA (pADPR) which then resulted in depletion of NAD+ and ATP intracellular (Pacher, P, 2005). It is with a substrate of xanthine oxidase would generate superoxide radicals, hydrogen peroxide and hydroxyl radicals.

STZ induction resulted inhibit aconitase activity and lead to DNA damage, with the end result β cell damage resulting in necrosis. B cell damage depends on the number of doses, route of administration and duration of administration. Impaired insulin secretion resulted in the number of insulin-receptor binding and glucose transporter / GLUT decline that resulted in disruption of
glucose uptake by cells. In the end there are barriers to transport glucose into cells/tissues. This causes glucose levels remain in the blood. In pancreatic β cells, a result of hyperglycemia resulted in increased ROS in the mitochondria that will suppress the first phase of glucose induced insulin secretion.

Similarly, in the fibroblast cells of hyperglycemia resulted in secretion of TNF-α that lead to increased ROS in the mitochondria. If it happens a long time, it can activate apoptosis signal-regulating kinase-1 (ASK1) and c-jun NH2-terminal kinase (JNK), increased serine phosphorylation of IRS-1 and decrease insulin stimulation by tyrosine phosphorylation of IRS-1, which then lead to insulin resistance occurs (Nishikawa, T, 2007). Hyperglycemia resulted in the formation of non-enzymatic glycation reactions that result in the formation of covalent bonds between the aldehyde group of glucose with free amino acid groups of proteins. AGEs form reaction products which have a chemical structure change. Changes in the structure of collagen and extracellular matrix causes the formation of AGEs (Lamster, 2008).

Synthesis of AGEs is a source of free radicals that affect connective tissue, react with receptors of macrophages, releasing cytokines, growth factors, stimulating the destruction and mitogenic processes. Reduction of vasodilators such as NO can cause decreased blood flow to the small blood vessels. Chronic hyperglycemia in DM is a source of free radicals can occur through several different pathways: autooxidation glucose, non-enzymatic glycation and activation sorbitol pathway (Pacher, 2005; Nishikawa, T, 2007). Several mechanisms can be a cause of disruption and damage the function of pancreatic β cells, including 1) the interaction of pancreatic β cells by T lymphocytes through MHC molecules, 2) induction of expression of nosocomial infection that catalyzes the formation of NO, 3) hyperglycemia increases mitochondrial trans membrane potential that would result in an increase superoxide / (oxygen-free radicals,) Nishikawa. T, 2007) and 4) activation of apoptosis program for β cell (Al-Mashat, 2006).

Induction periodontopathogen bacteria in gingival tissue triggers PMN cells greater macrophage cells to secrete more cytokines IL-1, TNF-α, ROS, ICAM-1 that would affect the inflammatory response, so that increase inflammatory processes (Alikhani Z, 2005). Periodontopathogen bacteria results chemotaxis products on neutrophils, activating proteinase cascade system, stimulate mast cells to release of biogenic amines, and stimulate inflammatory cells and activates cells to produce cytokines (Interleukin-1, tumor necrosis factor), platelet
activating factor and prostanoid (such as prostaglandins, leukotrienes, polymorphonuclear leukocytes (PMN) play an important role in the pathogenesis periodontitis (Liu R, 2006).

Levels of AGEs below normal in normal individuals. However, in conditions of hyperglycemia, there is an increase high and fast AGEs. AGEs form spontaneously increased due to elevated levels of glucose oxidation by fat in the blood. Influence on the formation of AGEs in diabetic complications such as renal complications, fibrosis, atherosclerosis, periodontal disease triggers, and reduced formation of tualang (Alikhani Z, 2005). Tissues damage found in the receptor of AGE is RAGE, which has to be identified called the signal-transducing receptor. There are several mechanisms whereby AGEs may affect the surrounding cells, such as trigger inflammation, stimulate apoptosis, or affect the production extracellular matrix. Inflammation resulting in the formation of AGEs from the effects of induction of bacteria that can cause bone damage on the network periodontal. Bacteria resulted in increased levels of cytokines IL-1, TNF-α which would increase the secretion matriksmetalloproteinase which could result in damage ekstraselulermatriks. In hyperglycemia there is decreasing of osteoblast differentiation and growth factor and reduced production ekstraseluler matriks (Liu R, 2006). Bone healing is diabetes is strongly influenced by the levels of AGEs. This is based on research results that provide application AGEs on marmot and found a decline in normal bone formation. It is found AGEs change cellular on Diabsetes such as reduced production ekstraseluler matrix, and related differentiation of osteoblast cell (Paragon Bioservices. 2009). Other effects AGEs can interfere wound healing process, because of induced apoptosis in extracellular matrix. Apoptosis decrease the number of osteoblast cell that can lead to resorption bone repair (Alikhani Z, 2005; Al-Mashat, 2005). Apoptosis in diabetes and periodontal tissue inflammation occurs due to expression of IL-1 or interferon-gamma triggers apoptosis, whereas there is damage to death receptor domains, by altering the expression of pro-apoptotic gene or trigger the production of radicals oksigen Advanced glycation end-products can also resulted in matrix-producing cells have apoptosis (Graves, D.T. 2006). Apoptosis is here associated with the onset of diabetic complications such as retinopathy, neuropathy, nephropati, and vasculopati. There are mechanisms of AGEs can trigger apoptosis: that is directly activate caspase, and indirect increases oxidative stress, or increase the expression of pro-apoptotik genes that regulate apoptosis. Experiments in vivo AGEs induce cell apoptosis of fibroblasts, via the enzyme
caspase-3 and also signals through the activation of caspase-8 and caspase-6.9 in vitro, AGEs have the effect of triggering mRNA levels of pro-apoptotic genes include several molecules, including ligands, receptors, molecules adapter, mitochondrial proteins and others (Lalla e, 2000; Al-Mashat, 2006). AGEs also stimulate the activation of NF-kB. AGEs and pro apoptotic molecule such as TNF, stimulate the anti-and pro-apoptotic, and the result depends on the balance between them. This balance can be affected by transcription factors FOXO1, globally induced gene expression pro-apoptotik and mechanism of stimulation of NF-kB related with antiapoptosis (Alkhani Z, 2005). In periodontium tissue occurs repair disruption that followed by the infection. In periodontitis, there is a connective tissue attachment loss of teeth which can not undergo the repair process because it is not capable of epithelial synthesis there is also damage bone tissue pathologically. One mechanism that could explain the inadequate repair of damaged cells that produce matrix. This is supported by apoptotic fibroblasts is high in patients with periodontitis, especially occurred in areas of inflammation. It was concluded that the inflammatory process caused a large induction of the inflammatory response in diabetic individuals and resulted in apoptosis of fibroblasts and osteoblasts.

Soft and hard tissue damage found in patients with diabetes. This is the same principle found that the decrease in apoptosis during the healing process is related to Quantitative and Qualitative healing process. On the contrary, conditions that trigger apoptosis is associated with impaired healing. Apoptosis cell in the matriksekstraseluter cell is the critical factor in the repair process of soft tissue and hard tissue and it is an important mechanism in diabetic and have an adverse effect on periodonsium tissue. HBOT 2.4 ATA for 3x30 minutes with 5 minutes intervals for 10 days in this study, can reduce cell apoptosis of fibroblasts and can accelerate the healing process of periodontitis. This is thought to decrease expression of apoptosis that is pre-apoptotic factors trigger the wound healing process. This condition is different by Gastman et al., 2003, that apoptosis can lead to disruption of healing process. The role of hyperbaric oxygen therapy to the decline of other apoptotic cells that diabetes is suspected that therapy can lower the activation polyl, pathway decreased the expression of TNF-α, lowering aktivase protein kinase C, and reduce oxidative stress reducing transcription factor NF-kB and HIF-1α (Mulawarmanti, D. 2008)

Decrease of the formation of ROS, TNF, AGEs, NF-kB and HIF-1α may affect the healing process or the response of bacteria of periodontitis by direct influence osteoblast cells or fibroblast cells,
such as the synthesis / expression of collagen, or indirectly reduce inflammation or apoptosis on matrix-producing cells. So the decrease of ROS, TNF, AGES, and NF-κB and HIF-1α in DM can help the healing process in periodontitis with hyperglycemia..

**SUMMARY**

Hyperbaric Oxygen Therapy 2.4 ATA 3x30 minute with interval 5 minute for 10 days can decrease apoptosis of fibroblast cell in periodontal tissues rat.

**REFERENCES**

7. Yuan, Li-Jen (Li); Niu, Chi-Chien (CC); Liu, Song-Shu (SS); Chan, Yi-Sheng (YS); Yang, Chuen-Yung (CY); Chen, Wen-Jer (WJ); Ueng, Steve W N (SW); 2003. Additive effects of hyperbaric oxygen and platelet-derived growth factor-BB in chondrocyte transplantation via up-regulation expression of platelet-derived growth factor-beta receptor. Journal of orthopaedic research. Nov; vol 27 (issue 11) : pp 1436-46