CURRENT RESEARCHES in HEALTH SCIENCES

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Chapter-1

IN VETERINARY MEDICINE: OXIDATIVE STRESS AND ANTIOXIDANTS*

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Oxidative Stress

The vast majority of eukaryotic organisms need atmospheric oxygen to survive. Oxygen may turn into free radicals, a very hazardous toxic form, during metabolic reactions in all cells of aerobic organisms. Free radicals are short-lived reactive atoms, ions, or molecules with one or more unpaired electrons in their outer orbitals (1). The main sources of cellular ROS production are mitochondria, peroxisomes, cytochrome P450 enzymes, and antimicrobial oxidative burst of phagocytic cells (2). Of these, mitochondria are a source of free radicals and therefore seen as a potential oxidative damage sitenge (3, 4). Most free radicals are oxygen (reactive oxygen species) or ROS derivatives and nitrogen (reactive nitrogen species) or RON derivatives (3). Molecules called ROS; superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (HOO^-), hypochloric acid (HOCl), singlet oxygen (O_2), ozone (O_3), alkyl radical (R), peroxyl radical (ROO^-) organic peroxide radical ($RCOO^-$), perhydroxyl radical (HO_2^-), alkoxyl radical (RO^-) (4-6). RNS molecules are nitric oxide (•NO), nitrogen dioxide ($•NO_2$), nitrous acid (HNO_2), dinitrogen tetroxide (N_2O_4), dinitrogen trioxide (N_2O_3), peroxynitrite (ONOO•), peroxynitrous acid (ONOOH), alkyl peroxynitrites (ROONO) and nitryl chloride (NO_2Cl) (7).

Reactive oxygen species are produced as a result of the activity of many enzymes such as Xanthine Oxidase (XOD), Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase, Neutrophil Myeloperoxidase (MPO) (8). Nicotinamide adenine dinucleotide phosphate (NADPH) is an enzyme that catalyzes the production of superoxide (O_2^-) from oxidase, oxygen, and NADPH. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is also found in phagocytes (neutrophils eosinophils, monocytes, and macrophages) (9, 10) and increases the production of superoxide on bacteria in these cells. Superoxide is converted to hydrogen peroxide (11) by the action of superoxide dismutases (SOD) and hydrogen peroxide passes easily through the plasma membrane (12). Hydrogen peroxide is consumed by myeloperoxidase (MPO) in neutrophils and oxidized with chloride ions to convert hypochloric acid (HOCl) (13). HOCl is the most bactericidal oxidant known to be produced by neutrophils. (14). Besides, hydrogen peroxide can be decomposed into hydroxyl radicals (HO^-) in the presence of conduction metals such as Fe^{2+} or Cu^{2+} through a series of reactions called Haber - Weiss and Fenton reactions (15, 16). HO^- radical is the most powerful oxidizing radical that can interact with DNA, proteins, lipids, amino acids, glucose and metals. Other free radicals, the simplest form of the peroxyl radical (ROO^-), the hydroperoxyl radical (HOO^-), play a role in lipid peroxidation (17).

Oxidants play a key role in the oxidation of various macromolecules, espe-

cially lipid, protein and DNA, and cause injury to various organs or systems (7, 18).

Oxidative Stress-Related Genetics, Physiology, Biochemistry and Pathology Mechanisms

ROS-Related DNA Oxidasyonu

ROS, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, can cause modification in bases or 2-deoxyribose moieties, single or double-stranded DNA breakage, cross-linking with proteins (2, 19). These type of DNA modifications are common in carcinogenesis, aging, and neurodegenerative, cardiovascular, and autoimmune diseases (20, 21)

Exposure of cells to H_2O_2 creates base modification products and increases DNA chain breakage. In fact, the DNA damage observed in the presence of H_2O_2 is due to the formation and activity of hydroxyl radicals produced from H_2O_2 by a Fenton reaction (2). Hydroxyl radicals can add guanine and adenine at positions 4, 5, or 8 on the purine ring, creating a large number of products. Two of the most common endogenous DNA base modifications are 8-hydroxy-2′-deoxyguanosine (8-OHdG) or 8-oxo-7,8-dihydroguanine (8-oxoG) (22, 23) and 2,6-diamino-4-hydroxy-5- formamidopyrimidine (FAPG). 8-oxoG is the most widely used biochemical and immunohistochemical marker of DNA oxidationoxidation (21, 26). Mitochondrial DNA is particularly susceptible to ROS attack due to its proximity to the O_2^- production site from the electron transport chain, lack of histone protection, and minimal repair mechanisms (19).

Ros in Lipid Peroxidation

Lipids are essential components of cell membranes that protect the structure of cells and control their function. Free radicals can directly attack polyunsaturated fatty acids in membranes and initiate lipid peroxidation (25). Both polyunsaturated fatty acids and cholesterol are oxidized by enzymatic and non-enzymatic means (26). It can be oxidized by enzymes such as lipids, lipoxygenases, cyclooxygenases, and cytochrome P450 (27). Free radical-mediated peroxidation of PUFA, (1) reaction of a carbon radical and molecular oxygen, (2) atom transfer of hydrogen from the substrate to the peroxyl-bearing chain, (3) cleavage of the peroxyl-bearing chain to give oxygen and a carbon radical, and a lipid radical, (4) rearrangement of peroxyl radicals and (5) cyclization of peroxyl radical proceeds with five basic reactions (28).

The primary effect of lipid peroxidation is the reduction in membrane flu-

idity, which changes the properties of the membrane and can significantly disrupt membrane-bound proteins. This effect acts as an amplifier, more radicals are formed and polyunsaturated fatty acids are reduced to various products (25). Aldehydes from these products are very reactive and can damage proteins (29). The main primary products of lipid peroxidation are lipid hydroperoxides (LOOH) (17, 30). Among many different aldehydes that can be formed as secondary products during lipid peroxidation, malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4- HNE) are the ones that have been studied extensively (31, 32). These reactive types of aldehydes are both cytotoxic and genotoxic (31, 33). MDA is widely used as a suitable biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids due to its easy reaction with thiobarbituric acid (TBA) (36).

Ros in Protein Oxidation

Free radicals cause a variety of damage, including oxidation of sulfhydryl groups, reduction of disulfides, reaction with aldehydes, modification of prosthetic groups or metal, protein-protein crosslinking, and peptide fragmentation (35, 36)Protein oxidation is induced directly by ROT (such as OH^- , H_2O_2) or indirectly as a result of the reaction with secondary products of oxidative stress. Direct protein carbonylation, oxidation of amino acid side chains with metals and hydrogen peroxide causes the formation of semialdehyde amino acids, and most of these reactions result from damage to lysine, arginine, and proline residues, resulting in protein carbonyl (PCO) products (37). The most commonly measured product of protein oxidation is protein carbonyls (38). Indirectly, protein carbonylation occurs by the action of aldehydes, the most reactive form of carbonyl groups, which are formed as a result of hydroxyl radical-mediated oxidation of lipids (18). In recent years, AOPP (advanced oxidized protein products), a new protein oxidation marker, has been identified. AOPP is defined as cross-linked protein products containing dithrosine and is thought to be a reliable marker for detecting protein damage (39). There are observed accumulation and damaging effects of oxidized proteins in various pathological conditions such as neurodegenerative diseases, diabetes and atherosclerosis and during aging (40).

Ros in Cell Signalling

Besides a direct role in cellular signaling, ROS can indirectly modulate cell function through the intervention of discrete quantities of products of their reactions with defined biomolecules (41). ROS production leads to the stimulation of various signaling systems such as mitogen-activated protein kinases (MAPKs)

and other stress-sensitive protein kinases (42).

MAPK is activated by several factors, including growth factors, cytokines, and oxidative stress, and regulates some critical cellular functions, including proliferation, differentiation, and cell death (43, 44). Mitogen-activated protein kinases (MAPK) form a family of serine/threonine kinases, including extracellular signal-regulated kinase 1/2 (ERK1 / 2), c-Jun N-terminal kinase (JNK), and p38 MAPK (44, 45). ERK, JNK / SAPK, and p38 can all be activated by a variety of stimuli including growth factors, cytokines, and different cellular stresses. Exogenous hydrogen peroxide in different cell types has a sensitivity to MAPK (p38, JNKs, ERKs) redox regulation. In addition, oxidative stress leads to significant activation of ERK1 / 2 (42). In ERK1 / 2 activation, the epidermal growth factor (EGF) receptor is usually activated by EGF ligand binding, resulting in receptor dimerization and phosphorylation (44). The protein SOS associated with the EGF receptor functions as a guanine nucleotide exchange factor for Ras, thus activating it, leading to ERK1 / 2 activation (46). Similarly, the platelet-derived growth factor receptor is activated by H_2O_2 and induces the activation of ERK1 / 2 (47). Also, both ERK and JNK / SAPK signaling pathways may play a role in NF-κB activation via phosphorylation of its inhibitor IκB (48).

NF-kB is usually the major regulator and signaling protein molecule of genetic function and is activated by cell damage and free radicals (49) and is therefore sensitive to redox. In non-stressful conditions, this protein is found in the cytoplasm associated with an inhibitory factor (IκB) (3). NFkB activation in response to a stimulus occurs as a result of phosphorylation and proteolytic degradation of IκB (50). NF-kB then enters the nucleus, attaches to DNA control elements and induces mRNA synthesis (51, 52). NF-kB plays a central role in the regulation of gene transcription and coding of inflammatory cytokines, growth factors, acute phase proteins, adhesion molecules, other transcription factors, and cell death regulators (3, 52).

AP-1 activity can be induced by H_2O_2 and also regulated by the redox state of cysteine in the cell. (53). Unlike NF-κB, AP-1 is basically a nuclear transcription factor. While the increase in GSH / GSSG ratio enhances AP-1 binding, GSSG inhibits DNA binding of AP-1 (54).

Oxidative Stress-Related Sytemic Pathology

Overproduction of oxidants and / or failure of the antioxidant defense system causes ROS to cause cellular and tissue damage (7). Organ systems especially affected by ROS include the kidney, liver, heart, CNS / nerve tissue, lung, pancreas (3).

Cardiovascular diseases can arise from complications of atherosclerosis. Oxidative stress plays an important role in the pathogenesis of atherosclerosis, especially vascular endothelial dysfunction (55). Reactive oxygen species have harmful effects on vascular function through a variety of mechanisms. In addition, ROS peroxidizes lipid components that lead to the formation of oxidized lipoproteins (LDL), one of the key mediators of atherosclerosis (56). Oxidized LDLs are pro-inflammatory and cause inhibition of endothelial nitric oxide synthase (eNOS), promote vasoconstriction and adhesion, stimulate cytokines such as interleukin-1 (IL-1), and increase platelet aggregation (57, 58).

The brain and nervous system are more susceptible to free radical damage than other organs (7, 59). ROS and RNS harm neuronal and glial cells and thus neuronal damage occurs (60). The brain contains membranes made up of proteins and large amounts of phospholipids. These phospholipids include oxidizable PUFAs with arachidonic acid and docosahexaenoic acid (61). PUFAs are vulnerable to free radical attack because they contain hydrogen ions (62). Hydroxynonenal, a lipid peroxidation product, is cytotoxic, especially for neurons, increases Ca^{2+} levels, inactivates glutamate transporters, and damages neurofilament proteins (63, 64).

The liver is a significant organ that is attacked by ROS (65). Parenchymal cells are primary cells in the liver that suffer damage caused by oxidative stress (66). Kupffer cells, hepatic stellate cells, and endothelial cells are potentially more exposed or sensitive to oxidative stress-related molecules. Various cytokines such as TNF-α produced in Kupffer cells caused by oxidative stress, and they can increase inflammation and apoptosis (67). Proliferation and collagen synthesis of hepatic stellate cells are triggered by lipid peroxidation caused by oxidative stress (68).

ROS plays a role in the pathogenesis of ischemic, toxic, and immunologically mediated kidney damage (69). The kidneys being rich in mitochondria indicate that they are highly vulnerable to damage caused by oxidative stress (70). Kidney damage can occur from many factors, including mitochondrial dysfunction, aging, diabetes mellitus, and inflammation (71). Also, oxidative stress increases in patients with chronic kidney disease, especially diabetic kidney disease (DCD). ROS-mediated renal inflammation and renal fibrosis can lead to diabetic kidney disease pathology through multiple signaling pathways including growth factor-β, connective tissue growth factor, Tnf-α, interleukin IL-1, IL-6, IL-18, and cell adhesion molecules contribute (72). Various markers of oxidative stress such as 8-hydroxy-2′-deoxyguanosine (8-OHdG), malondialdehyde (MDA), and advanced glycation end products (AGEs) are used to determine renal prognosis (73, 74).

The lung is exposed to high levels of oxygen, along with its large surface area and blood flow, and is susceptible to ROS-mediated injury (75). In allergic lung inflammation, ultrafine fraction plays an important role (76). Ultra-fine particles (UFPs, 0.1 mm diameter in thermodynamics) form during gas-to-particle conversion or incomplete fuel combustion processes (77). Compared to larger particles, they can accumulate at a higher rate in the peripheral lung and pass into the alveolar epithelium and produce reactive oxygen species (ROS) (78). Increased ROS levels trigger the release of the inflammatory mediator, induces, or increases nuclear factor-kB (NF-kB) activation of redox-sensitive transcription factors in the lung (79). Oxidative Stress also causes various lung diseases such as chronic obstructive pulmonary disease (COPD), bronchopulmonary dysplasia, asthma, idiopathic pulmonary fibrosis (IPF) and lung cancer (7).

Oxidative stress and accompanying inflammation play a critical role in the pathogenesis of pancreatitis (80). ROS / RNS directly attacks the cellular components of the pancreas and causes oxidative damage through activation of signaling cascades and long-term inflammatory cell uptake (81). Pro-inflammatory cytokines and oxidative stress stimulate common signal transduction pathways leading to amplification of the inflammatory cascade, mainly through activation of MAPK and NF-κB (82). Also, proinflammatory cytokines, specifically TNF-a and oxidative stress, promote each other by creating a vicious circle in acute pancreatitis.

ANTIOXIDANTS

Antioxidants are divided into two groups as enzymatic and non-enzymatic. The main enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX)(83). In addition to these major enzymes, thioredoxins (TRXs) such as heme oxygenase-1 (EC) and redox proteins, peroxiredoxins (PRXs), and glutathione reductase (GR), glucose 6-phosphate dehydrogenase (G6PDH), and glutathione sulfide transferase (GST) are antioxidants (84, 85). Non-enzymatic antioxidants include vitamins (α-tocopherol, ascorbic acid, and β-carotene), thiols (mainly glutathione, GSH), and various low molecular weight compounds such as lipoic acid, uric acid, and ubiquinone (17, 86).

Superoxide dismutase (SOD) is the first detoxification enzyme and the most powerful antioxidant in the cell (87). It catalyzes the conversion of two superoxide anion (O_2^-) molecules into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), making the potentially harmful superoxide anion less dangerous. SOD is a metalloenzyme and therefore requires a metal cofactor for its activity (88). Metal ions normally bound by SOD are iron (Fe), zinc (Zn) copper (Cu), and manganese (Mn) (89). Catalase and glutathione peroxidase help remove hydrogen peroxide. Catalase uses iron or manganese as its cofactor and catalyzes the reduction of hydrogen peroxide (H_2O_2) to water and molecular oxygen, ultimately completing the detoxification process created by SOD (90, 91). The Glutathione Peroxidase (GPx) system consists of several components, including glutathione peroxidase and glutathione reductase enzymes, and GSH and NADPH cofactors (92). These molecules together effectively remove hydrogen peroxide. The enzyme plays a more important role in preventing the lipid peroxidation process and therefore protects cells from oxidative stress (93).

Thioredoxins (Trx) are small redox-active proteins that complement the GSH system in protecting against oxidative stress (86). Oxidation of Trx1 or Trx2 releases kinase 1 (ASK1), which regulates apoptosis signaling and allows apoptosis initiation (94).

Glutathione is involved in two types of reactions during detoxification of ROS; (ı) GSH reacts non-enzymatically with radicals such as superoxide radical anion, nitric oxide, or hydroxyl radical (95), and (ıı) GSH is an electron donor for the reduction of peroxides in the GPx reaction (96). The final product of the oxidation of GSH is glutathione disulfide (GSSG) and intracellular GSH is regenerated from GSSG by the reaction catalyzed by glutathione reductase (GR) (97). Glutathione acts as a substrate or auxiliary substrate in enzymatic reactions and also reacts directly with free radicals and lipid peroxides (98). It is the main intracellular antioxidant in which oxidative stress is measured (3).

Vitamins directly cleanse ROS and regulate the activities of antioxidant enzymes. Among them, vitamin E has been recognized as one of the most important antioxidants. Vitamin E is a general term that includes all biologically active tocopherols and tocotrienols and their derivatives (99). Vitamin E inhibits the formation of lipid peroxyl radicals stimulated by ROS, thus protecting cells from peroxidation of PUFA in membrane phospholipids, oxidative damage of plasma, very low-density lipoprotein, cellular proteins, DNA, and membrane degeneration (100). It is α-tocopherol with the highest antioxidant effect. Also, vitamin E is traditionally thought to be the major non-enzymatic, small molecule antioxidant found in the lipid structures of cells (101).Vitamin C or ascorbic acid acts as a water-soluble free radical scavenger (102). Vitamin C protects against lipid peroxidation by clearing ROS in the aqueous phase before initiating lipid peroxidation (103). Vitamin C also performs against the oxidation of proteins and DNA and preserves their biological structures and functions (104). β-carotene reacts with peroxyl (ROO), hydroxyl (OH^-), and superoxide (O_2^-) radicals (105). Both carotenoids and retinoic acids (RAs) can regulate transcription factors. β -carotene inhibits the chain propagation effect of ROS by reacting with a peroxyl radical to form resonance-stabilized carbon-centered radicals within its conjugated alkyl structure (106). β-carotene inhibits oxidant-induced NF-kB activation and production of interleukin (IL)-6 and tumor necrosis factor-α (107).

Some diseases caused by Bacterial, Viral and Parasitic factors in animals and Antioxidants used in these diseases

Oxidative stress plays a role in various disease processes in animals, including sepsis, mastitis, acidosis, ketosis, enteritis, pneumonia, respiratory and joint diseases (108, 109). Microorganisms such as bacteria, viruses (110), and parasites (111) can increase the production of ROS in cells. Leptospirosis is considered to be a toxin-mediated disease leading to lipid peroxidation, as the membrane lipopolysaccharide plays a role in cytotoxicity (112). An increase in MDA and a decrease in GSH were observed in cattle affected by leptospirosis (113), indicating that oxidative stress plays a role in the pathogenesis of leptospirosis. It found a significant increase in malondialdehyde levels in sheep infected with *Pasteurella multocida* (114), as well as a significant decrease in superoxide dismutase, glutathione, and catalase levels. Also, Meral, Ercan (115) reported an increase in GPx, SOD, and total bilirubin activity in calves with septicemia due to *E. Coli.*

In dairy cows, infectious pathogens such as *Streptococcus agalactiae*, *Mycoplasma bovis* and *Staphylococcus aureus* and environmental pathogens such as *Escherichia coli* and *Klebsiella sp* cause mastitis (116). The pathogenesis of

mastitis involves an inflammatory reaction that results in response to many factors, including intra-mammary infections caused by microorganisms (117). During inflammatory conditions, phagocytes produce reactive oxygen species (ROS) necessary to kill bacteria (118). Oxidative stress can increase the adhesion of active neutrophils to mammary endothelial cells and intensify inflammation (119). Thus, mastitis can lead to increased formation of free radicals in milk that lead to oxidative stress (120). Also, during breastfeeding, the metabolic rate of mammary epithelial cells increases and therefore produces large amounts of reactive oxygen species (ROS) and lipid peroxides in vivo (121). In dairy cows, antioxidants and trace minerals play an important role in immune function (116). Micronutrients associated with antioxidant activity such as vitamin A, vitamin E, β-carotene, selenium, zinc, and copper have been examined in terms of their effects on mastitis (121, 122) and it has been reported that antioxidant supplementation may reduce the duration and severity of treatment in clinical mastitis (123, 124). In addition, Ascorbic acid is the most important water-soluble antioxidant in mammals (125).

Viruses can cause oxidative damage through direct effects on cells or indirect effects of host inflammatory responses (110). Bozukluhan, Merhan (126) found that haptoglobin, ceruloplasmin, urea, creatinine, MDA, and ALP, AST activities were significantly increased and albumin, Fe, and GSH concentrations were significantly decreased in sheep infected with sheep pox virus. Abou-Zeina, Nasr (127) determined that SOD and GPx activity and total antioxidant capacity (TAC) levels were significantly reduced in FMD infected sheep. Besides, Abou-Zeina, Nasr (127) reported that the administration of antioxidant preparations (zinc, methionine, Vitamin-E, selenium) to sheep with foot and mouth disease improves the overall health conditions and performance of the animals as it increases TAC and GPx activity and reduces DNA damage. Erkilic, Öğün (128) detected that while MDA and NO levels increased, GSH decreased in malignant catarrhal fever (MCF) disease in cattle. In dogs with distemper, Karadeniz, Hanedan (129) found significantly higher plasma concentrations of oxidant MDA, nitrates and nitrites, and ceruloplasmin, and detected that antioxidant concentrations were significantly reduced in the diseased group. In puppies infected with canine parvovirus, Elsayed, Kubesy (131) emphasized that MDA, H_2O_2, SOD, and GPX elevation and catalase, Zn, Cu, and iron decreased, and antioxidant supplementation could strengthen the body defense mechanism and reduce the stress state.

Parasitic diseases appear to be a causal source of oxidative stress in humans and animals (111, 132). Antioxidant systems containing vitamins have a cellular protective effect against oxidative stress caused by parasite infestation (133). The

intraerythrocytic parasite metabolizes hemoglobin and produces O_2^- in infection with *Theileria annulata* (134) and *Theileria sergenti* (135) in cattle, resulting in increased oxidative stress as shown by a significant increase in lipid peroxidation in erythrocytes (136). Grewal, Ahuja (134) reported a significant increase in erythrocytes G6PD and GSH-PX activities in cattle naturally infected with *T. annulata*. *Babesia spp*. is transmitted by ticks of small ruminants and causes fever, anemia, hemoglobinuria, and jaundice (137). Esmaeilnejad, Tavassoli (138) determined a significant decrease in GSH-Px, SOD, TAC, and CAT activities and a significant increase in MDA concentration in erythrocytes in sheep naturally infected with *Babesia ovis.* Kucukkurt, Cigerci (139) detected that DNA damage, MDA, protein carbonyl content (PCO), and nitric oxide (NOx) metabolites increased and total antioxidant activities (AOA) and glutathione (GSH) decreased in naturally infected goats with *Babesia ovis.* Esmaeilnejad, Tavassoli (140) determined an increase in SOD, MDA, protein carbonylation, DNA damage and a decrease in Catalase, G6PD, TAC levels in *Babesia bigemina* cattle. Vitamin E and selenium administration can be used as auxiliary therapeutic agents to regulate intravascular hemolysis caused by oxidative stress in bovine babesiosis. An increase in MDA level and a decrease in TAC and SOD levels were found in *Anaplasma ovis* (141) in goats and *Anaplasma marginale* (142) infections in cattle.

Production rates of free radicals increases in some parasitic infections, including *F. hepatica* infections in sheep (143). Saleh (143) found an increase in plasma MDA level and a decrease in plasma Albumin and ascorbate, and glutathione level in blood in sheep naturally infected with *F. Hepatica.* In naturally infected cattle with *Fasciola gigantica*, Bahrami, Esmaeilzadeh (144) determined an increase in liver tissue MDA level and a decrease in SOD and GPX. *Haemonchus contortus* (*H. contortus*) is one of the most pathogenic gastrointestinal nematodes of sheep and goats. Alam, Hassanen (145) found that serum total protein and albumin decreased and MDA increased in Haemonchus contortus in sheep and goats. Rashid and Irshadullah (146) found an increase in CAT, GST, glutathione GR, MDA, PC, and O2− levels and a decrease in GPx, GSH levels in the abomasal tissue of goats naturally infected with *Haemonchus contortus.*

References

1. Choi S, Benzie I, Collins A, Hannigan B, Strain J. Vitamins C and E: acute interactive effects on biomarkers of antioxidant defence and oxidative stress. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2004;551(1-2):109-17.
2. Altieri F, Grillo C, Maceroni M, Chichiarelli S. DNA damage and repair: from molecular mechanisms to health implications. Antioxidants & redox signaling. 2008;10(5):891-938.
3. Mandelker L. Oxidative stress, free radicals, and cellular damage. Studies on veterinary medicine: Springer; 2011. p. 1-17.
4. Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. Free radical biology and medicine. 2000;29(3-4):222-30.
5. Storz G, Imlayt JA. Oxidative stress. Current opinion in microbiology. 1999;2(2):188-94.
6. Betteridge DJ. What is oxidative stress? Metabolism-Clinical and Experimental. 2000;49(2):3-8.
7. Palipoch S, Koomhin P. Oxidative stress-associated pathology: a review. Sains Malaysiana. 2015;44(10):1441-51.
8. Finkel T. Oxidant signals and oxidative stress. Current opinion in cell biology. 2003;15(2):247-54.
9. Batot G, Martel C, Capdeville N, Wientjes F, Morel F. Characterization of neutrophil NADPH oxidase activity reconstituted in a cell-free assay using specific monoclonal antibodies raised against cytochrome b558. European journal of biochemistry. 1995;234(1):208-15.
10. Segal AW, Garcia R, Goldstone H, Cross A, Jones O. Cytochrome b-245 of neutrophils is also present in human monocytes, macrophages and eosinophils. Biochemical Journal. 1981;196(1):363-7.
11. Dupuy C, Virion A, Ohayon R, Kaniewski J, Deme D, Pommier J. Mechanism of hydrogen peroxide formation catalyzed by NADPH oxidase in thyroid plasma membrane. Journal of Biological Chemistry. 1991;266(6):3739-43.
12. Gutteridge J. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clinical chemistry. 1995;41(12):1819-28.
13. Harrison J, Schultz J. Studies on the chlorinating activity of myeloperoxidase. Journal of Biological Chemistry. 1976;251(5):1371-4.
14. Klebanoff SJ. Myeloperoxidase-halide-hydrogen peroxide antibacterial system. Journal of bacteriology. 1968;95(6):2131-8.

15. Haber F, Weiss J. The catalytic decomposition of hydrogen peroxide by iron salts. Proceedings of the Royal Society of London Series A-Mathematical and Physical Sciences. 1934;147(861):332-51.
16. Fenton H. LXXIII.—Oxidation of tartaric acid in presence of iron. Journal of the Chemical Society, Transactions. 1894;65:899-910.
17. Ozougwu JC. The role of reactive oxygen species and antioxidants in oxidative stress. International Journal of Research. 2016;1.
18. Grimsrud PA, Xie H, Griffin TJ, Bernlohr DA. Oxidative stress and covalent modification of protein with bioactive aldehydes. Journal of Biological Chemistry. 2008;283(32):21837-41.
19. Burton GJ, Jauniaux E. Oxidative stress. Best practice & research Clinical obstetrics & gynaecology. 2011;25(3):287-99.
20. Toyokuni S. Reactive oxygen species-induced molecular damage and its application in pathology. Pathology international. 1999;49(2):91-102.
21. Kasai H. Chemistry-based studies on oxidative DNA damage: formation, repair, and mutagenesis. Free Radical Biology and Medicine. 2002;33(4):450-6.
22. Kim J-E, Choi S, Yoo J-A, Chung M-H. 8-Oxoguanine induces intramolecular DNA damage but free 8-oxoguanine protects intermolecular DNA from oxidative stress. FEBS letters. 2004;556(1-3):104-10.
23. Bohr VA, Dianov GL. Oxidative DNA damage processing in nuclear and mitochondrial DNA. Biochimie. 1999;81(1-2):155-60.
24. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2′-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. Journal of environmental science and health Part C. 2009;27(2):120-39.
25. Cabiscol Català E, Tamarit Sumalla J, Ros Salvador J. Oxidative stress in bacteria and protein damage by reactive oxygen species. International Microbiology, 2000, vol 3, núm 1, p 3-8. 2000.
26. Niki E, Yoshida Y, Saito Y, Noguchi N. Lipid peroxidation: mechanisms, inhibition, and biological effects. Biochemical and biophysical research communications. 2005;338(1):668-76.
27. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative medicine and cellular longevity. 2014;2014.
28. Porter NA, Caldwell SE, Mills KA. Mechanisms of free radical oxidation of unsaturated lipids. Lipids. 1995;30(4):277-90.
29. Humphries KM, Szweda LI. Selective inactivation of α-ketoglutarate dehydrogenase and pyruvate dehydrogenase: reaction of lipoic acid with

4-hydroxy-2-nonenal. Biochemistry. 1998;37(45):15835-41.
30. Min B, Ahn D. Mechanism of lipid peroxidation in meat and meat products-A review. Food Science and Biotechnology. 2005;14(1):152-63.
31. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free radical Biology and medicine. 1991;11(1):81-128.
32. Sowell J, Conway HM, Bruno RS, Traber MG, Frei B, Stevens JF. Ascorbylated 4-hydroxy-2-nonenal as a potential biomarker of oxidative stress response. Journal of Chromatography B. 2005;827(1):139-45.
33. Benedetti A, Comporti M, Esterbauer H. Identification of 4-hydroxynonenal as a cytotoxic product originating from the peroxidation of liver microsomal lipids. Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism. 1980;620(2):281-96.
34. 3Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods in enzymology. 186: Elsevier; 1990. p. 407-21.
35. Fucci L, Oliver CN, Coon MJ, Stadtman ER. Inactivation of key metabolic enzymes by mixed-function oxidation reactions: possible implication in protein turnover and ageing. Proceedings of the National Academy of Sciences. 1983;80(6):1521-5.
36. Stadtman ER. Metal ion-catalyzed oxidation of proteins: biochemical mechanism and biological consequences. Free Radical Biology and Medicine. 1990;9(4):315-25.
37. Stadtman E, Berlett B. Fenton chemistry. Amino acid oxidation. Journal of Biological Chemistry. 1991;266(26):17201-11.
38. Stadtman ER, Levine RL. Protein oxidation. Annals of the New York Academy of Sciences. 2000;899(1):191-208.
39. Selmeci L, Székely M, Soós P, Seres L, Klinga N, Geiger A, et al. Human blood plasma advanced oxidation protein products (AOPP) correlates with fibrinogen levels. Free radical research. 2006;40(9):952-8.
40. Dean RT, FU S, Stocker R, Davies MJ. Biochemistry and pathology of radical-mediated protein oxidation. Biochemical Journal. 1997;324(1):1-18.
41. Poli G, Leonarduzzi G, Biasi F, Chiarpotto E. Oxidative stress and cell signalling. Current medicinal chemistry. 2004;11(9):1163-82.
42. Matsuzawa A, Ichijo H. Stress-responsive protein kinases in redox-regulated apoptosis signaling. Antioxidants & redox signaling. 2005;7(3-4):472-81.

43. Blenis J. Signal transduction via the MAP kinases: proceed at your own RSK. Proceedings of the National Academy of Sciences. 1993;90(13):5889-92.
44. Singh R, Czaja MJ. Regulation of hepatocyte apoptosis by oxidative stress. Journal of gastroenterology and hepatology. 2007;22:45-8.
45. Abe J-i, Kusuhara M, Ulevitch RJ, Berk BC, Lee J-D. Big mitogen-activated protein kinase 1 (BMK1) is a redox-sensitive kinase. Journal of Biological Chemistry. 1996;271(28):16586-90.
46. Zhougang S, Schnellmann RG. H2O2-induced transactivation of EGF receptor requires Src and mediates ERK1/2, but not Akt, activation in renal cells. American Journal of Physiology-Renal Physiology. 2004;286(5):F858-F65.
47. Knebel A, Rahmsdorf HJ, Ullrich A, Herrlich P. Dephosphorylation of receptor tyrosine kinases as target of regulation by radiation, oxidants or alkylating agents. The EMBO journal. 1996;15(19):5314-25.
48. Meyer CF, Wang X, Chang C, Templeton D, Tan T-H. Interaction between c-Rel and the mitogen-activated protein kinase kinase kinase 1 signaling cascade in mediating B enhancer activation. Journal of Biological Chemistry. 1996;271(15):8971-6.
49. Van den Berg R, Haenen G, Van den Berg H, Bast A. Transcription factor NF-κB as a potential biomarker for oxidative stress. British Journal of Nutrition. 2001;86(S1):121-7.
50. Moniruzzaman M, Ghosal I, Das D, Chakraborty SB. Melatonin ameliorates H2O2-induced oxidative stress through modulation of Erk/Akt/NFkB pathway. Biological research. 2018;51.
51. Genestra M. Oxyl radicals, redox-sensitive signalling cascades and antioxidants. Cellular signalling. 2007;19(9):1807-19.
52. Ward PA. Role of Complement, Chemokines, & Regulatory Cytokines in Acute Lung Injury. 1996.
53. Klatt P, Molina EP, de Lacoba MG, Padilla CA, Martínez-Galisteo E, Barcena J, et al. Redox regulation of c-Jun DNA binding by reversible S-glutathiolation. The FASEB Journal. 1999;13(12):1481-90.
54. Meyer M, Schreck R, Baeuerle PA. H2O2 and antioxidants have opposite effects on activation of NF-kappa B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. The EMBO journal. 1993;12(5):2005-15.
55. Singh U, Jialal I. Oxidative stress and atherosclerosis. Pathophysiology. 2006;13(3):129-42.

56. Bonomini F, Tengattini S, Fabiano A, Bianchi R, Rezzani R. Atherosclerosis and oxidative stress. Histology and histopathology. 2008;23:381-90.
57. Keaney Jr JF. Oxidative stress and the vascular wall: NADPH oxidases take center stage. Am Heart Assoc; 2005.
58. Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. Arteriosclerosis, thrombosis, and vascular biology. 2005;25(1):29-38.
59. Sun GY, Horrocks LA, Farooqui AA. The roles of NADPH oxidase and phospholipases A2 in oxidative and inflammatory responses in neurodegenerative diseases. Journal of neurochemistry. 2007;103(1):1-16.
60. Dincel GC, Atmaca HT. Role of oxidative stress in the pathophysiology of Toxoplasma gondii infection. International journal of immunopathology and pharmacology. 2016;29(2):226-40.
61. Halliwell B. Oxidative stress and neurodegeneration: where are we now? Journal of neurochemistry. 2006;97(6):1634-58.
62. Özmen İ, Nazıroğlu M, Alici HA, Şahin F, Cengiz M, Eren I. Spinal morphine administration reduces the fatty acid contents in spinal cord and brain by increasing oxidative stress. Neurochemical Research. 2007;32(1):19-25.
63. Ong W-Y, Lu X-R, Hu C-Y, Halliwell B. Distribution of hydroxynonenal-modified proteins in the kainate-lesioned rat hippocampus: evidence that hydroxynonenal formation precedes neuronal cell death. Free Radical Biology and Medicine. 2000;28(8):1214-21.
64. Mark RJ, Lovell MA, Markesbery WR, Uchida K, Mattson MP. A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid β-peptide. Journal of neurochemistry. 1997;68(1):255-64.
65. Sánchez-Valle V, C Chavez-Tapia N, Uribe M, Méndez-Sánchez N. Role of oxidative stress and molecular changes in liver fibrosis: a review. Current medicinal chemistry. 2012;19(28):4850-60.
66. Gebhardt R. Oxidative stress, plant-derived antioxidants and liver fibrosis. Planta medica. 2002;68(04):289-96.
67. Li S, Tan H-Y, Wang N, Zhang Z-J, Lao L, Wong C-W, et al. The role of oxidative stress and antioxidants in liver diseases. International journal of molecular sciences. 2015;16(11):26087-124.
68. Sakaguchi S, Takahashi S, Sasaki T, Kumagai T, Nagata K. Progression of alcoholic or non-alcoholic steatohepatitis; common metabolic aspects of innate immune system and oxidative stress. Drug metabolism and phar-

macokinetics. 2010:1-62.
69. Baud L, Ardaillou R. Involvement of reactive oxygen species in kidney damage. British medical bulletin. 1993;49(3):621-9.
70. Sureshbabu A, Ryter SW, Choi ME. Oxidative stress and autophagy: crucial modulators of kidney injury. Redox biology. 2015;4:208-14.
71. Honda T, Hirakawa Y, Nangaku M. The role of oxidative stress and hypoxia in renal disease. Kidney Research and Clinical Practice. 2019;38(4):414.
72. Elmarakby AA, Sullivan JC. Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. Cardiovascular therapeutics. 2012;30(1):49-59.
73. Xu G, Yao Q, Weng Q, Su B, Zhang X, Xiong J. Study of urinary 8-hydroxydeoxyguanosine as a biomarker of oxidative DNA damage in diabetic nephropathy patients. Journal of pharmaceutical and biomedical analysis. 2004;36(1):101-4.
74. Smit AJ, Gerrits EG. Skin autofluorescence as a measure of advanced glycation endproduct deposition: a novel risk marker in chronic kidney disease. Current opinion in nephrology and hypertension. 2010;19(6):527-33.
75. Park HS, Kim SR, Lee YC. Impact of oxidative stress on lung diseases. Respirology. 2009;14(1):27-38.
76. Alessandrini F, Schulz H, Takenaka S, Lentner B, Karg E, Behrendt H, et al. Effects of ultrafine carbon particle inhalation on allergic inflammation of the lung. Journal of Allergy and Clinical Immunology. 2006;117(4):824-30.
77. Lighty JS, Veranth JM, Sarofim AF. Combustion aerosols: factors governing their size and composition and implications to human health. Journal of the Air & Waste Management Association. 2000;50(9):1565-618.
78. Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, et al. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environmental health perspectives. 2003;111(4):455-60.
79. Shukla A, Timblin C, BeruBe K, Gordon T, McKinney W, Driscoll K, et al. Inhaled particulate matter causes expression of nuclear factor (NF)-κ B–related genes and oxidant-dependent NF-κ B activation in vitro. American journal of respiratory cell and molecular biology. 2000;23(2):182-7.
80. Robles L, Vaziri ND, Ichii H. Role of oxidative stress in the pathogenesis of pancreatitis: effect of antioxidant therapy. Pancreatic disorders & therapy. 2013;3(1):112.
81. Leung PS, Chan YC. Role of oxidative stress in pancreatic inflammation.

Antioxidants & redox signaling. 2009;11(1):135-66.
82. Pereda J, Sabater L, Aparisi L, Escobar J, Sandoval J, Viña J, et al. Interaction between cytokines and oxidative stress in acute pancreatitis. Current medicinal chemistry. 2006;13(23):2775-87.
83. Lubrano V, Balzan S. Enzymatic antioxidant system in vascular inflammation and coronary artery disease. World journal of experimental medicine. 2015;5(4):218.
84. Ji LL, Leeuwenburgh C, Leichtweis S, Gore M, Fiebig R, Hollander J, et al. Oxidative stress and aging: role of exercise and its influences on antioxidant systems. Annals of the New York Academy of Sciences. 1998;854(1):102-17.
85. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organization Journal. 2012;5(1):9-19.
86. Formigari A, Irato P, Santon A. Zinc, antioxidant systems and metallothionein in metal mediated-apoptosis: biochemical and cytochemical aspects. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2007;146(4):443-59.
87. Storey KB. Oxidative stress: animal adaptations in nature. Brazilian Journal of Medical and Biological Research. 1996;29:1715-33.
88. Santos-Sánchez NF, Salas-Coronado R, Villanueva-Cañongo C, Hernández-Carlos B. Antioxidant compounds and their antioxidant mechanism. Antioxidants: IntechOpen; 2019.
89. Ighodaro O, Akinloye O. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria journal of medicine. 2018;54(4):287-93.
90. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. Cellular and Molecular Life Sciences CMLS. 2004;61(2):192-208.
91. Marklund SL. Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. Biochemical Journal. 1984;222(3):649-55.
92. Cederbaum AI, Lu Y, Wu D. Role of oxidative stress in alcohol-induced liver injury. Archives of toxicology. 2009;83(6):519-48.
93. Kehrer JP. Free radicals as mediators of tissue injury and disease. Critical reviews in toxicology. 1993;23(1):21-48.
94. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, et al.

Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. The EMBO journal. 1998;17(9):2596-606.
95. Singh S, Wishnok J, Keshive M, Deen W, Tannenbaum S. The chemistry of the S-nitrosoglutathione/glutathione system. Proceedings of the National Academy of Sciences. 1996;93(25):14428-33.
96. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiological reviews. 1979;59(3):527-605.
97. Dringen R. Metabolism and functions of glutathione in brain. Progress in neurobiology. 2000;62(6):649-71.
98. Briviba K, Sies H. Nonenzymatic antioxidant defense systems. Natural antioxidants in human health and disease. 1994:107-28.
99. Vrolijk MF, Opperhuizen A, Jansen EH, Godschalk RW, Van Schooten FJ, Bast A, et al. The shifting perception on antioxidants: The case of vitamin E and β-carotene. Redox biology. 2015;4:272-8.
100. Topinka J, Binkova B, Sram R, Erin A. The influence of α-tocopherol and pyritinol on oxidative DNA damage and lipid peroxidation in human lymphocytes. Mutation Research Letters. 1989;225(3):131-6.
101. Burton GW, Joyce A, Ingold KU. Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? Archives of biochemistry and biophysics. 1983;221(1):281-90.
102. Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. Rsc Advances. 2015;5(35):27986-8006.
103. Conklin KA. Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects. Nutrition and cancer. 2000;37(1):1-18.
104. Rodrigo R, Guichard C, Charles R. Clinical pharmacology and therapeutic use of antioxidant vitamins. Fundamental & clinical pharmacology. 2007;21(2):111-27.
105. El-Agamey A, Lowe GM, McGarvey DJ, Mortensen A, Phillip DM, Truscott TG, et al. Carotenoid radical chemistry and antioxidant/pro-oxidant properties. Archives of biochemistry and biophysics. 2004;430(1):37-48.
106. Fang Y-Z, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002;18(10):872-9.
107. Niles RM. Signaling pathways in retinoid chemoprevention and treatment of cancer. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2004;555(1-2):97-105.
108. Celi P. Biomarkers of oxidative stress in ruminant medicine. Immunopharmacology and Immunotoxicology. 2011;33(2):233-40.

109. 109. Lykkesfeldt J, Svendsen O. Oxidants and antioxidants in disease: oxidative stress in farm animals. The veterinary journal. 2007;173(3):502-11.
110. Valyi-Nagy T, Dermody TS. Role of oxidative damage in the pathogenesis of viral infections of the nervous system. Histology and histopathology. 2005;20:957-67.
111. Stocker R, Hunt NH, Buffinton GD, Weidemann MJ, Lewis-Hughes PH, Clark IA. Oxidative stress and protective mechanisms in erythrocytes in relation to Plasmodium vinckei load. Proceedings of the National Academy of Sciences. 1985;82(2):548-51.
112. Kim YG, Jeon DY, Yang MK. Superoxide dismutase activity and lipid peroxidation in the liver of guinea pig infected with Leptospira interrogans. Free radical research. 1997;26(1):1-6.
113. Erdogan H, Karapehlivan M, Citil M, Atakisi O, Uzlu E, Unver A. Serum sialic acid and oxidative stress parameters changes in cattle with leptospirosis. Veterinary research communications. 2008;32(4):333-9.
114. El-Deeb WM, Elmoslemany A. The diagnostic accuracy of oxidative stress biomarkers in sheep with pneumonic pasteurellosis. Comparative Clinical Pathology. 2017;26(2):363-7.
115. Meral Ö, Ercan N, Fidancı UR. Septisemili buzağılarda lipid peroksidasyon düzeyi ve antioksidan enzim aktiviteleri. Ankara Üniv Vet Fak Derg. 2017;64:161-4.
116. Bruno DR, editor Mastitis, mammary gland immunity, and nutrition. Mid-South Ruminant Nutrition Conference; 2010.
117. 1Turk R, Piras C, Kovačić M, Samardžija M, Ahmed H, De Canio M, et al. Proteomics of inflammatory and oxidative stress response in cows with subclinical and clinical mastitis. Journal of proteomics. 2012;75(14):4412-28.
118. Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2000;279(6):L1005-L28.
119. Maddox JF, Aherne KM, Reddy CC, Sordillo LM. Increased neutrophil adherence and adhesion molecule mRNA expression in endothelial cells during selenium deficiency. Journal of leukocyte biology. 1999;65(5):658-64.
120. Gu B, Zhu Y, Zhu W, Miao J, Yu'E D, Zou S. Retinoid protects rats against neutrophil-induced oxidative stress in acute experimental mastitis. International Immunopharmacology. 2009;9(2):223-9.

121. Jin L, Yan S, Shi B, Bao H, Gong J, Guo X, et al. Effects of vitamin A on the milk performance, antioxidant functions and immune functions of dairy cows. Animal Feed Science and Technology. 2014;192:15-23.
122. Hogan J, Weiss W, Smith K. Role of vitamin E and selenium in host defense against mastitis. Journal of Dairy Science. 1993;76(9):2795-803.
123. Smith KL, Harrison J, Hancock D, Todhunter D, Conrad H. Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. Journal of dairy science. 1984;67(6):1293-300.
124. Abd Ellah MR. Role of free radicals and antioxidants in mastitis. Journal of Advanced Veterinary Research. 2013;3(1):1-7.
125. Sauberlich HE. Pharmacology of vitamin C. Annual review of nutrition. 1994;14(1):371-91.
126. Bozukluhan K, Merhan O, Gökçe Hİ, Öğün M, Atakişi E, Kızıltepe Ş, et al. Determination of Some Acute Phase Proteins, Biochemical Parameters and Oxidative Stress in Sheep with Naturally Infected Sheeppox Virus. Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 2018;24(3).
127. Abou-Zeina HA, Nasr SM, Nassar SA, Farag TK, El-Bayoumy MK, Ata EB, et al. Beneficial effects of antioxidants in improving health conditions of sheep infected with foot-and-mouth disease. Tropical animal health and production. 2019;51(8):2379-86.
128. Erkilic EE, Öğün M, Kırmızıgül AH, Adali Y, Ermutlu CŞ, Eroğlu HA, et al. Determination of some oxidative stress and inflammation markers in serum, blood and CSF in cattle with head-eye form of malignant catarrhal fever. Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 2017;23(4).
129. Karadeniz A, Hanedan B, Cemek M, Borku M. Relationship between canine distemper and oxidative stress in dogs. Revue Med Vet. 2008;159:462-7.
130. Aydoğdu U, Coşkun A, Başbuğ O, Ağaoğlu ZT. Parvoviral enteritisli köpeklerde total oksidan-antioksidan durum ile oksidatif stres indeksinin değerlendirilmesi. FÜ Sağlık Bil Vet Derg. 2018;32(3):161-4.
131. Elsayed NM, Kubesy A, Salem NY. Altered blood oxidative stress biomarkers in association with canine parvovirus enteritis. Comparative Clinical Pathology. 2020;29(2):355-9.
132. Selkirk ME, Smith VP, Thomas GR, Gounaris K. Resistance of filarial nematode parasites to oxidative stress. International journal for parasitology. 1998;28(9):1315-32.
133. Smith N, Bryant C. Free radical generation during primary infections

with Nippostrongylus brasiliensis. Parasite immunology. 1989;11(2):147-60.
134. Grewal A, Ahuja C, Singha S, Chaudhary K. Status of lipid peroxidation, some antioxidant enzymes and erythrocytic fragility of crossbred cattle naturally infected with Theileria annulata. Veterinary Research Communications. 2005;29(5):387-94.
135. Shiono H, Yagi Y, Chikayama Y, Miyazaki S, Nakamura I. Oxidative damage and phosphatidylserine expression of red blood cells in cattle experimentally infected with Theileriasergenti. Parasitology Research. 2003;89(3):228-34.
136. Ginsburg H, Atamina H. The redox status of malaria-infected erythrocytes: an overview with an emphasis on unresolved problems. Parasite. 1994;1(1):5-13.
137. Razmi G, Naghibi A, Aslani M, Dastjerdi K, Hossieni H. An epidemiological study on Babesia infection in small ruminants in Mashhad suburb, Khorasan province, Iran. Small Ruminant Research. 2003;50(1-2):39-44.
138. Esmaeilnejad B, Tavassoli M, Asri-Rezaei S, Dalir-Naghadeh B. Evaluation of antioxidant status and oxidative stress in sheep naturally infected with Babesia ovis. Veterinary parasitology. 2012;185(2-4):124-30.
139. Kucukkurt I, Cigerci IH, Sinan I, Kozan E, Aytekin I, Eryavuz A, et al. The effects of babesiosis on oxidative stress and DNA damage in Anatolian black goats naturally infected with Babesia ovis. Iranian journal of parasitology. 2014;9(1):90.
140. Esmaeilnejad B, Tavassoli M, Dalir-Naghadeh B, Samiei A, Rajabi S, Mohammadi V, et al. Status of oxidative stress, trace elements, sialic acid and cholinesterase activity in cattle naturally infected with Babesia bigemina. Comparative Immunology, Microbiology and Infectious Diseases. 2020:101503.
141. Jalali SM, Bahrami S, Rasooli A, Hasanvand S. Evaluation of oxidant/antioxidant status, trace mineral levels, and erythrocyte osmotic fragility in goats naturally infected with Anaplasma ovis. Tropical animal health and production. 2016;48(6):1175-81.
142. Esmaeilnejad B, Tavassoli M, Samiei A, Hajipour N, Imani-Baran A, Farhang-Pajuh F. Evaluation of oxidative stress and antioxidant status, serum trace mineral levels and cholinesterases activity in cattle infected with Anaplasma marginale. Microbial pathogenesis. 2018;123:402-9.
143. Saleh MA. Circulating oxidative stress status in desert sheep naturally infected with Fasciola hepatica. Veterinary parasitology. 2008;154(3-4):262-9.

144. Bahrami S, Esmaeilzadeh S, Oryan A. Role of oxidative stress in concomitant occurrence of Fasciola gigantica and leiomyoma in cattle. Veterinary Parasitology. 2014;203(1-2):43-50.
145. Alam RT, Hassanen EA, El-Mandrawy SA. Heamonchus Contortus infection in Sheep and Goats: alterations in haematological, biochemical, immunological, trace element and oxidative stress markers. Journal of Applied Animal Research. 2020;48(1):357-64.
146. Rashid S, Irshadullah M. Evaluation of antioxidant and oxidant status of goats (Capra aegagrus hircus) naturally infected with Haemonchus contortus. Journal of helminthology. 2019;94:1-6.

Chapter-2

SELF-WORTH CONCEPT

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Individuals can maintain a healthy psychological life terms are expected to have a strong self-worth doubt. A healthy self-worth development is of great importance for individuals to have a better quality and effective life in personal, social, academic and professional life. It is thought that having a theoretical knowledge on this subject will benefit the lives of individuals in order to gain awareness about self-worth and to develop healthy self-value. Therefore, in this section, the subject of self-value will be tried to be presented in all aspects.

1. SELF-WORTH

The concept of self-worth has an important place in our lives and is the meaning we attach to our place in life. Self-worth is the belief that one's own mind and skills are sufficient for a happy life. Individuals' sense of self-worth is the personal evaluation of the individual's reaction to what they think (1).

Self-worth is an individual's self-evaluation and reflection in line with his abilities and limitations. People strive to maintain, strengthen and protect their self-esteem and for this they identify areas that will support their own value. The person tries to see himself / herself successful in areas where his / her own importance and value determines. That is, a person's opinion of his / her own worth is a situational x is the domain where one attaches its value when one expresses "I am valuable if I succeed at x" or "I am worthless if I fail at x" (1-3).Considering that the person feels good with the increase in self-worth or the person feels bad with the decrease in Self-worth, Self-value is one of the elements that provide psychological well-being for us.

Self-worth; It affects our self-esteem, desires, perception, emotions, behavior, and provides us with standards for what we should do to feel valued. For example, a high school student who feels valuable with the approval of others may smoke in order to be among his friends, or a woman who cares very much about her physical appearance may go to continuous exercise (1, 2, 4, 5).

2. OVERVIEW OF THE THEORIES RELATED TO SELF-WORTH

Martin Covington mentioned Self-Value Theory in 1992. According to this theory, putting the sense of self at the center of the desires and behaviors of human beings and the meaning of these in their own right as self-value, he mentioned that human beings always seek the approval of others in order to find meaning in their lives. Covington said that the individual tends to present a positive self image while seeking the approval of others (6).

According to self-worth theory, self-worth is measured by academic success. Self-worth is a structure that increases the quality of school success, provides

positive motivation, and also increases the desire to learn and teach. Individuals tend to attribute their success to themselves and their failures to external factors. When a person experiences an academic failure, he / she engages in various behaviors including attributing this failure to others, using the denial defense mechanism, comparing his / her performance with those who perform better than him / her (6).

Halter (1990) expresses the concept of self-worth as an individual's awareness of his / her own skills and self-evaluation in line with these skills. William James (1890) emphasized that human beings want to know what they are as human beings and their worth, and that they strive to achieve success and avoid failure. Croker and Wolfe structured James's thought and introduced the conditional-value model in 2001. According to this model, they defined the variability in self-esteem as the result of positive and negative events experienced in the fields of conditional self-worth. According to the Self-Worth Model they developed, individuals structure their self-worth in certain areas, and self-worth mostly affects self-evaluations related to these areas. These areas in which the individual constructs self-worth are closely related to the individual's personal standards and goals (1). For example; Individuals who structure their self-worth in the field of academic achievement attach great importance to academic achievement in the process of structuring self-worth, and it is very important for people to evaluate the grades they get from the lessons and the academic level of their teachers (1, 7).

Croker and Wolfe (2001) explained that a person's sense of self-worth is not only dependent on success and failure in these areas, but also by complying with the standards he sets for self-worth areas. For example, if a person has structured his self-worth in the field of virtue, he will follow the moral rules and shape his life through these rules (1).

According to Crocker et al. (2003), there are seven possible areas in which an individual can structure self-worth. These areas are; Obtaining approval (Other's approval), family support, physical appearance (Appearence), competition (Competition), academic competence (Academiccompetency), God's love and virtue (Virtue). The area structured according to this model varies from individual to individual, and the person can structure self-worth in one area as well as in several areas (7).

Getting approval (Other's approval): Those who construct self-value in this area are people who need to be accepted and approved by others. Therefore, they are concerned with the thoughts of others about themselves (1, 7). VanDellen et al. (2009), in his study, argued that it connects directly or indirectly to the social evaluation of others, regardless of the field in which it constructs self-worth (8).

Developmentally, a child needs parental approval, but as a person matures, he or she must believe that their success comes from their own competence rather than the approval of others. Psychological problems arise when the person overlaps the self-worth field with the approval of others. In a study, it was found that narcissists are structured on getting their self-worth of approval (9). When he is intensely involved with gaining approval from others, fear of negative evaluation begins and the person may experience social anxiety disorder and timidity.

Family support: The people who structure their self-worth into family support are those who care about their family's love and support (1, 7). As a matter of fact, family support is very important in terms of mental health. There are studies showing a significant relationship between depression and depression (10-12).

Physical appearance (Appearence): The people who construct self-worth in this area are people who attach great importance to their external view. Individuals are very concerned with the style, weight, height of their hair and their facial beauty. The more satisfied a person is with his or her appearance, the more Self-worth is (1). People who attribute the source of self-worth to their physical appearance are people who are less satisfied with their appearance and constantly watch over the bodies (13). Studies have found a significant relationship between structuring self-worth into physical appearance and eating disorder, depression (14-16).

Competition: Individuals who structure their self-worth in the field of competition are more concerned with the success of others than their own competences. He constantly compares himself to others. In a study by Eroğlu and Güler (2015), the field of competition, bullying and victimization were positively interpreted, and these individuals carried their competition to the virtual environment and their efforts to cyber bullying against their competitors and they may be exposed to cyberbullying in this process (17).

Academic competency: Studies have found that people who condition their self-worth to academic competence focus on performance / achievement rather than learning (18). It is emphasized that they feel worthless and inferior (1, 9, 19).

God's love: People who structure their self-value according to the love of God measure their values with the love of God. The individual will feel valuable and psychologically well when he believes that God loves and values him (1, 7, 20).

Virtue (Virtue): Individuals who structure their self-worth in this area measure their values with virtuous behavior. A person feels worthless when he acts contrary to his own set of moral standards. The field of virtue provides regulatory criteria for what an individual should do in order to feel valued. These individuals tend to behave positively in social terms (1, 7, 20).

Conditional self-worth has important effects on the achievement of one's goals and self-regulation. Because, in order to increase self-esteem, the individual tries to escape from any situation that would endanger his self-worth and not fail, so that his condition self-value can be highly motivating and allows the person to move towards his goals (21).

Externally conditioned self-worth is highly dependent on the satisfaction of others (gaining approval, academic achievement, physical appearance), while intrinsic conditional self-worth depends on one's own inner qualities (love of God, family support, and virtue). Since intrinsic conditional self-worth is not tied to external conditions, it has been observed that people who attribute self-worth to internal conditions, such as love of God, have higher psychological well-being and self-esteem (9)..Individuals who structure self-worth according to external domains have been reported to have low mental well-being, more prone to depression, and behave unstable because they attribute self-worth to someone else's approval (2, 9).

When the literature is examined, it has been argued that when constructing the self-worth of the individual, it can be added in different fields than these seven fields. For example, wanting to be good in romantic relationships, being able to maintain the relationship, may mean that that person structures their self-worth in the field of competence in romantic relationships. (22).

3. SELF-WORTH DEVELOPMENT

3.a HEALTHY SELF-WORTH DEVELOPMENT

Baumeister (1998) suggested that individuals who develop a healthy sense of self-worth face failure or continue to feel valuable after failure. It is suggested that individuals develop their sense of self-worth, determined at the end of the socialization process, and that cultural norms, developmental experiences, peer influence, modeling and parent-child relationships are effective in this process. In the first 5-6 years, the child's self-worth is shaped by the family. As the child starts school, external forces (friends, teachers, etc.) start to be effective in the structuring of self-worth. The experiences of the individual determine the area in which he constructs his self-worth, and he seeks a social environment that will feel valuable with the effect of these experiences (1).

The fact that the factors that individuals take into account and are affected by while constructing their ego are different determines under which conditions a person will feel valuable and thus finds his self-worth in which area to be structured. A healthy sense of self-worth will develop in the individual by analyzing

his / her weaknesses and strengths correctly, based on his / her abilities and establishing harmonious relationships with the social group they are in (1).

The attachment theory developed by Bowlby in 1969; It is an approach that explains that the bond between the child and the parents affects the child's sense of trust and psychological development. According to this theory, parental behaviors and interaction style guide one's expectations, beliefs and attitudes in close relationships in later years. According to Bowlby, when a secure bond is established between mother and child, the feeling of "precious self" will develop in the child. Therefore, a healthy sense of self-worth will be created. In a study conducted by Park et al. (2004), it was argued that individuals' attachment styles are influenced in the self-value structuring process. He found that people with secure attachment style are people who do not attach their values to external areas and that these individuals structure their self-worth most in the area of family support. These individuals build their self-worth in the field of family support, as they are able to establish close relationships and interpersonal relationships that are not dependent on the behavior and reactions of others. People with obsessive attachment style will structure their self-worth in the field of getting approval, as they are people who feel worthless, do not feel worthy of love, and always expect positive evaluations in their close relationships. As a matter of fact, in the study conducted by Park et al. (2004), it was found that obsessive attachment people structured their self-worth in the field of getting approval and physical appearance. Individuals with indifferent attachment style are individuals who avoid establishing relationships. Therefore, they are not expected to construct their self-values into areas of family support, approval, and love of God. In addition, it was found in the article that individuals with avoidant attachment style have a low level of structuring their self-worth in the areas of gaining approval and love of God (23) . It was found that people with anxious attachment style attribute their self-worth to social approval (23, 24).

Culture is one of the factors that affect in which area one constructs self-worth. For example, in a society where family is important, individual self-worth will be built on family support, and love of God and virtue will be more important for individuals living in religious communities (3). Liu et al. (2017) conducted a study based on the low subjective well-being of Easterners from the West and one of the factors affecting this is related to getting approval from others. As a result; They found that getting approval from self-worth domains was higher for Taiwanese respondents than American respondents, and subjective well-being was lower (25).

3.b UNHEALTHY SELF-WORTH DEVELOPMENT

Individuals generally try not to fail in areas where they attach their self-worth and effectively fulfill their associated goals in these areas (1). Gecas (1982) reported that individuals who develop a healthy sense of self-worth protect their self-worth when they feel threatened and create a consistent self-image (26).

When the person fails in the field in which he attaches his self-value, his immediate abandonment of the field in which he constructs his self-value or his focus on the area where he has structured his self-value is a sign that the sense of self-value develops unhealthy. This situation makes it difficult for the individual to adapt to life and causes psychological problems. One should strive to be successful in the field in which he structures his self-worth, struggle when faced with failure, but should not see this situation as a threat to his self and be overly attached to it. An example may be that a child who fails in his lessons leaves school immediately.

In studies on self-worth, it was found that people who develop healthy and high self-worth have positive affect, have high life satisfaction and hopelessness, and are happy (Crocker ,et al.,2004).

When the literature is examined, there are different reasons for unhealthy self-worth development. These reasons; It takes the form of structuring self-value in the wrong domain, structuring self-value into external fields, the number of fields in which self-value is structured, and unstable structuring of self-value.

1. Constructing self-worth in the wrong domain: The domain in which the individual structures self-worth provides regulatory standards for what an individual must do to feel valued and important. Therefore, the person will not comply with these standards that you have configured in the wrong field, will face constant failure and feel inadequate (Crocker & Park, 2004).

2. Structuring self-value into external domains: The unstable structure of self-worth feelings of individuals who structure their self-value in the external domain is displayed. The unstable nature of self-worth leads to depression, stress, and a decrease in the ability to control life events in individuals who structure self-worth in external domains (Sargent et al.,2006). It has also been argued that it does not mean that it will have value. Individuals who construct their self-worth in the field of obtaining approval will feel valuable when they manage to influence others or fill their surroundings with people who admire them, and their self-worth will gain a stable appearance (Crocker &Wolfe, 2001).

3. Number of domains that construct self-worth: Croker and Wolfe (2001) stated that structuring self-value in many areas will result in a healthier sense of self-worth and be more functional. But he emphasized that this is related to the

level of self-worth. In other words, for individuals with a high level of self-worth, having more than one self-value field is functional and will make them feel better psychologically. But the individual with a low sense of self-worth will have to deal with many areas that will make him feel worthless.

4. Unstable structuring of self-value: The reason for the unstable structuring of self-value is that self-value depends on the attitudes and behaviors of others. The value and importance of the person should not change according to the behavior of others. In cases where self-worth is dependent on the individual's own attitudes and behaviors, self-worth can maintain its stable structure for a long time since it is up to the individual to control what form the self-worth will take (Crocker & Wolfe, 2001). An individual with an unstable self-worth may display problematic behaviors when confronted with any situation that threatens his self-worth.

4. RECOMMENDATIONS FOR COPING WITH UNHEALTHY SELF-WORTH AND HEALTHY SELF-WORTH DEVELOPMENT

Efforts to cope with unhealthy self-value developments and develop a healthy self-value are considered very important and valuable in order for individuals to lead a more organized life and have stronger self-perceptions. In this context, the interventions and suggestions presented below on the subject will contribute to healthy self-value development.

Practical suggestions for dealing with unhealthy self-worth:

Since the unhealthy and low sense of value is a learned concept, it can be forgotten and replaced. This learning chance can be used anytime from birth to death. That is, one can learn to have higher value at any point in his life. However, as we get older, it gets harder to do this, and sometimes it can take longer. Knowing that change is possible and deciding to achieve it is the first big step. Some of us learn slowly, but we all learn. An individual who fails to analyze correctly the factors stemming from low and unhealthy self-worth will face the danger of failing again in the future and feeling yourself worthless and insignificant.

Practical suggestions for developing a healthy sense of self-worth:

1. Correct determination of the self-worth area of the person: According to everyone, what success is and the meaning it attributes to success is different. Individuals with low self-worth will constantly change the field they encounter with failure and determine a field according to their own abilities, which will make the person feel more important and valuable. For example, I am not a successful student, but it can develop a purpose as a moral person.

2. Making a list of the characteristics that the person likes: Seeing the positive

and beautiful features of the person is a way for the person to feel valuable. While making the list, getting support from our family and friends will allow us to see many features of ourselves that we do not notice.

3. Being aware of the jobs that the person is successful in: When people feel worthless and insignificant, they expect to be deceived, oppressed, humiliated by others. This paves the way for them to become victims. Because they constantly expect the worst, they usually attract the worst situations and sometimes succeed. The situations in which the person performs well and is successful in this bad situation often go out of his mind. For this reason, reminding the person about successful events from the past to this day and making a list will create a sense of self-confidence. Even praising the person for these achievements will make him feel valuable.

4. Providing social support to the person: Especially for those who build their self-worth on family support, social support is an important reason for the person to feel valuable and important. Feeling worthless, the person hides behind a wall of insecurity to defend themselves, leaving them with terrible feelings of loneliness. Thus, they become indifferent by moving away from other people, and they treat others as they treat themselves.

5. Stop measuring one's self-worth according to others: It is expected that people who constantly leave their self-worth to the approval of others feel worthless and their self-worth is low. In this case, the self-worth of the person will constantly change according to what people think and how they behave. Therefore, one should not attribute his own self-worth to the approval of others, but must shape his self-worth by using his own inner resources.

6. Sparing time for himself: One should spare time for himself to feel valuable, and time for the things he likes.

KAYNAKLAR

1. Crocker J, Wolfe CT. Contingencies of self-worth. Psychological review. 2001;108(3):593-623.
2. Crocker J, Luhtanen R, Sommers S. Contingencies of self-worth: Progress and prospects. European review of social psychology. 2004;15(1):133-81.
3. Crocker J, Park LE. The costly pursuit of self-esteem. Psychological bulletin. 2004;130(3):392-414.
4. Crocker J, Knight KM. Contingencies of self-worth. Current directions in psychological science. 2005;14(4):200-3.
5. Crocker J. Contingencies of self-worth: Implications for self-regulation and psychological vulnerability. Self and Identity. 2002;1(2):143-9.
6. Wentzel KR, Miele DM. Handbook of Motivation at School NewYork2009. 141-70 p.
7. Crocker J, Luhtanen R, Cooper ML, Bouvrette A. Contingencies of self-worth in college students: theory and measurement. Journal of personality and social psychology. 2003;85(5):894-908.
8. vanDellen MR, Hoy MB, Hoyle RH. Contingent self-worth and social information processing: Cognitive associations between domain performance and social relations. Social Cognition. 2009;27(6):847-66.
9. Luhtanen RK, Crocker J. Alcohol use in college students: effects of level of self-esteem, narcissism, and contingencies of self-worth. Psychology of Addictive Behaviors. 2005;19(1):99-103.
10. Doğan T. Psikolojik belirtilerin yordayıcısı olarak sosyal destek ve iyilik hali. Türk Psikolojik Danışma ve Rehberlik Dergisi. 2008;3(30):30-44.
11. Arslantaş H, Ergin F. Yalnızlık, Depresyon, Sosyal Destek ve Etki Eden Faktörler Turkish Journal of Geriatrics. 2011;14(2):135-44.
12. Rueger S, Malecki CK, Pyun Y, Aycock C, Coyle S. A meta-analytic review of the association between perceived social support and depression in childhood and adolescence. American Psychological Association. 2016;142(10):1017 - 67.
13. Overstreet N, Quinn D. Contingencies of self-worth and appearance concerns: Do domains of self-worth matter? Psychology of Women Quarterly. 2012;36(3):314-25.
14. Bardone-Cone AM, Lin SL, Butler RM. Perfectionism and Contingent Self-Worth in Relation to Disordered Eating and Anxiety. Behavior Therapy. 2017;48(3):380-90.
15. Sanchez DT, Crocker J. How investment in gender ideals affects well-be-

ing: the role of external contingencies of self-worth. Psychology of Women Quarterly. 2005;29(1):63-77.
16. Sargent JT, Crocker J, Luhtanen RK. Contingencies of self–worth and depressive symptoms in college students. Journal of social and clinical psychology. 2006;25(6):628-46.
17. Eroğlu Y, Güler N. Koşullu öz-değer, riskli internet davranışları ve siber zorbalık/mağduriyet arasındaki ilişkinin incelenmesi. Sakarya University Journal of Education. 2015;5(3):118-29.
18. Park L, Crocker J, Kiefer A. Contingencies of self-worth, academic failure, and goal pursuit. Personality and Social Psychology Bulletin. 2007;3(11): 1503-17.
19. Crocker J, Karpinski A, Quinn DM, Chase SK. When grades determine self-worth: consequences of contingent self-worth for male and female engineering and psychology majors. Journal of personality and social psychology. 2003;85(3):507.
20. Wolfe C, Crocker J. What does the self want? Contingencies of self-worth and goals. 2003.
21. Crocker J, Brook A, Niiya Y, Villacorta M. The Pursuit of Self-Esteem: Contingencies of Self-Worth and Self-Regulation. Journal of personality. 2006;74(6):1749-72.
22. Sanchez D, Kwang T. When the relationship becomes her: Revisiting women's body concerns from a relationship contingency perspective. Psychology of Women Quarterly. 2007;31(4):401-14.
23. Park L, Crocker J, Mickelson K. Attachment styles and contingencies of self-worth. Personality and Social Psychology Bulletin. 2004;30(10):1243-54.
24. Cheng S, Kwan K. Attachment dimensions and contingencies of self-worth: The moderating role of culture. Personality and Individual Differences. 2008;45(6):509-14.
25. Liu C, Chiu YC, Chang JH. Why Do Easterners Have Lower Well-Being Than Westerners? The Role of Others' Approval Contingencies of Self-Worth in the Cross-Cultural Differences in Subjective Well-Being. Journal of Cross-Cultural Psychology. 2017;48(2):217-24.
26. Gecas V. The self-concept. Annual review of sociology. 1982;8(1):1-33.

Chapter-3

COVID-19 AND DIABETIC COMPLICATIONS

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Introduction

The new corona virus Covid-19 pandemic caused a worldwide health problem, following its first detection in Wuhan, China in December 2019 (1). Covid-19, also known as SARS-CoV2, mainly causes lung pneumonia, but can cause disease in multiple organ systems (2). The Covid-19 Pandemic is a disease that started in the city of Wuhan in late 2019 and soon spread to other parts of China and the world and could have fatal consequences caused by a new type of Coronavirus. The World Health Organization declared the Covid-19 outbreak as a global pandemic on March 11, 2020. The cause of this disease was SARS-CoV-2 due to its similarity to Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and the disease was named as Coronavirus Disease 2019 (Covid-19) by the World Health Organization on January 7, 2020, by the Chinese Center for Disease Control and Prevention has been named (5). The new type of coronavirus is an RNA-type virus that is highly contagious and causes serious respiratory infections. It is stated that the infection is transmitted by droplets, by touching surfaces containing virus and then by touching the mucous membranes of the mouth, nose and eyes. Although the contagious period of the disease is not known exactly, it is thought that it can start 1-2 days before the symptomatic period and continue until the 14th day after the disease is infected. In mild cases of the disease, symptoms such as cough, fever, and difficulty breathing are observed; severe acute respiratory tract infection and high mortality can be seen. New type of coronavirus; It threatens individuals with chronic diseases such as diabetes, hypertension, respiratory and kidney disease, especially the elderly (5).

Diabetes Mellitus

Diabetes Mellitus is a life-long disease characterized by defects in carbohydrate, fat and protein metabolism, with high blood glucose levels and affecting many systems in the body. In all diabetic patients, when blood sugar (plasma glucose) levels are not under control, various system, organ or tissue damage may occur in the short (acute) and long (chronic) periods. These damages are called “secondary diseases (complications) related to diabetes”. Hypoglycemia (low blood sugar), ketoacidosis (diabetic coma), lactic acidosis, bacterial / fungal infections, hyperglycemic nonketotic coma are acute complications of diabetes. Among the chronic complications, microvascular damage is the most common; retinopathy (damage to the eyes), neuropathy (damage to the nerves), nephropathy (damage to the kidneys) and macrovascular damage are the most common; Accelerated arteriosclerosis, diabetic foot, coronary artery disease, and impotence (sexual reluctance or inability) are present. These complications can occur in

people with both Type 1 and Type 2 diabetes. Especially chronic ones can progress without symptoms over the years. Therefore, even if all patients diagnosed with diabetes do not have any complaints, examinations and tests should be performed at least once a year in terms of complications.

Pathophysiology of Diabetes and Covid-19

Infectious diseases that may occur after the pathophysiological changes of diabetes mellitus cause hyperglycemia. As a result, it leads to complications that develop with infection. In the natural response of the body against infection, the response that occurs on the basis of chronic diabetes leads to dysregulation, endothelial dysfunction, hypercoagulability, and proinflammatory and various infections with impaired barrier structure and appear more severe (6). The pro-inflammatory state that occurs with diabetes is also inevitable. Viral and bacterial respiratory infections are common in diabetics due to reasons such as decreased T cell response, neutrophil dysfunction, and irregular humoral immunity. The relationship between increased pneumonia, morbidity and mortality in diabetic patients is also known (6). Interferon, which responds to all viral infections and is important in combating, is suppressed in Covid-19 patients. In addition, the resulting maladaptive delayed and exaggerated interferon response caused a cytokine storm and the risk of organ damage was found to be high. Cytokine storm triggers impaired endothelial-epithelial barrier functions. However, it causes a poor prognosis of the disease by causing hypercoagulability in the microvascular environment (7, 8). Pathological changes in diabetes specific organs come together with cellular mechanisms affected by Covid-19. This increases the likelihood of a cytokine storm that will end in multiple organ failure and damage. Interleukin-6 (IL-6), fibronogen, ferritin, D-dimer, and C-reactive protein levels were found to be significantly higher in individuals with diabetes infected with Covid-19 compared to non-diabetic individuals (7). Especially, diabetic individuals had very high serum lactate dehydrogenase (LDH), CRP, ferritin and D-dimer levels. In addition, low lymphocyte count and more common computer tomography (CT) findings stand out as an indicator of the poor prognosis of the disease (9). Adipocyte dysfunction and high-grade inflammation that occur with comorbidities such as diabetes, obesity, hypertension and ischemic heart disease lie behind the factors that lead to cytokine storm with Covid-19. These comorbidities are responsible for the high mortality. If comorbidities coexist with cytokine storm, mortality rates increase exponentially. The expression of angiotensin converting enzyme 2 (ACE2) decreases in people with diabetes, and ACE2, which is found in many organs including the lungs, pancreas, kidneys, vascular system, and intestinal endothelium,

has important roles such as anti-inflammation and anti-oxidation (7). The physiological condition of this enzyme, which is disrupted by diabetes mellitus, increases the risk of severe lung damage such as acute respiratory distress syndrome (acute respiratory distress syndrome, ARDS) in case of being infected with Covid-19 (10). Previous studies on SARS have shown that it causes acute hyperglycemia by connecting to ACE2 and this situation is responsible for mortality. The ability of ACE2 to be expressed in the pancreas may cause the virus to enter the pancreatic islets, causing acute beta cell dysfunction, resulting in an acute hyperglycemic state (7, 11). There is a similar situation in Covid-19, where ACE2 expression is intense in pancreatic islets as well as in the exocrine pancreas. This virus, which penetrates the pancreatic tissue, is highly likely to cause islet damage. It has been previously shown that SARS-CoV binds to ACE2 in pancreatic islet cells, damages them and possibly causes acute hyperglycemia (11). This suggests that it contributes to increased mortality even in people without diabetes (11). The increase in pancreatic enzymes is considered as a supportive finding that this virus causes secondary DM (8, 12).

Guo et al. (7), in their study in which they evaluated 24 patients, reported that a severe clinical picture occurred in terms of organ damage, inflammatory factors or hypercoagulability and worsened the prognosis in SARS-CoV-2 pneumonia patients with diabetes compared to patients without diabetes, whether or not there were other comorbidities. It is clear that people with diabetes are at high risk for Covid-19 infection and for disease-related medical complications. This suggests that more sensitivity should be exercised in diagnosis, treatment and follow-up for Covid-19 in approaching diabetic patients (13). During the pandemic period, many diabetic patients had to cancel their routine checks in diabetes clinics. This situation, together with the increased stress associated with social isolation and lack of physical activity, has paved the way for worsening glycemic and blood pressure control, which makes diabetic patients more susceptible to Covid-19 infections (13).

Covid-19 and Diabetic Retinopathy

An average of 25% of registered diabetes patients worldwide have any degree of diabetic retinopathy (DR). DR is the most common, treatable, chronic complication of diabetes (14). It is responsible for approximately 12% of new one-year blindness in the US working population (20-74 years old) (14). The prevalence of DR varies mainly depending on whether the diabetes is due to insulin, and the age and duration of diabetes onset. More than 90% of all diabetics develop retinopathy at some point in their lives. It was found that diabetics have a 25-fold hig-

her risk of blindness compared to non-diabetics (15). Covid-19's challenge to the health community has delayed diagnosis and treatment efforts. While the disease destroyed respiratory functions, various ocular injuries were accompanied. Covid-19 RNA virus could be detected in the tears of infected patients. It is known that the entry points of the ocular space cannot resist viral infections and serve as reservoirs. Clinically, Covid-19 has been associated with mild conjunctivitis, which may be the first and only symptom of the disease (16). Mild retinal changes such as hyperreflective lesions in the inner layers, cotton-wool spots, and micro-bleeds have also been reported on optical coherence tomography (OCT) (16). It is said to give severe ocular symptoms associated with the increased incidence of systemic diseases such as Covid-19, diabetes mellitus and Kawasaki disease (16). The prone position, invasive mechanical ventilation and exposure to various resistant bacteria lead to increased risk factors in patients receiving treatment in the intensive care unit. These effects lead to ocular surface disorders in the eye, secondary infections, and less commonly, ocular complications such as acute ischemic optic neuropathy and increased intraocular pressure (16). The well known retinal toxic effects of hydroxychloroquine derivative drugs used in the treatment of Covid-19 are alarming. High-dose antimalarial agents used in the treatment of Covid-19 carry the risk of short-term retinal toxicity. Ocular side effects are frequently encountered in drugs such as Ritonavir-Lopinavir and many other drugs such as interleukin-1, interleukin-6 and interferon inhibitors. In addition to all these treatment processes, we believe that retinopathies caused by diabetes mellitus will increase especially with curfews and limitation of movement. In healthy individuals, a decrease in daily walking from 10,000 steps to 1,500 steps may lead to impaired insulin sensitivity and slowing of lipid metabolism, increased visceral fat and decreased lean body mass, and worsening cardiovascular performance (17). As a result, it should be thought that it will have negative effects on public health such as worsening of the picture in newly diagnosed diabetes. It is also inevitable that the tendency towards ophthalmologists for eye complications due to diabetes during or after Covid-19 treatment is inevitable (17).

Covid-19 and Diabetic Neuropathy

It is seen in more than 50% of diabetic patients over the age of 60. It increases the formation of diabetic foot and foot ulceration 7 times. Neuropathic pains that are difficult to treat may occur. Motor, sensory or autonomic effects occur according to the damaged nerve function (18, 19, 20). Microvascular damage develops in the vascular structures supplying the nerve due to compression and blood flow is impaired. Damage occurs in the myelin sheath as a result of com-

pression, edema and ischemia. While edema develops due to venous return in a mild compression, arterial ischemia develops if the degree of compression increases. Changes that occur as a result of nerve compression are directly related to the degree of pressure and the duration of the press. Prolonged or repeated compression may result in endothelial dysfunction, edema, inflammation, fibrosis and demyelination. Edema and fibrosis further increase the mechanical pressure. As a result of decreased myelinization, axonal conduction velocity decreases at the affected nerve level. Partial or complete block of nerve conduction may develop due to increased demyelination in the future. If the pressure is continuous, axonal degeneration may develop, that is, in the ongoing pressures, axolysis develops distally and wallerian degeneration is observed. In this case, the prognosis may be poor and recovery may take weeks or even months even if the pressure is removed (21). Patients with diabetic neuropathy are more susceptible to compression because the accumulation of the glucose metabolite sorbitol causes endoneural edema. This enables neural damage to develop more easily (22). We believe that the Covid-19 pandemic will be more affected by the viral load of patients with diabetic neuropathy and will have adverse effects such as tissue failure. However, no new studies have been conducted on this subject yet. In the future, the effects of larger-scale covid-19 on patients with diabetic nephropathy should be investigated.

Covid-19 and Diabetic Foot

It is a more common complication of diabetes mellitus in men. Symptoms include foot rash, common non-healing wounds and ulcers. The most feared complication of diabetic foot disease is amputation, which is 10-30 times more common in diabetic patients than in the general population. More than 80% of non-traumatic amputations constitute diabetes, 85% of which have foot ulcers (23, 24). Exposure to high blood sugar for a long time causes damage to the nerves and vessels, especially in the feet. In this way, the feeling of pain in the feet of diabetic patients decreases; the patient cannot realize the injuries. As a result of neuropathy, standing dryness and cracks in the skin occur. Patients who cannot sense heat cause severe burns while trying to warm their cold feet. As a result of damage to small and large vessels, the blood flow to the foot is reduced. All sorts of wounds on the foot become ulcerated; it gets better too late or not at all. The most important factors that cause ulcer development are ischemia and neuropathy. The neuropathic foot is generally hot, dry, painless, and the ulcer is located on the toes. Foot veins are plump and pulse. Neuroischemic foot is characterized by cold, painful heel ulcers and absence of pulse. Atherosclerosis, which is promi-

nent in diabetic cases, affects the distal peripheral arteries (20, 23). In addition to atherosclerosis, microvascular changes such as basement membrane thickening and increased capillary fragility, thromboses and endothelial dysfunction may also cause ischemia. The presence of ischemia is one of the main reasons that delay the healing of the ulcer and increase the risk of amputation. Patients with diabetes mellitus are in the high risk group for Covid-19 pandemic (25). First of all, patients with diabetic foot have worse clinical pictures with increasing cytokine levels (interleukin-6, interleukin-10 and tumor necrosis factor-α) and Covid-19 (26). Although cytokine changes and increases are observed in patients with diabetic foot ulcers, cytokine storm may occur (27, 28). Imbalance in pro-inflammatory cytokines plays a role in the pathogenesis of Charcot osteoarthropathy and creates potential bidirectional relationships between Covid-19 and the diabetic foot (29). Neuropathy is the most important factor in the development of diabetic foot lesions and may reduce the inflammatory response to infections (28, 29, 30). In patients with diabetic foot, the combination of severe neuropathy with a condition of covid-19 infection may have an effect on the production of proinflammatory cytokines. In addition, ischemia caused by peripheral artery disease is also important in the etiology of diabetic foot ulcers (31). It has also been reported that patients with severe ischemia cannot reach lower extremity infections with intravenous antibiotics (28). This adverse effect is also thought to be associated with other areas of infection due to atherosclerosis in patients with peripheral artery disease (31). Based on this idea, it is alarming to know that intravenous antibiotics used in the Covid-19 pandemic cannot reach their target area. In addition to all these negativities, the decrease in the daily activities of patients with shortness of breath helps the diabetic foot to be loaded and may contribute to faster healing rates of neuropathic ulcers. Although the idea of a potential relationship between Covid-19 and the diabetic foot does not seem very attractive, at least in theory, it seems premature as to whether the pandemic will have any implications for wound healing and hence the diabetic foot (32, 33).

Covid-19 and Diabetic Nephropathy

In the structures called glomeruli in the kidneys, there are millions of capillary tangles, and the blood passing through these structures is constantly subjected to cleaning. In the purification process, useless molecules are excreted in the urine, while blood cells and necessary molecules are retained in the body. In diabetic patients, the function of the glomeruli deteriorates over the years as a result of continuous high blood sugar and damage to capillaries (34, 35). As a result, sugar and some proteins in the blood cannot be retained and become excreted in

urine. Small amounts of protein excretion with urine is called microalbuminuria (30-300 mg of albumin in 24 hour urine). When the disorder in the kidneys progresses, blood urea rises and blood pressure rises (34). Swelling (edema) begins in the body and especially in the feet; the amount of urine decreases over time. Recently, the patient has to survive with hemodialysis. Dialysis is a treatment that is both expensive and significantly restricts the user's life.

Covid-19 morbidity and mortality increase in patients with diabetes and kidney disease with unknown mechanisms. ACE2 is needed for Covid-19 to enter the cells. Since ACE2 is a susceptibility factor for infection, nephropathic kidney biopsies in diabetic nephropathy and how drugs alter ACE2 receptor expression in the kidneys are consistent with detecting ACE2 expression mainly in proximal tubular epithelial cells (PTEC). In a study, cell-specific localization was confirmed by in situ hybridization (36). ACE2 expression levels were not altered in diabetic nephropathy due to exposure to renin angiotensin aldosterone system inhibitors. Inductive molecular network modules expressing ACE2 have also been identified in diabetic nephropathy, which is generally associated with viral entry, immune activation, and endomembrane reconstruction (36). The diabetic nephropathy ACE2 modulator overlaps with patterns seen in cells infected with Covid-19. Similar cellular orientations were detected in ACE2-positive PTEC obtained from urine samples of 13 Covid-19 patients hospitalized, and an ACE2-core regulated PTEC expression program that could interact with Covid-19 infection processes was proposed (36). For this reason, no research has yet been done on Covid-19 receptor networks, whether there is a risk link between diabetic nephropathy and therapeutic strategies. Viral entry plays an active role in the immune system, endomembrane organizations and RNA processing events. Since the ACE2-positive PTEC module for diabetic nephropathy overlaps with cell models infected with Covid-19, it is thought that it may reveal conditions that may result in tissue damage through diabetic nephropathy in severe cases.

The purpose of this book section is to prevent the negative conditions of Covid-19 infection in complications caused by diabetes mellitus and to offer solutions. Covid-19 infection produces an inflammatory response with cytokine storm, and cytokines can induce insulin resistance and direct beta cell damage, leading to worsening of dysglycemia. A serious course and late recovery from SARS-CoV infection in DM patients is an inference we have learned from the past. Now, Covid-19 shows serious symptoms with similarly increased risk of death in diabetics. Therefore, DM patients as well as the high-risk UDM group should implement isolation, social distancing, and personal hygiene safety measures to prevent morbidity and mortality.

Result

Although Covid-19 is initially located in the respiratory system, it affects the cardiovascular system through ACE2 and the renin-angiotensin-aldosterone system imbalance and causes adverse effects and causes problems such as endothelial dysfunction and microvascular damage. Additional studies are needed on these mechanisms. Can investigate ways to prevent myocardial disease. High troponin levels are significantly associated with fatal outcomes in patients with Covid-19. The mechanisms that cause myocardial damage are still not proven. Patients with cardiovascular disease have a worse prognosis when caught with Covid-19. Therefore, cardiovascular risks should be considered during the treatment of Covid-19. In addition, while talking about the side effects of Covid-19 drugs on the eye, we believe that eye complications due to diabetes during or after Covid-19 treatment will increase diabetic retinopathy. In diabetic neuropathy, we believe that the Covid-19 pandemic will be affected more at the rate of viral load and will have negative effects such as tissue failure. The reduction in daily activities in the diabetic foot helps less strain on the diabetic foot and may contribute to faster healing rates of neuropathic ulcers. A potential relationship between Covid-19 and the diabetic foot seems premature as to whether the pandemic will have any implications for wound healing and hence the diabetic foot. It is possible to talk about similar implications in diabetic nephropathy. There is a need for studies that will determine the risk link between Covid-19 receptor networks, diabetic nephropathy and therapeutic strategies. Since viral entry will play an active role in the immune system, endomembrane organizations and RNA processing events, we believe that in diabetic nephropathy, patients infected with Covid-19 may result in tissue damage in severe cases. All these inferences have given us an idea to consider the possibilities of a negative picture between Covid-19 and diabetic complications and to open the door to new studies. It may be advisable for individuals with diabetes mellitus complications to be more careful about adhering to Covid-19 pandemic measures.

References

1. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. Journal of autoimmunity. 2020;109:102433.
2. Hu Y, Sun J, Dai Z, et al. Prevalence and severity of corona virus disease 2019 (COVID-19): A systematic review and meta-analysis. Journal of clinical virology. 2020;127:104371.
3. Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, et al. World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). International Journal of Surgery (London, England).2020; 76: 71–76.
4. FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic. 2020; http://www. klinikarastirmalar.org/upload/documents/Clinical-Trial-Conductduring-COVID-19-Direct-to-Final-3-17-20.pdf
5. Jordan RE, Adab P, Cheng KK. Covid-19: risk factors for severe disease and death. BMJ 2020 Mar 26;368:m1198.
6. Kornum JB, Thomsen RW, Riis A, Lervang HH, Schonheyder HC, Sorensen HT. Type 2 diabetes and pneumonia outcomes: A population-based cohort study. Diabetes Care. 2007;30(9):2251-2257.
7. Guo W, Li M, Dong Y, et al. Diabetes is a risk factor for the progression and prognosis of COVID-19. Diabetes Metab Res Rev. 2020;e3319.
8. COVID-19 Pandemi diyabet izlem ve tedavi kriterleri uzlaşı raporu. Türk Diyabet Vakfı, Mayıs 2020.
9. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;382(18):1708-1720.
10. Pal R, Bhansali A. COVID-19, diabetes mellitus and ACE2: The conundrum. Diabetes Res Clin Pract. 2020;162:108132.
11. Yang JK, Lin SS, Ji XJ, Guo LM. Binding of SARS coronavirus to its receptor damages islets and causes acute diabetes. Acta Diabetol. 2009;47(3):193-199.
12. Drucker DJ. Coronavirus Infections and Type 2 Diabetes- Shared Pathways with Therapeutic Implications. Endocr Rev. 2020;41(3):bnaa011.
13. Hill MA, Mantzoros C, Sowers JR. Commentary: COVID-19 in patients with diabetes. Metabolism. 2020;107:154217.
14. Chew EY, Ferris FL. Nonproliferative diabetic retinopathy. In: Ryan SJ, Ogden TE, Hinton DR, Schachat AP (eds). Retina. 3rd ed. Vol 2. Philadelphia: Mosby. 2001; 1295- 1308.

15. Kahn HA, Hiller R. Blindness caused by diabetic retinopathy. Am J Ophthalmol. 1974; 78: 58-61.
16. Bertoli F, Veritti D, Danese C, Samassa F, Sarao V, Rassu N, et al. Ocular Findings in COVID-19 Patients: A Review of Direct Manifestations and Indirect Effects on the Eye. J Ophthalmol. 2020; 2020: 4827304.
17. Krogh-madsen R, Thyfault JP, Broholm C, Mortensen OH, Olsen RH, Mounier R, et al. A 2-wk reduction of ambulatory activity attenuates peripheral insulin sensitivity. Journal of Applied Physiology. 2010;108(5):1034-40.
18. Khanolkar MP, Bain SC, Stephens JW. The Diabetic Foot Q J Med. 2008; 101: 685-695.
19. E. Tamır, Treating The Diabetic Ulcer: Practical Approach And General Consepts. Isr Med Assoc J. 2007;9(8):610-5.
20. Boulton AJM, Vinik AI, Arezo JC, Bril V, Feldman EL, Freeman R, et al. Diabetic neuropathies: a statement by the American Diabetes Association. Diabetes Care. 2005;28(4):956-62.
21. Doughty CT, Bowley MP. Entrapment Neuropathies of the Upper Extremity. Med Clin North Am. 2019;103(2):357-370.
22. Terzi M, Cengiz N, Onar MK. Diyabetik Nöropati. O.M.Ü. Tıp Derg.2004; 21(1):39-49
23. Garrow AP, Boulton AJM. Vibration perception threshold- a valuable assessment of neural dysfunction in people with diabetes. Diabet Metab Res Rev.2006; 22:411–441.
24. Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. Jama.2005; 293:217–228.
25. Iacobellis G. COVID-19 ve diyabet: DPP4 inhibisyonu bir rol oynayabilir mi? Diabetes Res Clin Pract. 2020; 162:108-125.
26. Pedersen SF, Ho YC. SARS-CoV-2: a storm is raging. J Clin Invest. 2020; 130(5):2202-2205.
27. Zubair M, Ahmad J. Role of growth factors and cytokines in diabetic foot ulcer healing: A detailed review. Rev Endocr Metab Disord. 2019; 20: 207 - 217
28. Papanas N, Maltezos E. Growth factors in the treatment of diabetic foot ulcers: new technologies, any promises? Int J Low Extrem Wounds. 2007;6(1):37-53.
29. Papanas N, Maltezos E. Etiology, pathophysiology and classifications of the diabetic Charcot foot. Diabet Foot Ankle. 2013;21:4. doi: 10.3402/dfa.v4i0.20872.
30. Bönhof GJ, Herder C, Strom A, Papanas N, Roden M, Ziegler D. Emer-

ging Biomarkers, Tools, and Treatments for Diabetic Polyneuropathy. Endocr Rev. 2019;40(1):153-192.
31. Armstrong DG, Boulton AJM, Bus SA. Diabetic Foot Ulcers and Their Recurrence. N Engl J Med. 2017; 376: 2367-2375 .
32. Papanas N, Papi M, Rerkasem K. Progress in Wound Healing: Wisdom Not Consumed in Confidence? Int J Low Extrem Wounds. 2019 Mar;18(1):5.
33. Papanas N, Papi M, Rerkasem K. The Science of Wound Healing: Perhaps, “More Lovely and More Temperate”? Int J Low Extrem Wounds. 2019;18(2):110-111.
34. Pharmacotherapy of Diabetes: New Developments. Improving Life and Prognosis for Diabetic Patients. Edited by Carl Erik Mogensen. Denmark, Mart 2007.
35. Wells BG, Schwinghammer TL, Malone PM, Koleser JM, Rotschafer JC, Dipiro JT. Pharmacotherapy principles & practise.2007; 501-511.
36. Menon R, Otto EA, Sealfon R, Nair V, Wong AK, Theesfeld CL, et al. SARS-CoV-2 receptor networks in diabetic and COVID-19 associated kidney disease. MedRxiv. Preprint. 2020 May 13. doi: 10.1101/2020.05.09.20096511

Chapter-4

EVALUATION OF STIGMATIZATION CAUSED by THE COVID-19 OUTBREAK

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Introduction

Stigmatization can be defined as "a simplified, standardized image of some people's shame in general by a community with a common sense of thought" (1,2). In other words, stigmatization is the negative evaluation of a person or group as defective or disgraced based on characteristics such as mental illness, ethnic group, drug abuse, or physical inability (2–4). It is emphasized with stigmatization that the person or group stigmatized is different, and because of this difference, many negative characteristics are attributed to the stigmatized people. Since early ages, stigmatization is a phenomenon that people are exposed to for many facts and situations. The stigmatization definition includes three elements of the social cognitive structure. These include negative emotional reactions about a minority group caused by the endorsement of stereotypes or negative beliefs, and prejudices held by many members of the social group, discrimination or behaviour motivated by these prejudices (5,6). As a result of both cognitive and emotional reactions, people who are stigmatized lose their position in society and can no longer be in their old positions in it. In addition to the tendency of society to stay away from these people, it also wants to keep patients away from themselves and the society. As a result, patients are exposed to clear discrimination and exclusion.

Stigmatization Process

Stigmatization is a process that occurs as a result of many successive mental stages and the components of this process can be summarized as follows. First, stigmatization is often triggered by a person diagnosed with or labelled as a mental illness. Labelling, on the other hand, brings stereotypes formed by prejudices to the agenda in the society (7–9). Second, stereotypes of how people with the disease are is a concept that determines the definitions that society has previously agreed on. The term of stereotype consists of combining the words "stereos" (rigidity) and "tupos" (trace) in Latin and is used in the meaning of "never changing after it is formed" (7,9). Third, prejudices support stereotypes and together cause some emotional reactions as a result. As a result of prejudices that develop, feelings of fear and anger are mostly fed to individuals with the disease. The individual's being considered dangerous is the main reason for fear. Anger is caused by the disturbance of the peace of the society, the need for help and care, the interruption of work and the increase in the workload of other individuals (8,9). Fourth, emotions towards patients as a result of prejudices are generally fear and anger. The most important cause of fear, as one can easily guess, is the thought that patients are dangerous people. At the root of the anger is that patients are con-

sidered as useless, incompetent and unable to take care of themselves, as well as being regarded as people who disturb the peace of society. As a result, sick people often cause anger and fear in society (8,10 Fifth, as a result of emotional and cognitive reactions, individuals are exposed to a loss of status in the society. Society tends to both stay away from these individuals and isolate them from society. As a result of this attitude reflected in all areas of human relations, segregation or discrimination becomes inevitable (9,10) Sixth, discrimination is the deprivation of some basic rights and interests by individuals or groups in the society due to stigmatization and prejudice. Patients are exposed to many constraints in social life first and then they are excluded from society in concrete or figurative terms. Discrimination includes the processes of restricting, preventing and excluding patients from the society, starting from the situation of not wanting to be close and being against (11,12).

The concept of stigmatization is generally examined under three headings in the literature. Social stigmatization, self-stigmatization and structural discrimination (13,14).

Social stigmatization: It is the reaction of the society against a certain group or person based on assumptions that exists due to a certain characteristic (14).

Self-stigmatization: This concept, which is also called internalized stigmatization, is the social stigmatizing attitude and prejudiced thoughts of stigmatized people (14).

Structural discrimination: It is the systematic effect of social rules and regulations on individuals with mental illness (14).

COVID-19 and stigma

There is a long history of stigmatization in the conceptualization of the disease when the disease differs from the healthy one. Responses to diseases are shaped by their unpredictability and perceived infectiousness. Considering illnesses as both bad and personal responsibilities contributes to social stigmatization (15). Jones (16) states that the responses to epidemic diseases have become clear with the failure to recognize the seriousness of the problem at first, and then community interventions based on moral and mechanical interpretations. For example, the severe acute respiratory syndrome (SARS) outbreak in China in 2002 caused global concern. Unfortunately, the fear caused by the epidemic has led to a lot of stigma (17). Although there were no associated SARS cases in the United States during this period, many citizens began to avoid Chinese-Americans in China and other Asian-American communities, including the Japanese, Korean and Vietnamese peoples. Because these groups were believed to be at greater risk

for the spread of SARS disease (18).

In December 2020, in the pneumonia epidemic, where the city of Wuhan in China is the center, pneumonia developing due to the newly defined SARS-CoV-2 factor was defined as Coronavirus disease 2019 (COVID-19). It has been observed that the clinical findings of SARS-CoV-2 infection in hospitalized patients in Wuhan range from mild manifestations such as asymptomatic disease and mild upper respiratory tract infection to severe viral pneumonia accompanied by respiratory failure and may result in death (19,20). The outbreak of public health emergencies like COVID-19 is stressful times for people and communities. COVID-19 causes the death of many patients, pain due to loss, fear and anxiety, economic and psychological crises. Therefore, the fear, anxiety and uncertainty caused by the disease can lead to the emergence of social stigmatization against people, societies, places or objects (6,21)worries, and anxiety among individuals worldwide. The present study developed the Fear of COVID-19 Scale (FCV-19S. For the outbreak process, the most recent recommendations from WHO (World Health Organization) and CDC (Centers for Disease Control and Prevention) include promoting improved hygiene practices, ensuring that the most up-to-date and accurate information is available to the public, actively correcting misinformation, and most importantly, eliminating stigma, especially in countries where outbreaks occur (22).

Social stigmatization in health is a negative relationship between a person or a group that shares certain characteristics and a certain disease. Social stigmatization can mean that in times of epidemics, people are tagged, exposed to stereotypes, discriminated against, treated separately, and/or faced loss of status because of a perceived link to a disease (23). Such treatment can adversely affect those exposed to the disease and their caregivers, families, friends and communities. People who do not have the disease but share other characteristics with this group may also experience stigmatization. The current COVID-19 outbreak has caused social stigmatization and discriminatory behaviour against people of certain ethnic backgrounds and anyone thought to be in contact with the virus (23,24). For example, people have associated COVID-19 disease with a population or nationality, and everyone in the population or in that area has faced stigmatization and discrimination, whether they are at risk for the disease or not. People of Asian descent, people traveling, emergency responders or healthcare professionals are part of the discriminated group. In addition, patients diagnosed with COVID-19 experienced stigmatization during the period of quarantine and after being released from quarantine (18).

The level of stigmatization associated with COVID-19 is based on three main

factors: The disease being new and still not fully known, frequent fear and anxiety towards the unknown and this fear is easy to associate with "others" (25). When confusion, anxiety and fear among the people are fed by harmful stereotypes, the severity of stigmatization takes on a different dimension. The stigmatization that occurs weakens social cohesion and drives people to social isolation independent of quarantine. It can cause more serious health problems and difficulties in controlling the epidemic (26,27).

Stigmatization can cause individuals to hide their illness symptoms, not benefit from health services urgently, and not adopt healthy lifestyle behaviours in order to prevent discrimination (25). It is also possible to encounter situations such as health, education, denial of housing or employment, physical violence, etc. Accordingly, stigmatization hurts everyone in psychological and economic understanding by creating fear or anger towards other people. Thus, it becomes inevitable that stigmatization negatively affects the emotional and mental health of stigmatized groups and the communities they live in. Stopping stigmatization is important in making communities and community members resilient (27).

Studies conducted show clearly that the stigmatization and fear caused by infectious diseases prevent elimination of disease spread (17,28). In this case, it is about trusting reliable health services and advice, showing empathy to affected individuals, understanding the disease, and taking effective, practical measures so that people can keep their loved ones safe. How we communicate about COVID-19 is important to support people in taking effective action to help combat disease and avoid inciting fear and stigmatization. An environment should be created in which the disease and its impact can be discussed and addressed openly, honestly and effectively (29).

Reducing Stigmatization in the COVID-19 Pandemic Period

First, while social distance is an important point in the spread of COVID-19, it must be prevented that physical distance increase marginalization, avoidance and maltreatment against people associated with COVID-19. In addition to maintaining social distance during the pandemic period, it is important to give stigma-reducing messages that are transformed into a continuous practice. Thus, empathy will be developed towards people who are infected with COVID-19 or who are at risk of transmission (30,31).

Second, during the COVID-19 pandemic period, travel restrictions, curfews and quarantine are implemented in dozens of countries (18,32). These approaches help prevent the spread of COVID-19 and provide convenience to the healthcare system with increased intensity. Yet COVID-19 travel restrictions can also faci-

litate stigmatization by reproducing the social construction of the disease as an occupation, which can strengthen social hierarchies and power inequalities - sometimes in authoritarian ways (32). The enforcement of travel bans, movement restrictions and quarantines can disproportionately affect already stigmatized persons, including homeless persons (imprisoned persons, immigrants and refugees, and racial minorities) (6). COVID-19 may include education of public messaging and legal authorities on travel bans and quarantine, anti-stigmatization (6,32,33). In addition, UNAIDS recommends that instead of punishing COVID-19 for violating public health policies, approaches focus on empowering communities on their own and to protect each other's health (33).

Third, misinformation and lack of awareness about COVID-19 needs to be addressed (34). While misinformation is the driver of fear and stigma, other underlying facilitators cause stigmatization, and removal of false information and raising awareness should be considered in reducing stigma (35). Studies report that awareness and knowledge of COVID-19 is low and fear of the disease is high (17,36). In the period of the COVID-19 pandemic, potentially socioeconomically disadvantaged, racial minority groups at risk for disease, or people with more limited health literacy are considered at risk for disease and stigmatization. When the pandemic occurs, we need to take action to ensure that all citizens are adequately informed of the seriousness of the threat, with great openness and attention to health literacy best practices, and to explain the specific steps that must be taken to avoid harm (37).

Fourth, we must involve those most affected by COVID-19 in developing stigma reduction strategies (27,38). For example, gender-based roles as family caregivers may increase women's exposure to COVID-19 and may require a gender-based analysis of the social and health effects of public health measures such as quarantine (38). Because of this, global and national strategic plans for COVID-19 stigmatization must be grounded in strong gender analysis and must ensure meaningful participation of affected groups, including women and girls, in decision-making and implementation (39).

Healthcare professionals and communicators provide some general recommendations against stigmatization in the COVID-19 pandemic process (27):

- ✓ Protecting the privacy and confidentiality of those who want to receive health services,
- ✓ Quickly sharing with the public that relations with products, people and places are whether risky or not.
- ✓ Raising awareness about COVID-19 without increasing fear,

- ✓ Sharing accurate information about how the virus spreads,
- ✓ Taking measures against negative behaviours, including negative statements about groups of people on social media or exclusion of individuals,
- ✓ Being careful about shared images and making sure that these images do not reinforce stereotypes,
- ✓ Communicating face-to-face with stigmatized groups through media channels including news and social media,
- ✓ Meeting the emerging need for social support of people who are concerned about friends or relatives in the affected areas.

Conclusion

The stigmatization that occurs weakens social cohesion and drives people to social isolation independent of quarantine. It can cause more serious health problems and difficulties in controlling the epidemic. Acordingly, by developing effective behavioral and health education strategies and providing timely attention to the special needs of affected populations, we can limit stigmatization at the time of the COVID-19 pandemic.

References

1. Smith RA. Language of the Lost: An Explication of Stigma Communication. Commun Theory. 2007;17(4):462–85.
2. Sartorius N. Stigmatized illnesses and health care. Croat Med J. 2007;48(3):396–7.
3. Corrigan PW, editor. The stigma of disease and disability: Understanding causes and overcoming injustices. [Internet]. Washington, DC, US: American Psychological Association; 2014. xi 319.
4. Fischer LS, Mansergh G, Lynch J, Santibanez S. Addressing Disease-Related Stigma During Infectious Disease Outbreaks. Disaster Med Public Health Prep. 2019;13(5–6):989–94.
5. Smith RA, Hughes D. Infectious Disease Stigmas: Maladaptive in Modern Society. Commun Stud. 2014;65(2):132–8.
6. Logie CH, Turan JM. How Do We Balance Tensions Between COVID-19 Public Health Responses and Stigma Mitigation? Learning from HIV Research. AIDS Behav. 2020;1–4.
7. Link BG, Phelan JC. Conceptualizing Stigma. Annu Rev Sociol. 2001;27(1):363–85. https://doi.org/10.1146/annurev.soc.27.1.363
8. Link BG, Phelan JC. Stigma and its public health implications. Lancet. 2006;367(9509):528–9.
9. Cam O, Cuhadar D. Stigma Process and Internalized Stigma among Individuals with Mental Illness. J Psychiatr Nurs 2011;2(3):136–40.
10. Fung KMT, Tsang HWH, Corrigan PW, Lam CS, Cheung W. Measuring self-stigma of mental illness in China and its implications for recovery. Int J Soc Psychiatry. 2007 Sep;53(5):408–18.
11. Ucok A. Why is a person with schizophrenia stigmatized? J Clin Psy. 2003;(Ek 1):3–8.
12. Ucok A. Schizophrenia: Stigma, Myths and Realities. Psikiyatri Dunyasi. 1999;3(3):67–71.
13. Baysal GÖD. Stigmatization and mental health. Vol. 22, Archive Medical Review Journal. Çukurova Üniversitesi; 2013. p. 239–51.
14. Karagöl A, Çalişkan D, Beyazyuz M. In terms of public health, mental disorders with three dimensions stamping. J Contin Med Educ. 2013;22:96–101.
15. Whittle HJ, Palar K, Ranadive NA, Turan JM, Kushel M, Weiser SD. "The land of the sick and the land of the healthy": Disability, bureaucracy, and stigma among people living with poverty and chronic illness in the United States. Soc Sci Med. 2017;190:181–9.

16. Jones DS. History in a Crisis — Lessons for Covid-19. N Engl J Med. 2020;382(18):1681–3.
17. Person B, Sy F, Holton K, Govert B, Liang A, Garza B, et al. Fear and Stigma: The Epidemic within the SARS Outbreak. Emerg Infect Dis. 2004;10(2):358–63.
18. Villa S, Jaramillo E, Mangioni D, Bandera A, Gori A, Raviglione MC. Stigma at the time of the COVID-19 pandemic. Clin Microbiol Infect. 2020;26(11):1450–2.
19. Li R, Pei S, Chen B, Song Y, Zhang T, Yang W, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). Science (80-). 2020;368(6490):489–93.
20. Abdulamir AS, Hafidh RR. The Possible Immunological Pathways for the Variable Immunopathogenesis of COVID—19 Infections among Healthy Adults, Elderly and Children. Electron J Gen Med. 2020;17(4):em202.
21. Ahorsu DK, Lin C-Y, Imani V, Saffari M, Griffiths MD, Pakpour AH. The Fear of COVID-19 Scale: Development and Initial Validation. Int J Ment Health Addict. 2020;1–9.
22. CCP. CCP Creates Clearinghouse for COVID-19 Resources. 2020 [cited 2020 May 28]. Available from: https://ccp.jhu.edu/2020/03/04/clearinghouse-covid-19-resources-sbcc/
23. UNICEF. Social stigma associated with the coronavirus disease (COVID-19). 2020 [cited 2020 May 25]. Available from: https://www.unicef.org/documents/social-stigma-associated-coronavirus-disease-covid-19
24. NHP. Social stigma and COVID-19. 2020 [cited 2020 Jul 15]. Available from: https://www.nhp.gov.in/healthlyliving/social-stigma-and-covid-19
25. Zvolensky MJ, Garey L, Rogers AH, Schmidt NB, Vujanovic AA, Storch EA, et al. Psychological, addictive, and health behavior implications of the COVID-19 pandemic. Behav Res Ther. 2020;134:103715.
26. Khan N, Fahad S, Naushad M, Faisal S. Effects of Social Stigma on the Sick People of COVID-2019 in the Community of the World. SSRN Electron J. 2020; Available from: https://www.ssrn.com/abstract=3600579
27. CDC. (Centers for Disease Control and Prevention) Reducing Stigma. 2020 [cited 2020 May 25]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/reducing-stigma.html
28. Des Jarlais DC, Galea S, Tracy M, Tross S, Vlahov D. Stigmatization of Newly Emerging Infectious Diseases: AIDS and SARS. Am J Public Health. 2006;96(3):561–7. Available from: http://ajph.aphapublications.org/doi/10.2105/AJPH.2004.054742

29. Habersaat KB, Betsch C, Danchin M, Sunstein CR, Böhm R, Falk A, et al. Ten considerations for effectively managing the COVID-19 transition. Nat Hum Behav. 2020;4(7):677–87.
30. Lewnard JA, Lo NC. Scientific and ethical basis for social-distancing interventions against COVID-19. Lancet Infect Dis. 2020;20(6):631–3.
31. The Lancet. COVID-19: learning from experience. Lancet. 2020;395(10229):1011.
32. WHO. Public health considerations while resuming international travel. 2020. [cited 2020 Apr 15]. https://www.who.int/news-room/articles-detail/public-health-considerations-while-resuming-international-travel
33. UNAIDS. Rights in the time of COVID-19-Lessons from HIV for an effective, community-led response. 2020 [cited 2020 Jul 13]. Available from: https://www.unaids.org/en/resources/documents/2020/human-rights-and-covid-19
34. R J, D B, Waran K. Social Media Reigned by Information or Misinformation About COVID-19: A Phenomenological Study. Soc Sci Humanit Open. 2020; Available from: https://www.ssrn.com/abstract=3596058
35. Stangl AL, Earnshaw VA, Logie CH, van Brakel W, C. Simbayi L, Barré I, et al. The Health Stigma and Discrimination Framework: a global, cross-scutting framework to inform research, intervention development, and policy on health-related stigmas. BMC Med. 2019;17(1):31.
36. Hossain MA, Jahid MIK, Hossain KMA, Walton LM, Uddin Z, Haque MO, et al. Knowledge, attitudes, and fear of COVID-19 during the Rapid Rise Period in Bangladesh. Pakpour AH, editor. PLoS One. 2020;15(9):e0239646. Available from: https://dx.plos.org/10.1371/journal.pone.0239646
37. Wolf MS, Serper M, Opsasnick L, O'Conor RM, Curtis LM, Benavente JY, et al. Awareness, Attitudes, and Actions Related to COVID-19 Among Adults With Chronic Conditions at the Onset of the U.S. Outbreak. Ann Intern Med. 2020;M20-1239. Available from: https://www.acpjournals.org/doi/10.7326/M20-1239
38. Mabuie MA. Human rights violations, gender inequality and social stigma in the context of COVID-19: A call for action. Am Res J Humanit Soc Sci. 2020;3(9):8–14.
39. United Nation. Progress Towards Gender Equality Under Threat, World Leaders Warn as General Assembly Marks Twenty-Fifth Anniversary of Landmark Women's Rights Conference. 2020 [cited 2020 Apr 25]. Available from: https://www.un.org/press/en/2020/ga12275.doc.htm

Chapter-5

MOLECULAR METHODS in the DIAGNOSIS AND TYPING of LEPTOSPIROSIS

Assoc. Prof. Dr. Tülin Güven Gökmen

Leptospirosis is a zoonotic infection that affects over 150 mammalian species, caused by *Leptospira* spp. and is usually transmitted by direct contact through injured skin or mucosal membrane [1,2]. In the spread of leptospirosis, renal carrier animals that carry microorganisms in their urine play a very important role. *Leptospira* spp., which have both saprophytic and pathogenic species in nature, have a thin, spirally motile, hook-shaped morphology. Recent studies have shown that 66 different species including more than 300 serovars have been identified for *Leptospira*. While *Leptospira biflexa* is a saprophytic species, *Leptospira interrogans, Leptospira kirschneri* and *Leptospira noguchii* are important infectious agents[3].

Various conventional and molecular techniques are used in the diagnosis of leptospirosis, with the most common being dark-field microscopy (DFM), microagglutination test (MAT), culture, and polymerase chain reaction (PCR). Culture analysis is a method with high specificity and accepted as a reference when combined with MAT. However, it takes a long process, such as eight weeks, and its sensitivity has been found to be low. Although direct DFM provides a rapid diagnosis, it requires experienced eyes and is also reported as "compatible spirochetes were seen". MAT is a reference method that can only be carried out by reference laboratories since it is difficult to maintain the culture of *Leptospira* species[2,4,5].

While molecular methods, such as PCR, PCR-based typing methods, pulsed-field gel electrophoresis (PFGE), and sequencing were previously used as a complementary approach to culture and serological testing, they are now seen as an important alternative to the above-mentioned methods. A rapid diagnosis is especially important in the prognosis and treatment of leptospirosis. Considering the disadvantages of conventional methods, the most effective techniques in terms of rapid diagnosis are molecular methods. Although not cost-effective, molecular methods can detect the disease in the acute stage, can be used as confirmatory tests due to their higher sensitivity and specificity, and can provide epidemiological data in surveillance studies[6].

Molecular Identification Methods of *Leptospira* spp.

PCR

The PCR detection of Leptospira DNA dates back to 1989[7]. PCR is a very valuable method as it can detect DNA in the first five to 10 days when the bacterial load is between 10^5 and 10^9 leptospires/L in the blood[8]. It has 90% sensitivity in dogs in the first five days of the disease. This method includes both traditional and real-time tests and is often used to detect pathogenic leptospiral serovars; howev-

er, it cannot adequately distinguish between serogroups or serovars. Recent epidemiological studies have shown that the use of the PCR method is limited to the typing of *Leptospira* species[3]..

16S rRNA-PCR is a highly sensitive and specific method that targets the 16S ribosomal RNA subunit and distinguishes between pathogenic and saprophytic species. It can detect approximately 10 genome equivalents (GE)/mL of whole blood in the identification of *L. interrogans* and *L. biflexa* serovars. These serovars are distinguished by DNA sequencing or restriction fragment length polymorphism (RFLP)[2,9].

Sequence-based techniques have made significant contributions especially to *Leptospira* taxonomy and molecular epidemiology. 16S rDNA sequencing allows to classify *Leptospira* as nonpathogenic, pathogenic, and intermediate pathogenic species. However, the number of different nucleotides among some species is as low as two to three bases. One insertion/deletion and one base difference provide the distinction between *L. interrogans* and *L. kirschneri* [10,11].

The RFLP method can be used to distinguish between non-pathogenic and pathogenic strains at a lower cost than sequencing. *Apo*I enzyme is used in studies as a restriction enzyme[2].

23S rRNA-PCR identifies 23 strains containing six pathogenic genospecies of *Leptospira,* and eight *L. biflexa* strains and signature sequences are determined to distinguish pathogens from saprophytes. This method is sensitive in detecting 200 copies, with two copies of 23S rDNA corresponding to approximately 100 cells[12].

***flaB*-PCR** was developed for the identification of the *flaB* gene-encoding flagellar protein found in *Leptospira* spp. Pathogenic and saprophytic differentiation is provided by different primers. The sensitivity of *flaB*-PCR has been estimated as 100 cells per ml of whole blood[13].

***LipL32*-PCR** targets the LipL32 surface protein gene region on pathogenic *L. interrogans* DNA. It can detect 100 bacteria per ml in plasma, serum or whole blood, and 0.7 GE in paraffin-embedded tissues[2,14].

***OmpL1*-PCR** can distinguish seven groups of *L. interrogans* (Intergroup A, Intergroup B, Borgpeter, Kirschneri, Santarosai, Noguchii, and Weilii) using species-specific PCR primer sets. In pathogenic *Leptospira* strains, outer membrane proteins are the most important and highly conserved gene regions. Trans-membrane outer membrane protein L1 is one of the most sensitive proteins for antigen-antibody immune response[2,15] .

***lig*-PCR** targets the leptospiral immunoglobulin-like (lig) proteins. LigA and LigB proteins are adhesins that bind to fibronectin, collagen, laminin, and elastin and found only in pathogenic species. lig-PCR is sensitive to detection of 10 to

10^7 copies when targeting the conserved regions of LigA and B. It has a sensitivity value that is very similar to 16S rRNA-PCR[16].

***gyrB*-PCR** is an alternative target to 16s rRNA-PCR. The DNA Gyrase Subunit B gene has been successfully used for the identification of *Leptospira* spp. *gyrB*-PCR shows higher nucleotide/evolutionary divergence from 16rRNA-PCR, a highly sensitive method, if sequencing is performed after PCR[17].

Molecular Typing Methods of *Leptospira* spp.

Leptospirosis is still recognized as an important zoonosis worldwide. Molecular typing studies are essential to establish monitoring and outbreak surveillance systems aimed at characterizing human reported cases and agents in wild and domestic animal populations, such as pigs, dogs, cattle, and rats. Serological typing methods provide only subspecies discrimination but cannot reveal the molecular characteristics of the isolates. For this purpose, many methods are used, including ribotyping, southern blot hybridization, whole-genome analysis, sequencing of genes encoding rRNA, arbitrarily primed PCR (AP-PCR), randomly amplified polymorphic DNA (RAPD), multiple-locus variable-number tandem repeats analysis (MLVA), multi-locus sequence typing (MLST), pulsed field gel electrophoresis (PFGE), multispacer sequence typing (MST), and amplified fragment length polymorphisms (AFLP), CRISPR-Cas system, and whole-genome sequence typing (WGST) [5,18].

AP-PCR

This method classifies 48 *Leptospira* reference strains, including *L. interrogans, L. Kirschneri, L. borgpetersenii,* and *L. santarosai*, and has been reported to yield results consistent with 16S rRNA gene sequencing. The ability of AP-PCR to discriminate species has been validated by several studies[18,19,20]. The AP-PCR fingerprint analysis is a method that can be preferred in epidemiological studies due to its speed. It can be used to compare the DNA patterns of a large number of isolates and determine their geographic distribution.

Primer selection is very important for accurate evaluations. In studies comparing primers, it has been shown that the discrimination power of the M16 primer is suitable for AP-PCR[21,22]. However, it is not suitable for large-scale studies due to poor reproducibility and difficulty to compare inter-laboratory data.

RAPD

RAPD-PCR has been shown to be a useful technique in investigating the molecular epidemiology of leptospirosis. Many studies have shown that RAPD-

PCR has the ability to distinguish between species and even serovar-level strains. However, it has only moderate repeatability. RAPD-PCR can be an alternative method for subtyping *Leptospira* isolates because it provides very rapid results that are easy to interpret[23].

PFGE

This method is based on the comparison of DNA patterns that are cut from the whole genome by restriction enzymes. The principle of this method is the cut of DNA isolated from bacteria embedded in agarose by restriction enzymes with a 5-6 base long recognition site. Then, polymorphic DNA patterns are separated to fragments in an electrical field in a mobile matrix gel based on movement according to their molecular weight/size[24]. The *NotI* restriction enzyme is generally used in PFGE studies for the identification of *Leptospira* spp. The genome size of *L. interrogans* serovars varies between 3.96 and 4.62 Mb[25]. The switch time is set between 2-10 and 35-60 according to the genome size[26,27].

PFGE is highly compatible with the MAT, which is the gold standard method. Providing similar results to MAT and having high discrimination power and reproducibility, PFGE has also started to be considered as the gold standard among molecular typing methods. The use of PFGE has been shown to have excellent inter-laboratory reproducibility in the molecular typing of *Leptospira* serovars. This high reproducibility ensures inter-laboratory standardization and consistency in data. Due to this compatibility between laboratories, it provides the creation of databases and sharing of data internationally. PFGE is a very reliable method to investigate the molecular epidemiology of leptospirosis and identify potential new serovars and species.

Computer-based gel analyses allow to evaluate the clonal relationship between strains, compare data, and generate dendrograms. However, there is only limited data due to the labor intensive work schedule and time-consuming nature of the method[16,28].

AFLP

In this method, genomic DNA is cut by different restriction enzymes. Subsequently, the cut fragments are amplified with primers matching adapters. Amplicons are then separated on polyacrylamide gels and analyzed with various software.

Although AFLP has been used in molecular typing, in particular for the examination of clonal relationship in epidemics, its use remains limited since it requires large amounts of purified genomic DNA, labeled primers, and an automated DNA sequencer[5,29,30].

MLVA

Polymorphic tandem repeats, also called variable sequential repeats (VNTR), are commonly used for fingerprinting in many microorganisms. This easy-to-use, rapid, cost-effective, and highly discriminative method has a potential application in understanding the leptospiral molecular epidemiology. The *L. interrogans* genome contains short repeat DNA sequences with sequence motifs less than 100 bp in length. These repeats are well suited for PCR-based polymorphism analyses.

In studies, the number of repeat regions is determined according to the size of amplicons, and the differences between the isolates are determined based on the number of repeat regions. The MLVA method is used for four pathogenic species: *L. interrogans, L. santarosai*, *L. borgpetersenii,* and *L. kirschneri*. In related studies, when MLVA was performed for *L. interrogans*, seven (VNTR-4, VNTR-7, VNTR-9, VNTR-10, VNTR-11, VNTR-19, and VNTR-23) and six (V8, V27,V29, V30, V36, and V50) loci were targeted. In the MLVA method, the amplification process is performed in 90 cycles. Studies on *L. kirschneri* have focused on the VNTR-32, VNTR-33 and VNTR-42 loci. The primers of the VNTR-4, VNTR-7, VNTR-10, VNTR-Lb4, and VNTR-Lb5 loci are used for seven serogroups (Icterohaemorrhagiae, Canicola, Pomona, Grippotyphosa, Autumnalis, Sejroe, and Ballum)[31,32,33].

MLVA is capable of distinguishing serovars belonging to pathogenic *Leptospira* species. Epidemiological studies examine the stability and geographic distribution of these repeat sequences over time. With computer-based programs similar to the PFGE system, data exchange between laboratories, geographic distribution, and differences can be determined. The most important advantage of MLVA over PFGE is that it can be undertaken using samples without the need for culture[16,32,34].

CRISPR-Cas System

This is known as an immune system against bacteriophages and plasmids and consists of the CRISPR-associated (Cas) protein and the clustered regularly interspaced short palindromic repeat (CRISPR) sequence. In the CRISPR-Cas system, Cas proteins target foreign nucleic acids and protect bacteria from mobile genetic elements. The CRISPR-Cas system is only found in pathogenic and intermediate pathogenic *Leptospira* species.

In studies conducted, the diversity of the CRISPR-Cas system has been determined in 41 *Leptospira* genomes. There are usually two different types of the CRISPR-Cas system (subtype I-B and subtype I-E) in pathogenic strains. When

the subtype I-B spacer sequence and content in *L. interrogans* were examined, it was preserved at the serovar level and hypervariable at the serotype level. Therefore, the CRISPR-Cas system is a very useful method in the genotyping of the *L. interrogans* strain and differentiation of its serotypes[35,36].

MLST

MLST consists of a sequence analysis of a variety of housekeeping genes, but its discrimination is high since it also detects synonymous mutations. It is generally characterized by the sequencing of the 450-550 bases region of six to 10 housekeeping genes. The allele number of each locus is determined, and the allelic profile or sequence type (ST) is created based on the combination of alleles at all loci. The use of large numbers of housekeeping genes prevents misinterpretation due to the horizontal DNA transfer common in *Leptospira*.

Three MLST schemes are loaded in the pubmlst.org database: N7LMLST, R7L-MLST, and MLST scheme 6. Seven loci of MLST (R7L-MLST) have high discriminatory power, describing 90 of the 96 STs generated. The R7L-MLST scheme includes housekeeping gene loci, namely *adk* (adenylate kinase), *glmU* (UDP-N-acetylglucosamine pyrophosphorylase), *icdA* (isocitrate dehydrogenase), *lipL32* (outer membrane lipoprotein LipL32), *lipL41* (outer membrane lipoprotein LipL41), *mreA* (rod-shape-determining protein rodA), and *pntA* (NADP transhydrogenase subunit alpha).

Determining STs allows not only an epidemiological analysis but also the definition of the serogroups. The most important limitation of MLST is the low sequence variation in housekeeping genes for some species. For example, Pomona and Canicola strains cannot be distinguished by the MLST method. However, this discrimination can be achieved when MLVA is added to the MLST data[5,35,37,38].

Some studies aimed to increase the discrimination power of MLST through modifications. In the core genome MLST, the method is applied to a higher number of genes of the core genome, reaching 500. Similarly, a fixed number of genome loci are examined, and an allele numbering system is created and evaluated using various software. It can be used very effectively to investigate epidemiological relationship and surveillance analysis[39].

MST

This is a simple method that uses PCR and sequencing methods together. MST is based on comparing the nucleotide sequences of several intergenic sites. Differences in spacers that are less exposed to selection pressure than coding genes are examined. MST has high discrimination and reproducibility, and can describe

phylogeographic lineages.

In addition to identifying the *L. interrogans* serogroup and Icterohaemorrhagiae and Copenhagen serovars, MST has been shown to reveal differences at the strain level; therefore, it has excellent potential in epidemiological applications[40,41,42].

WGST

With the development of technology and software and the decrease in costs, it has now become possible to perform whole-genome analyses providing complete genetic data. However, the probability of error increases in the interpretation and statistical evaluation of large data.

WGST is very suitable for the determination of geographic differences, biological diversity, host adaptation of *Leptospira* spp., and comparative analysis of the genome of serovars. Studies on WGST have determined that *L. interrogans* and *L. borgpetersenii* genomes have approximately 3,400 and 2,800 protein-encoding genes, respectively, of which 656 are pathogen-specific. Many of these genes (59%) are considered to indicate the existence of pathogenic mechanisms, and their roles are known. It has been found that strains with a shortening of their genome in the evolutionary process increase host dependency to survive in different environmental conditions[5,35,43,44].

Consequently, today, conventional methods are being gradually replaced by molecular level studies. Molecular methods used for diagnosis and typing of leptospirosis can also provide information on prognosis and surveillance, and the correct management and treatment of the disease can be successfully performed.

REFERENCES

1. Miotto BA, Tozzi BF, Penteado MS, Guilloux AGA, Moreno LZ, Heinemann MB, Moreno AM, Lilenbaum W, Hagiwara MA. Diagnosis of acute canine leptospirosis using multiple laboratory tests and characterization of the isolated strains. BMC Vet Res. 2018; 14:222.
2. Gökmen TG, Soyal A, Kalayci Y, Onlen C, Köksal F. Comparison of 16S rRNA-PCR-RFLP, LipL32-PCR and OmpL1-PCR methods in the diagnosis of leptospirosis. Rev Inst Med Trop Sao Paulo. 2016; 58:64
3. Sykes JE, Hartmann K, Lunn KF, Moore GE, Stoddard RA, Goldstein RE. 2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. J Vet Intern Med 2011; 25:1.
4. Limmathurotsakul D, Turner EL, Wuthiekanun V, Thaipadungpanit J, Suputtamongkol Y, Chierakul W, Smythe LD, Day NPJ, Cooper B, Peacock SJ. Fool's gold: Why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. Clin Infect Dis. 2012; 55:322-31.
5. Ahmed A, Grobusch MP, Klatser PR, Hartskeerl RA. Molecular approaches in the detection and characterization of Leptospira. J Bacteriol Parasitol 2012; 3:1000133.
6. World Health Organization. Report of the second meeting of the leptospirosis burden epidemiology reference group. Geneva: WHO; 2011. Available from: http://apps.who.int/iris/bitstream/10665/44588/1/9789241501521_eng.pdf
7. Van Eys GJ, Gravekamp C, Gerritsen MJ, Quint W, Cornelissen MT, Schegget JT, Terpstra WJ. Detection of leptospires in urine by polymerase chain reaction. J Clin Mic. 1989; 27: 2258-2262.
8. Musso D, Scola B. Laboratory diagnosis of Leptospirosis: A challenge. J Microbiol, Immunol Infect. 2013; 46: 245-252.
9. Natarajaseenivasan K, Raja V, Narayanan R. Rapid diagnosis of leptospirosis in patients with different clinical manifestations by 16S rRNA gene based nested PCR. Saudi J Biol Sci. 2012; 19:151-5.
10. Fenner JS, Anjum MF, Randall LP, Pritchard GC, Wu G, Errington J, Dalley CG, Woodward MJ. Analysis of 16S rDNA sequences from pathogenic Leptospira serovars and use of single nucleotide polymorphisms for rapid speciation by D-HPLC. Res Vet Sci. 2010; 89:48–57.
11. Morey RE, Galloway RL, Bragg SL, Steigerwalt AG, Mayer LW, Levett PN. Species-specific identification of Leptospiraceae by 16S rRNA gene sequencing. J. Clin. Microbiol. 2006; 44(10): 3510–6.

12. Woo TH, Patel BK, Smythe LD, Symonds ML, Norris MA, Dohnt MF. Identification of pathogenic Leptospira genospecies by continuous monitoring of fluorogenic hybridization probes during rapid-cycle PCR. J Clin Microbiol. 1997; 35(12):3140-3146.
13. Kawabata H, Dancel LA, Villanueva SY, Yanagihara Y, Koizumi N, Watanabe H. flaB polymerase chain reaction (flaB-PCR) and its restriction fragment length polymorphism (RFLP) analysis is an efficient tool for detection and identification of Leptospira spp. Microbiol Immunol. 2001; 45:491–6.
14. González S, Geymonat JP, Hernández E, Marqués JM, Schelotto F, Varela G. Usefulness of real-time PCR assay targeting lipL32 gene for diagnosis of human leptospirosis in Uruguay. J Infect Dev Ctries. 2013; 7:941-5.
15. Hung NT, Huy NV, Hieu ND, Quynh DT, Thuy VTB, Minh NN. Expression and Purification of the Recombinant OmpL1 Protein for Potential Vaccine Production against Leptospirosis. Int J Zoo Animal Biol. 2020, 3(6): 000252.
16. Palaniappan RU, Chang YF, Chang CF, Pan MJ, Yang CW, Harpending P, McDonough SP, Dubovi E, Divers T, Qu J, Roe B. Evaluation of lig-based conventional and real time PCR for the detection of pathogenic leptospires. Mol Cell Probes. 2005; 19(2):111-7.
17. Slack AT, Symonds ML, Dohnt MF, Smythe LD. Identification of pathogenic Leptospira species by conventional or real-time PCR and sequencing of the DNA gyrase subunit B encoding gene. BMC Microbiol. 2006; 6: 95.
18. Cerqueira GM, Picardeau M. A century of Leptospira strain typing. Infect Genet Evol. 2009; 9: 760-768.
19. Brown PD, Levett PN. Differentiation of Leptospira species and serovars by PCR-restriction endonuclease analysis, arbitrarily primed PCR and low-stringency PCR. J Med Microbiol. 1997; 46: 173–181.
20. Ciceroni L, Ciarrocchi S, Ciervo A, Petrucca A, Pinto A, Calderaro A, Viani I, Galati L, Dettori G, Chezzi C, Differentiation of leptospires of the serogroup Pomona by monoclonal antibodies, pulsed-field gel electrophoresisand arbitrarily primed polymerase chain reaction. Res Microbiol. 2002; 153: 37–44.
21. Roy S, Biswas D, Vijayachari P, Sugunan AP, Sehgal SC. A 22-mer primer enhances discriminatory power of AP-PCR fingerprinting technique in characterization of leptospires. Trop Med Int Health. 2004; 9(11):1203-1209.

22. Pereira MM, Matsuo MG, Bauab AR, Vasconcelos SA, Moraes ZM, Baranton G, Girons IS. A clonal subpopulation of Leptospira interrogans sensu stricto is the major cause of leptospirosis outbreaks in Brazil. J Clin Microbiol. 2000; 38: 450–2.
23. Benacer D, Mohd Zain SN, Sim SN, Mohd Khalid SZ, Galloway RL, Souris M, Thong KL. Determination of Leptospira borgpetersenii serovar Javanica and Leptospira interrogans serovar Bataviae as the persistent Leptospira serovars circulating in the urban rat populations in Peninsular Malaysia. Parasit Vect. 2016; 9 (1): 117.
24. Gökmen TG, Kızılyıldırım S. Pulsed Field Jel Elektroforezi (PFGE). Hast İnf Der. 2011; 15(1): 1-10.
25. Nieves C, Ferrés I, Díaz-Viraqué F, Buschiazzo A, Zarantonelli L, Iraola G. Draft Genome Sequences of 40 Pathogenic Leptospira Strains Isolated from Cattle in Uruguay. Microbiol Resour Announc. 2019; 8(47): 21.
26. Galloway RL, Levett PN. Evaluation of a modified pulsed-field gel electrophoresis approach for the identification of Leptospira serovars. Am J Trop Med Hyg. 2008; 78: 628–632.
27. Koizumi N, Uchida M, Makino T, Taguri T, Kuroki T, Muto M, Kato Y, Watanabe H. Isolation and characterization of Leptospira spp. from raccoons in Japan. J Vet Med Sci. 2009; 71(4): 425-429.
28. Mende K, Galloway RL, Becker SJ, Beckius ML, Murray CK, Hospenthal DR. Interlaboratory agreement of pulsed-field gel electrophoresis identification of Leptospira serovars. Am J Trop Med Hyg. 2013; 89(2): 380–384.
29. Nalam K, Ahmed A, Devi SM, Francalacci P, Baig M. Genetic affinities within a large global collection of pathogenic Leptospira: implications for strain identification and molecular epidemiology. PLoS One. 2010; 5: 12637.
30. Vijayachari P, Ahmed N, Sugunan AP, Ghousunnissa S, Rao KR, Hasnain SE, Sehgal SC. Use of fluorescent amplified fragment length polymorphism for molecular epidemiology of leptospirosis in India. J Clin Microbiol. 2004; 42: 3575–3580.
31. Majed Z, Bellenger E, Postic D, Pourcel C, Baranton G, Picardeau M. Identification of variable-number tandem-repeat loci in Leptospira interrogans sensu stricto. J Clin Microbiol. 2005; 43: 539–545.
32. Slack AT, Dohnt MF, Symonds ML, Smythe LD. Development of a multiplelocus variable number of tandem repeat analysis (MLVA) for Leptospira interrogans and its application to Leptospira interrogans serovar Aus-

tralis isolates from far North Queensland, Australia. Ann Clin Microbiol Antimicrob. 2005; 4: 10.
33. Salaun L, Merien F, Gurianova S, Baranton G, Picardeau M. Application of multilocus variable-number tandem-repeat analysis for molecular typing of the agent of leptospirosis. J Clin Microbiol. 2006; 44: 3954–3962.
34. Pailhoriès H, Buzelé R, Picardeau M, Robert S, Mercier E, Mereghetti L, Lanotte P. Molecular characterization of Leptospira spp. by multilocus variable number tandem repeat analysis (MLVA) from clinical samples: a case report. Int J Inf Dis. 2015; 37: 119-121.
35. Caimi K, Ruybal P. Leptospira spp., a genus in the stage of diversity and genomic data expansion. Infect Genet Evol. 2020; 81: 104241.
36. Xiao G, Yi Y, Che R, Zhang Q, Imran M, Khan A, Yan J, Lin X. Characterization of CRISPR-Cas systems in Leptospira reveals potential application of CRISPR in genotyping of Leptospira interrogans. Apmis 2019; 127: 202–216.
37. Ahmed N, Devi SM, Valverde M, Vijayachari P, Machang'u RS, Ellis WA, Hartskeerl RA. Multilocus sequence typing method for identification and genotypic classification of pathogenic Leptospira species. Ann Clin Microbiol Antimicrob. 2006; 5: 28.
38. Varni V, Ruybal P, Lauthier JJ, Tomasini N, Brihuega B, Koval A, Caimi K. Reassessment of MLST schemes for Leptospira spp. typing worldwide. Infect Genet Evol. 2014; 22: 216–222.
39. Guglielmini J, Bourhy P, Schiettekatte O, Zinini F, Brisse S, Picardeau M. Genus-wide Leptospira core genome multilocus sequence typing for strain taxonomy and global surveillance. PLoS Negl Trop Dis. 2019; 13: e0007374.
40. Ayral F, Zilber AL, Bicout DJ, Kodjo A, Artois M, Djelouadji D. Distribution of Leptospira interrogans by Multispacer Sequence Typing in Urban Norway Rats (Rattus norvegicus): A Survey in France in 2011-2013. PLOS ONE. 2015; 10(10): e0139604.
41. Zilber AL, Picardeau M, Ayral F, Artois M, Demont P, Kodjo A, Djelouadji Z. High-resolution typing of Leptospira interrogans strains by multispacer sequence typing. J Clin Microbiol. 2014; 52: 564–571.
42. Le Guyader M, Fontana C, Simon-Dufay N, Balzer HJ, Pantchev N, Thibault JC, Cupillard L, Bomchil N, Kodjo A. Successful Leptospira genotyping strategy on DNA extracted from canine biological samples. J Micbiol Methods. 2020; 176: 106007.
43. Xu Y, Zhu Y, Wang Y, Chang YF, Zhang Y, Jiang X, Zhuang X, Zhu Y,

Zhang J, Zeng L, Yang M, Li S, Wang S, Ye Q, Xin X, Zhao G, Zheng H, Guo X, Wang J. Whole genome sequencing revealed host adaptation-focused genomic plasticity of pathogenic Leptospira. Sci Rep. 2016; 6: 20020.

44. Cosate MR V, Soares SC, Mendes TA, Raittz RT, Moreira EC, Leite R, Fernandes GR, Haddad JPA, Ortega JM. Whole-genome sequence of Leptospira interrogans serovar Hardjo subtype Hardjoprajitno strain Norma, isolated from cattle in a leptospirosis outbreak in Brazil. Genome Announc. 2015; 3: 1–2.

Chapter-6

MANAGEMENT OF HEMORRHOIDAL DISEASE

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PREFACE

Hemoroidal disease is a very common medical problem. It is estimated that half of the population has the hemorroidal symptoms. The symptoms of hemorroidal disease are very common and require elimination of other anorectal pathologies especially tumors.

Patients generally prefer using medications by themselves which can cause diagnostic errors. Contributing factors for increased incidence of symptomatic hemorrhoids include conditions that elevate intra-abdominal pressure such as pregnancy and straining, or those that weaken supporting tissue.

The management of hemorroidal disease includes medical treatment, dietary regulation, local procedures, mucosal fixation techniques, or excision surgery.

Nowadays, there are lots of new technologies which give us to provide wide range of treatment modalities according to patients' clinical situations.

MANAGEMENT OF HEMORRHOIDAL DISEASE

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The management of hemorroidal disease includes medical treatment, dietary regulation, local procedures, mucosal fixation techniques, or excision surgery.

Hemorrhoidal disease is requiring surgical management in approximately 10% of cases. Despite its long history and high prevalence, we are still trying to identify the best treatment. Earlier surgical approaches were soon abandoned and now only detain an historic significance. For long, proctologists have given their preference to hemorrhoidectomy that was gradually perfected through the years. The true innovation came in 1937, with the famous Milligan-Morgan hemorrhoidectomy, still one of the leading interventions for treatment of hemorrhoids. Less fortune encountered alternative techniques, such as the Whitehead hemorrhoidectomy, and closed and semi-closed techniques. Later on, the advent of a new concept of the pathogenesis of hemorrhoidal disease has brought to the development of stapled prolassectomy techniques. This approach has encountered both supporters and detractors between the experts in this field and has received a strong impulse by the emerging trend towards “day-surgery”. Today the search for the best surgical technique for hemorrhoidal disease is far from being over and witnesses the introduction of new techniques for hemorrhoidal dissection. The choice of the best strategy remains in the hands of the clinician in the modern conception of tailored surgery[1] .

In the healthy body, hemorrhoids contribute to continence by enhancing anal closure, and therefore treatment that includes the excision of hemorrhoidal tissue should be avoided or should be the treatment of last resort, because of the risk of secondary fecal incontinence. In addition, complications such as secondary bleeding, anal sphincter injury, and stenosis may occur after invasive surgery[2,3]. The treatment of hemorrhoids should focus on eliminating the symptoms and minimizing postoperative pain, complications, and recurrences. Against this back ground, noninvasive surgical treatments of hemorrhoids have shown great advances in recent years. Multiple techniques are now available. Rubber band

ligation (RBL), sclerotherapy, and infrared coagulation are the most common, but other techniques are also employed: cryosurgery, bipolar diathermy, laser coagulation, the Ultroid (Ulterior Technologies, Tampa, FL, USA) approach, and anal dilatation. As noted in recent reviews, each of the above methodologies has its advocates, and there is no perfect technique. Randomized controlled trials have compared each method with some others, but there is no overarching study that has compared all the techniques with each other [4,5].

DEFINITION

Hemorrhoids is a very common anorectal disease defined as the symptomatic enlargement and/or distal displacement of anal cushions, which are prominences of anal mucosa formed by loose connective tissue, smooth muscle, arterial and venous vessels[6].

Hemorrhoids are two distinct vascular structures: the internal hemorrhoidal plexus, which is submucosal, and the external hemorrhoidal plexus, which is subcutaneous.

The term internal hemorrhoids is used above all in relation to the internal plexus.

Hemorrhoids are normal anatomical structures[1].

ANATOMY

ANAL CANAL

It is the final part of digestive tract which passes through the perineum. It measures 3 to 4 cm. The anal canal is divided into two part by the dentate line (or pectinate line), situated at the juntion of the lower 1/3 and middle 1/3, and runs obliquely upwards and backwards.

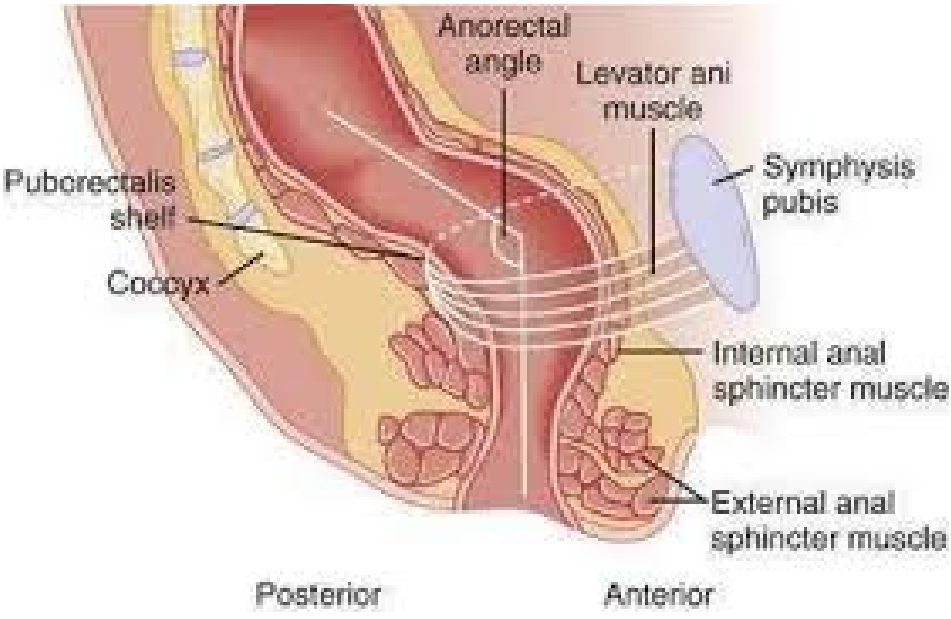


Diagram 1: Orientation of anal canal.

The mucosa of the anal canal below the dentate line forms the pecten, powewrfully adherent to deep layers by virtue of the presence of Parks' ligament. Above, the mucosa is raised in longitudinal folds, the rectal columns or columns of Morgagni, which cover the internal hemorrhoidal plexus.

ANAL SPHINCTER

The mucosa of the anal canal is surrounded by two cylindirical cuffs. The fist internal cylinder is internal sphincter which results from thickening and from downward prolongation of the internal circular muscle layer of the rectum. The external cylinder is formed by the external sphincter which is continuity above with the pelvic floor (puborectalis) and a subcutaneous superficial band.

In the lithotomy position, the subcutaneous band is reflected at the peryphery of the margin, the lower edge of the internal sphincter then lying close proximity to the skin. The finger feels the intersphincteric grooves which separate the internal sphincter from the subcutaneous band of the external sphincter. This forms the intersphinteric space. It is occupied by the conjoined longitudinal muscle, with fibers from puborectalis and from the longitudinal muscle of the rectum. This fibromuscular complex passes under the internal sphincter to form Parks' ligament which adheres to pecten.

INTERNAL HEMORRHOIDS

Internal hemorrhoids are blood cavities. They are lined by venous endothelium or by a capillary wall (photo 1) .

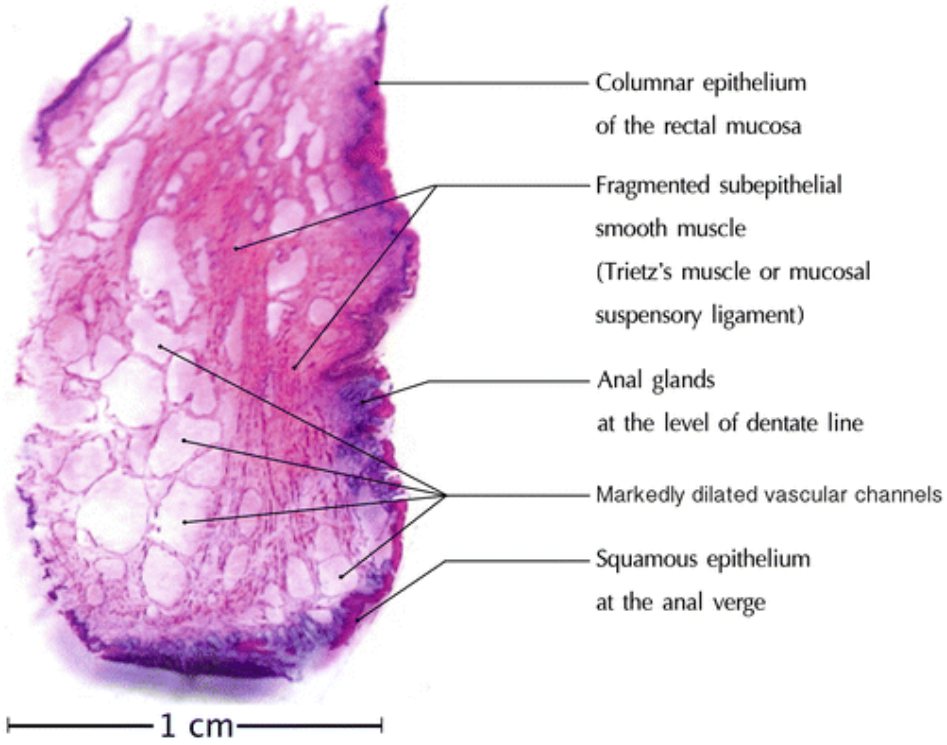


Photo 1: Blood pools of hemorrhoids (Histopathologic changes in advanced hemorrhoids)

Furthermore, arteiovenous anastomoses exist within hemorrhoids, giving the tissue cavernous type appearance.

Internal hemorrhoids are surrounded by non glandular transitional or non-keratinized squamous epithelium.

This vascular tissue is held in place above Parks' ligament, by musculoconnective fibers, a true suspensory ligament, arising from the submucosae ani, from the in the internal sphincter and from the conjoined longitudinal muscle.

EXTERNAL HEMORRHOIDAL PLEXUS

This consists of small subcutaneous veins which drain the margin of the anus. They are situated in the immediate proximity of the subcutaneous band of the external sphincter.

They are located in the marginal space.

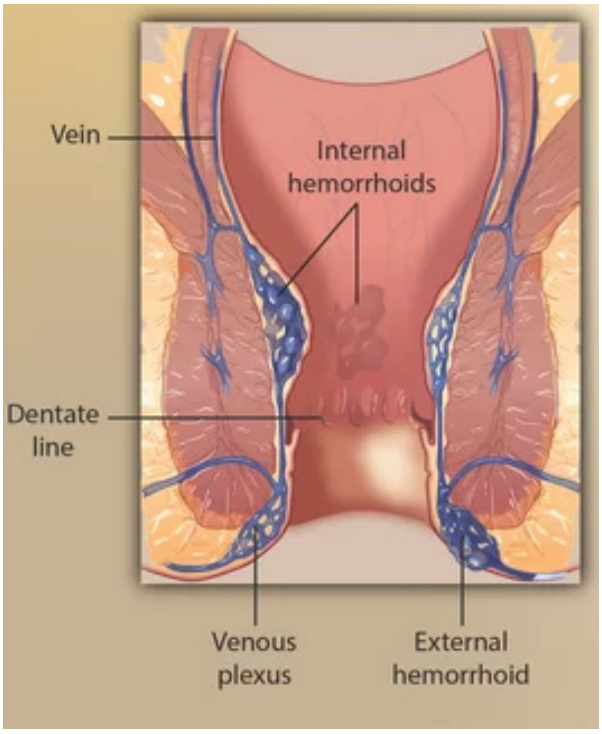


Diagram 2: Saggital section of anal canal.

BLOOD SUPPLY OF HEMORRHOIDS

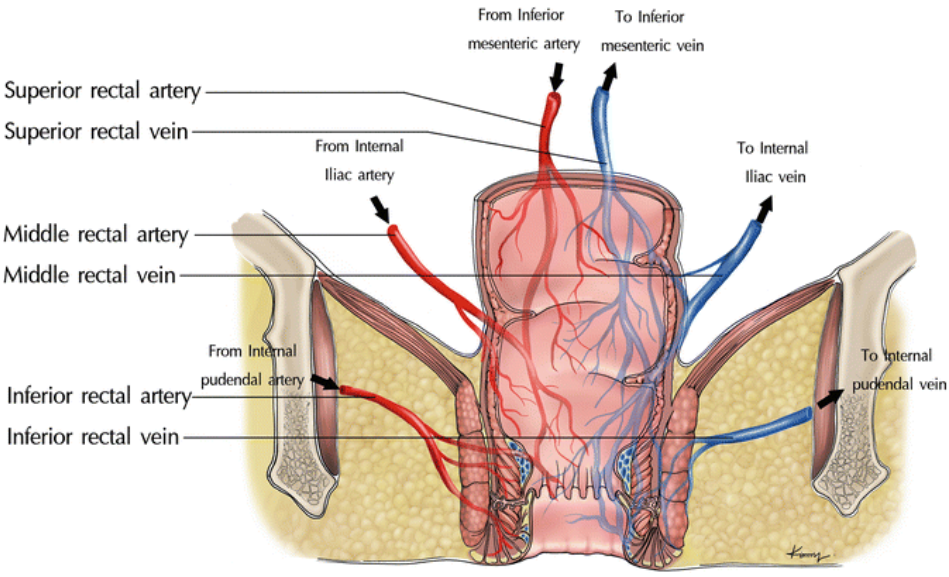


Diagram 3: Anatomy of anorectal vasculature[2]

The rectum and anal canal are supplied by 3 arteries:

1. Superior rectal artery; terminal branch of the inferior mesenteric artery
2. Middle rectal artery; arising from the internal iliac artery
3. Inferior rectal artery; arising from the pudental artery.

Extensive anastomoses exist between them. Arterial flow in the internal hemorrhoids is eesentially of submucosal origin and comes from the superior rectal artery.

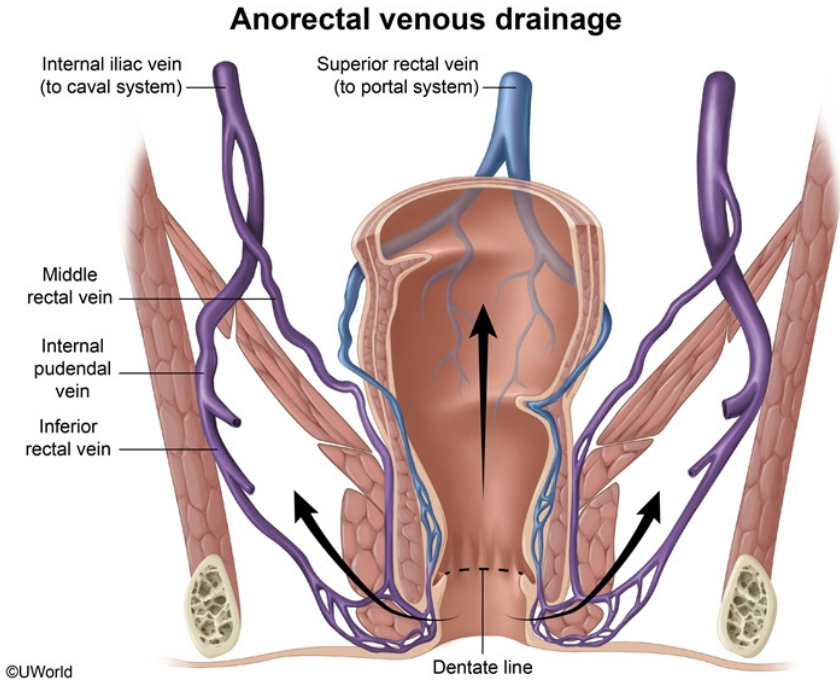


Diagram 4: Venous drainage rectum and hemorrhoids

Veins are satellite to arteries and have identical names. Internal hemorrhoids drain into;

superior rectal vein, which finally drain into portal vein,

The middle rectal vein drain into inferior vena cava.

Internal hemorrhoids are anatomical portocaval anastomoses.

The inferior rectal vein arises from external subcutaneous plexus. A fine submucosal vascular system forms a commucation between internal and external hemorrhoidal plexi.

NERVE SUPPLY

The dentate line separates the internal and external hemorrhoidal plexus. It is an important landmark because it serves as a guide to the site where the sesation of pain is perceived. Pain is not perceived 1 to 2 cm above the dentate line.

PATHOPHYSIOLOGY OF HEMORRHOIDS

Hemorrhoids are normal structures. They become pathological if symptoms occur.

It is currently thought that the cushion arrangement of hemorrhoidal tisuue enables it to ensure fine occlusion of the anal canal and thus to play role in con-

tinence. The vascular component enables hemorrhoids to adjust and adapt to the size of anal canal.

PATHOGENESIS

The exact pathophysiology of symptomatic hemorrhoid disease is poorly understood. Previous theories of hemorrhoids as anorectal varices are now obsolute as shown by Goenka et al, patients with portal hypertension and varices do not have an increased incidence of hemorrhoids [7]. Currently, the theory of sliding anal canal lining, which proposes that hemorrhoids occur when the supporting tissues of the anal cushions deteriorate, is more widely accepted. Advancing age and activities such as strenuous lifting, straining with defecation, and prolonged sitting are thought to contribute to this process. Hemorrhoids are therefore the pathological term to describe the abnormal downward displacement of the anal cushions causing venous dilatation[5]. On histopathological examination, changes seen in the anal cushions include abnormal venous dilatation, vascular thrombosis, degenerative process in the collagen fibers and fibroelastic tissues, and distortion and rupture of the anal subepithelial muscle. In severe cases, a prominent inflammatory reaction involving the vascular wall and surrounding connective tissue has been associated with mucosal ulceration, ischemia, and thrombosis[8].

There are several mechanism to explain the onset of hemorrhoidal disease.

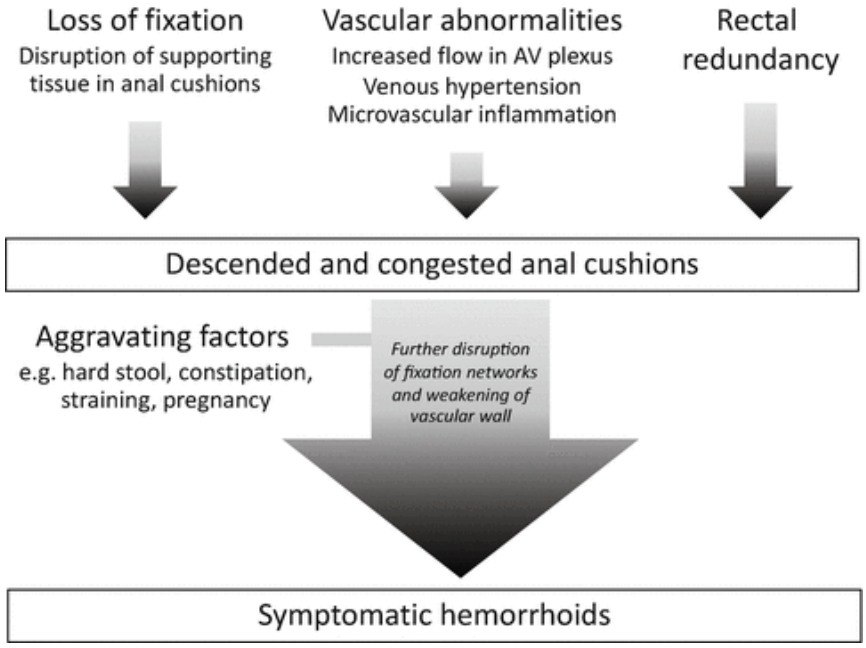


Diagram 5: Pathophysiology of hemorrhoids[2]

Mechanical theory

The musc ulofibroelastic tisuue supporting internal hemorrhoids and holding them in place tends to degenerate with age. Abnormal laxity of the means of fixation of hemorrhoids which are no longer firmly attached to the deep layers

This laxity results in the greater mobility of hemorrhoids, which can then move when the intrarectal pressure tends to increase (constipation). At the maximum point the suspensory ligament and Parks' ligament are ruptured and internal hemorrhoids permanently prolapsed at the anal verge.

Laxity of support tissues also enables distension of the vascular component and cause increase in the size of hemorrhoids.

Mobilization and distension of hemorrhoids result in fragilization of the mucosa covering the hemorrhoids which cause bleeding of hemorrhoids.

Genetic determination of deterioration of the support tissues of internal hemorrhoids would offer an explanation of the high incidence of hemorrhoidal disase in certain families.

Hemodynamic theory

The pathogenesis of internal hemorrhoids involves three vascular structures: veins, arteries, and arteriovenous shunts. They are implicated to some extent in the onset of the disease and its symptoms.

a. Role of venous circulation

Internal hemorrhoids may be caused by a backflow of venous blood. This backflow could be result of increased intraabdominal pressure (pregnancy, straining of stools).

Portal hypertension is a more theoretical cause. Lots of studies have failed to demonstrate any increase in the incidence of hemorrhoids in patients suffering from portal hypertension.

The distension of hemorrhoids could be enhanced by vascular stasis secondary to impaired venous return:

by mechanical obstruction: difficult defecation and persistence of fecesin the rectal ampulla.

by functional obstruction: defect in venous drainage due to failure of internal sphincter to relax during defecation

b. Role of arterial circulation

An incrase in blood flow in the splanchnic territory, affecting the inferior mesenteric artery, could cause dilatation of internal hemorrhoids. Postprandial hemorrhoidal symtoms are attributed to this by certain authors.

c. Role of arteiovenous shunts

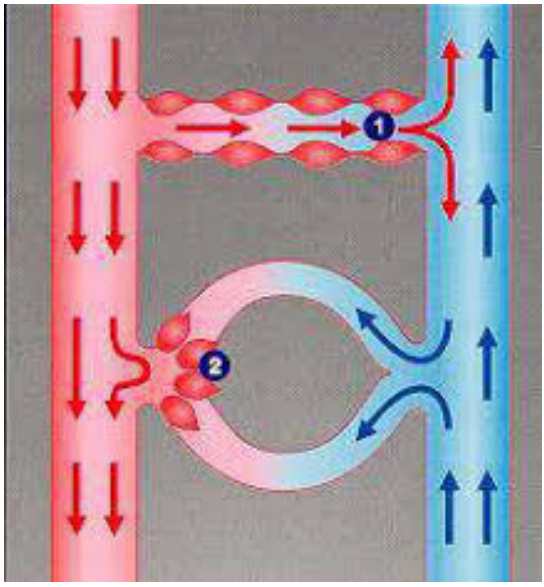


Diagram 6: Role of atreiovenous shunts 1. Opening of aretiovenous shunts 2. contraction of precapillary sphincter.

Hypothetically existence of the precapillary sphinvter capable of reacting to hormonal or neurophysiological stimuli is compatible with this hypothesis and could be an explanation for the fluctuating nature of the manifestations of hemorrhoids. No definitely confirmed mechanisms have been demonstrated up to now on the basis of what can be found in the literature.

Sphincter factors

Several types of abnormality have been shown by anorectal manometry in patients suffering from hemorrhoidal disease. The main finding is increase in the resting pressure of anal canal.

This raising in pressure appears to be more in relation wtih filling of hemorrhoids than to any increase in sphincter activity. This spasticity disappears after hemorrhoidectomy.

Finally, there is no unanimous theory exists to explain the onset of pathological internal hemorrhoids. The various theories are not mutually exclusive and is reasonable to assume that the pathophysiology of internal hemorrhoids dost not involve one single factor only [1].

FACTORS TRIGGERING HEMORRHOIDAL SYMPTOMS

The mechasnisms described before enable understanding of the formation of pathological hemorrhoids.

Certain triggering factors are well defined.

. Disturbances of intestinal function (constipation, diarrhea)

. Difficulties in emptying the rectum

. Pregnancy, labor

. Local medications (suppositories, enema)

Other suggested factors

. Menstruation

. Sedentary life style

. Sports (horseback riding, cycling, diving)

. Alcohol

. Spices

Summary of Different Concepts Regarding the Pathophysiology of Hemorrhoids and Related Therapeutic Approaches

Concept	**Short description**	**Therapeutic approach**
Sliding anal cushions (loss of fixation network)	Hemorrhoids develop when the supporting tissues of anal cushions disintegrate or deteriorate	Rubber band ligation, plication of hemorrhoids, hemorrhoidectomy
Vascular abnormality	The high arterial blood flow and venous hypertension of anorectal vascular plexus and/or structural changes of anorectal vasculature lead to the formation of hemorrhoids	Oral or topical phlebotonics, injection sclerotherapy, laser treatment, Doppler-guided hemorrhoidal artery ligation
Rectal redundancy	Prolapsing hemorrhoids are associated with an internal rectal prolapse	Stapled hemorrhoidopexy or procedure for prolapse and hemorrhoids
An increased pressure on anorectal vascular plexus	An increased pressure on anorectal vascular plexus results in the development of hemorrhoids or aggravates the symptoms of hemorrhoids	

Table 1: Summary of Different Concepts Regarding the Pathophysiology of Hemorrhoids and Related Therapeutic Approaches [9]

ANORECTAL EXAMINATION AND THE DIAGNOSIS OF INTERNAL HEMORRHOIDAL DISEASE

Proctological examination is well tolerated by patients if performed gently and if it is explained in advance, with mention being made of its succesisve phases. It is important to be able to convince the patient, notably because this examination is irreplaceable. Colonoscopy or rectosigmodoscopycannot take its place since they do not provide a view of this region.

The diagnosis of hemorrhoids pure clinical, the same applying to a wide range of proctological diseases.

CLINICAL PRESENTATION

The diagnosis of hemorrhoids is made by patients in general, but any anorectal symptomatology is synonymous with hemorrhoids. Statements by the patients do not suffice to make the diagnosis. It is essential to specifically define the presenting symptoms of the patient in order to detect any possible abnormalities which could not be related to hemorrhoids.

Bleeding:

It is estimated thet 10% of an adult population has minimal anal bleeding. This bleeding is due to hemorrhoids 70 to 80 % of cases, and 15 % of cases to a fissure. Thus, in the majority of the cases, the origin of anal bleeding is confirmed by clinical examination.

Investigation of the colon (barium enema, flexible sigmoidoscopy or colonoscopy) is necessary only to eliminate other causes of bleeding.

Children	**Adults**	**Elderly people**
Meckel's diverticulum	Inflammatory bowel disease	Diverticular disease
Juvenil polyps	Adenamatous polyps	Angiodysplasia
Inflammatory bowel disease	Carcinoma	
	Arteriovenous malformations	
	Small intestinal neoplasia	
	Hereditary telangiectasia	
	Infective colitis	
	Hemorrhoids	
	Solitary rectal ulcer	
	Anal fissure	

Table 2: Causes of rectal bleeding

Hemorrhoidal bleeding is typically associated with defecation consisting of bright red blood, separate from stools and often dripping immediately after defecation. Blood is sometimes streaked on the stool. More rarely, blood has stagnated in the rectum and the presence of clots and of dark red blood is then possible.

The extent of bleeding varies considerably. There may merely be bleeding visible on toilet paper or, on the contrary, it may spatter the toilet bowl.

In all cases, it is essential to eliminate any other cause of bleeding, and tumors in particular, before attributing bleeding to hemorrhoids.

Pain:

The type of pain must be defined, with its acute or chronic nature and the changes which occur in it at the time of rectal emptying.

Internal hemorrhoids are not usually painful. When pain is the chief complaint, another source should be sought.

Internal hermorrhoids are painful if they are associated with:

. a thrombosed perianal varyx

. a fissure

. thrombosed hemorrhoidal prolapse

. an internal thrombosis

Painless	**Painful**
Internal hemorrhoids	Thrombosed external hemorrhoids
Anal fistula	Mixed hemorrhoids
Polyps (anal or rectal)	Anal fissure
Early cancer	Anal trauma
Radiation telangiectasia	Advanced cancer
Kaposi sarcoma	Rectal abscess
Solitary rectal ulcer syndrome	Anal warts
Rectal varices	Rectal prolapse
Postsurgical anastomotic ulcer	Proctitis ani
Postpolipectomy ulcer	Proctitis or colitis

Table 3: Causes of rectal pain and painless pathologies

Swelling and prolapse:

Mobilization of internal hemorrhoids and their exteriorization at the anal verge may be felt by the patient. The latter describes the development of a mass at the time of defecation: prolapse.

It is sometimes associated with feeling of discomfort, described as a desire to defecate, feeling of fullness in the perineum or anus, or heaviness. Pain may occur if patients strain excessively, but another cause should the be sought, in particular a fissure.

Patients may also merely describe a feeling of swelling or heavinessduring rectal emtying.

Patients may finally describe a permanent swelling at the anal verge. This may be due to prolapsed hemorrhoids.

Swellings of the anal canal:	
acute	thrombosed external hemorrhoids abscess
chronic	condyloma tags carcinoma
Prolapse:	
	hemorrhoids rectal prolapse anal tumors hypertrophic anal papilla rectal tumors

Table 4: Swelling and prolapses of the anal canal

Discharge:

In the cases of hemoorhoids, it involves a watery or mucoid discharge. It may be responsible merely for a feeling of dampnessat the anal verge, or a discharge staining clothing.

It occurs in the presence of prolapsed hemorrhoids, even when intermittant.

. Watery:	- hemorrhoids
	- total prolapse of rectum
	- mucosal prolapse
	- condyloma
	- eczema
	- fissure
	- carcinoma
	- inadequate hygiene
	- villous adenoma
	- irritable bowel syndrome
	- solitary rectal ulcer
	- inflammatory bowel disease
. Purulent:	- abscess
	- fistula
	- fissure (infected)
	- hidradenitis
	- furuncle
	- inflammatory bowel disease
	- sexually transmitted disease
. Fecal:	- inadequate hygiene
	- fecal incontinence

Table 4: Causes of anal discharge

Pruritus ani:

This is an extremely frequent symptom. Pruritus may occur during hemorrhoidal disease. It is usually associated with prolapse and discharge, which favor local maceration. Othet then tis situation, pruritus is no connected with hemorrhoids.

The cause of pruritus ani are listed:

Infections	. parasites . Bacxterial infections (group A, beta-hemolytic streptococcus, Staphylococcus aureus) . Fungal infections (particularly Candida) . Sexually transmitted diseases (condyloma, herpes, syphilis, gonorrhea)
Fecal soiling	. Encopresis . Incontinence (usually post anorectal or bowel surgeries) . Chronic diarrhea . Poor hygiene . Transient internal relaxation of the anal canal sphincter . Anatomic abnormalities (e.g. proplapsed internal hemorrhoids, rectal prolapse)
Local irritation	. excessive use of soap and detergents . use of local creams and medications
Dietary agents	. coffee . cola . beer . tomatoes . chocolate . tea . citrus fruits . milk
Anorectal disease	. abcess . fistula . fissure . dermatologic disease . psoriasis . contact dermatitis . atopic dermatitis . hydradenitis suppurativa . paget disease
Systemic disease	. Diabetes . Hepatic disease . Leukemia . thyroid disorder . psychological disorders
Other	. chemotherapy

Table 5: Causes of pruritus ani

Fecal incontinence

It is a more common symptom than might be suspected on the basis presenting symptomatology.

It has been found more than 11-15 % of an adult population living at home [10].

It is not directly related with hemorrhoids. It is essential to seek it out since patients may not complain of its spontaneously. Its presence indicates the the need for caution concerning indications for surgery hemorrhoidectomy may worsen it in the presence of pelvic floor diseaseor of previous surgery.

Disturbances of rectal emptying and intestinal function

Bowel habits must be accurately defined and analayzed. Correction of disturbances in intestinal function (constipation in particular) and of difficult rectal emptying is the first stage of any treatment of hemorrhoids.

STAGING OF HEMORRHOIDS

It is possible to grade hemorrhoids on the basis of findings by this clinical examination.

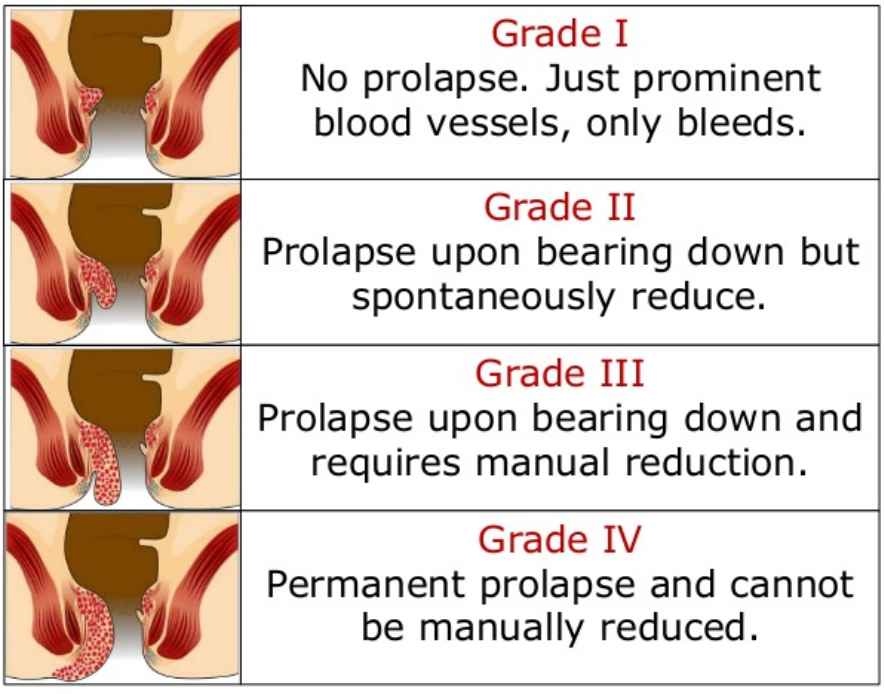


Table 6: Grading of hemorrhoids

EXTERNAL HEMORRHOIDAL THROMBOSIS (EHT)

It is extremely common condition. It is due to formation of a clot in one of the veins of the subcutaneous perianal plexus, still referred to as the external hemorrhoidal plexus.

It is located under the epidermis of the anal verge.

Pathology takes the form only of thrombosis. Thrombosis may affect the entire circumference of the anal verge, but most often causes a hemispherical swelling at one point on the anal verge.

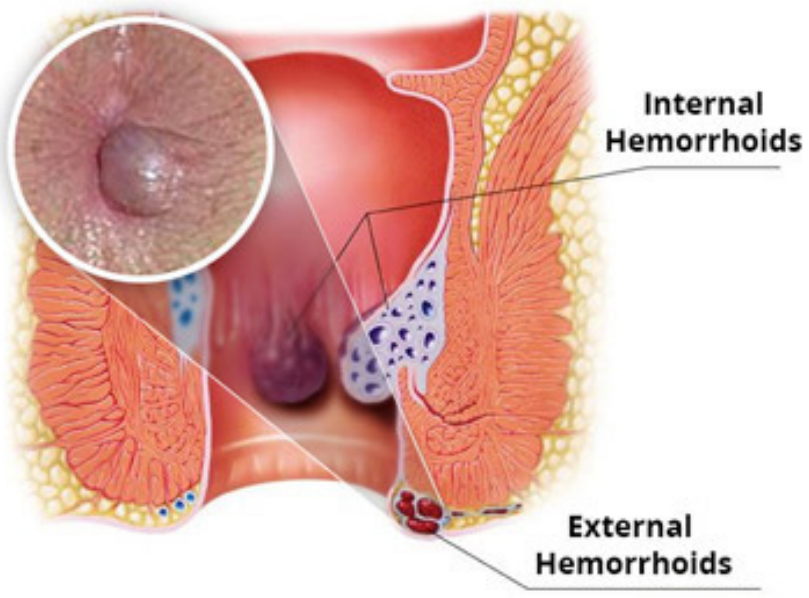


Photo 2: External thrombosed hemorrhoid (EHT)

PATHOPHYSIOLOGY

The pathophysiology of external hemorrhoids is not fully elucidated. During obstructed defecation and constipation with straining at stool, increased local venous pressure could lead to mechanical impairment of venous return, thereby possible favoring dilatation of the external hemorrhoidal plexuses and the onset of an EHT[1].

Apart from constipation, other situations accompanied by increased intarabdominal pressure (pregnancy, labor) alseo classically favor the onset of an EHT.

Symptoms

Typically, the patient presents with pain in the form tension, of sudden onset

and recent onset, located at the margin of the anus.

This pain, typically very severe, is permanent, nonpulsatile, and not chronically related to defecation as in the case of anal fissure.

This pain is concomittant wtih the onset of painful and tender swelling of the anal verge. This is a single irreducible swelling, accompanied by neither discharge nor bleeding.

Ther is no relation between the size of the swelling and the severity of pain, which may be very intense and virtually intolerable, or on the contrary only very slight.

OUTCOME

Immediate: The outcome is spontanously favorable, pain disappearing in 2 to 7 days. Swelling of hemorrhoids goes down within 1 to 6 weeks.

Thrombosis may necrose and spontaneous bleeding is responsible for immediate relief of symptoms.

Late: External hemorrhoidal thrombosis may leave late skin sequelae in the form of skin tags. These painless skin folds are often improperly referred to as external hemorrhoids.

TREATMENT

Excision of external hemorrhoid

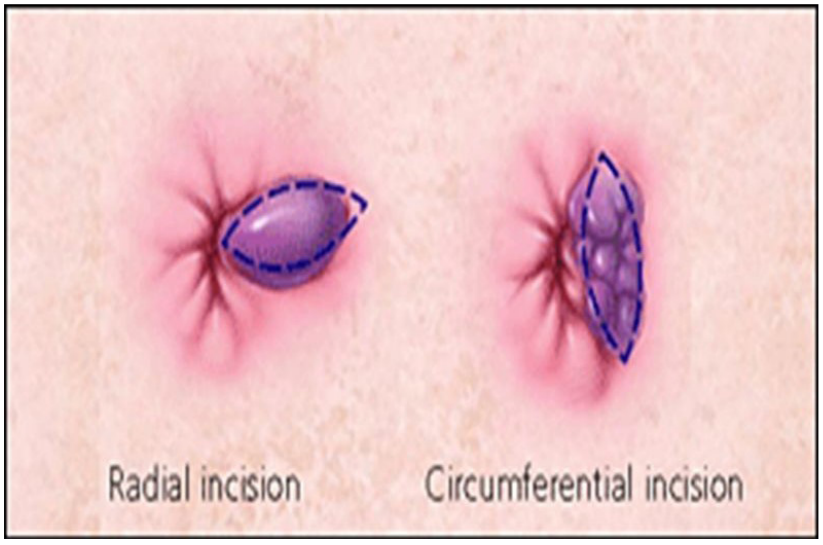


Diagram 7: Excision of external hemorrhoid

After local anesthesia by intra- and perithrombotic infiltration of an anesthetic, either the thrombosis is incised vertically, enabling evacuation of the clot, or the entire swelling is excised.

Excision of EHT is patricularly indicated in the presence of a painful and visible thrombus, or where there is persistent swelling.

TREATMENT OF INTERNAL HEMORRHOIDS

PRINCIPLES OF TREATMENT

Normal anatomical structures, internal hemorrhoids should be treated only if they are responsible for adverse manifestations [1].

The demand for treatment should come from the patients themselves, and this is an important point to be taken into account.

Hemorrhoidal disease is benign, with no mortality associated with it, this is not necessarily applying to treatment and in particular when surgical.

Hemorrhoids have a physiological role in continence, notably of flatus and of liquids.

Treatment must leave anatomical structures as intact as possible.

It is only in the case of failure of medical and instrumental treatment methods that the removal of actual hemorrhoid tissue should be envisaged [1].

The aim of treatment is to influence the mobilization of hemorrhoids and fragilization of mucosa.

1. Medical teratment

Medical treatment is always indicated. It is adjusted in terms of the extent of pain, size of thrombosis, and underlying condition (pregnant or breastfeeding women in particular) .

It includes:

. warm sitz baths

. standard analgesics (paracetamol)

. topical anti-inflammatory agents, containing a corticosteroid in some instances

. oral anti-inflammatory drugs

In all cases, such treatment must be accompanied by regularization of intestinal function.

Such treatment is indicated in the following situations:

. relatively painless thrombosis of moderate size,

. thrombosis accompanied by edema.

Regularization of intestinal funciton and of defecation:

This is the basic treatment for hemorrhoidal disease. It is possible to influence the volume and degree of hydration of feces.

A wide range of laxatives can be suggested:

. bran,

. mucilages,

. liquid paraffin,

. nonabsorbable sugars (lactulose).

General dietary advice:

The aim is dealing with constipation; icreased daily fiber intake, drinking of water, regularity of defecation.

Suggest avoidance of colonic srimulants (coffee, tea, to avoid alcohol and spicy foods).

These various food products do not cause hemorrhoidal disease but may lead to its revealing itself.

Flavonoids

They are widely prescribed for treating hemorrhoidal disease since they represent the only oral treatment available.

Their mode of aciton has not been completely elucidated in hemorrhoidal diseas to which the concepts of venous disease in general are not transposable.

The flavonoid derivatives act by decreasing capillary fragility and by anti inflammatory activity.

Clinical efficacy of certain flavonoid derivatives has been rigorously demonstrated in the symptomatic treatment of acute hemorrhoidal attacks provided they are used in high doses.

When prescribed in the long term some flavonoid derivatives are able to prevent the recurrence of acute attacks.

Topical agents

Large number of topical agents exist, many of them on sale over the counter. They consists of various combinations of:

. anti-inflammatory agents, steroids in particular,

. local anaesthetics,

. oily substances,

. various vasculotropic derivatives.

They act by lubricating the anal canal, facilitating fecal evacutaion. They are often used in ointment form with a finger-stool, rather than as suppositories. They have the advantage in the eyes of patients of being applied directly to the site of pain.

2. Instrumental treatment (Nonsurgical Office-based Procedures)

The common principle is to induce fibrotic scarring at the apex of the internal hemorrhoidal plexus. Whatever the technique used, one must privilege single-use devices; when used, reusable equipment must be sterilized after each use according to standard procedures[11].

For internal hemorrhoids, rubber band ligation, sclerotherapy, and infrared coagulation are the most common procedures but there is no consensus on optimal treatment. Overall, the goals of each procedure are to decrease vascularity, reduce redundant tissue, and increase hemorrhoidal rectal wall fixation to minimize prolapse [9]. This is a complement to medical treatment.

a. Injection sclerotherapy

Principle:

The fibrous scar is obtained following an inflammatory reaction induced by various substances: quinine-urea hydrochloride, 5% phenol, 0,5 or 1 % polidocanol, and hypertonic saline.

Techniques:

Equipment necessary: 10 ml syringe, one amp compound, one small caliber eg, 28 G needle and a needle extension, a proctoscope.

The procedure itself: the injection is strictly submucosal through the proctoscope and the summit of clumps of hemorrhoidal tissue. Hemorrhoids are identified by withdrawal of the apparatus which is then reinserted for a few milimeters.

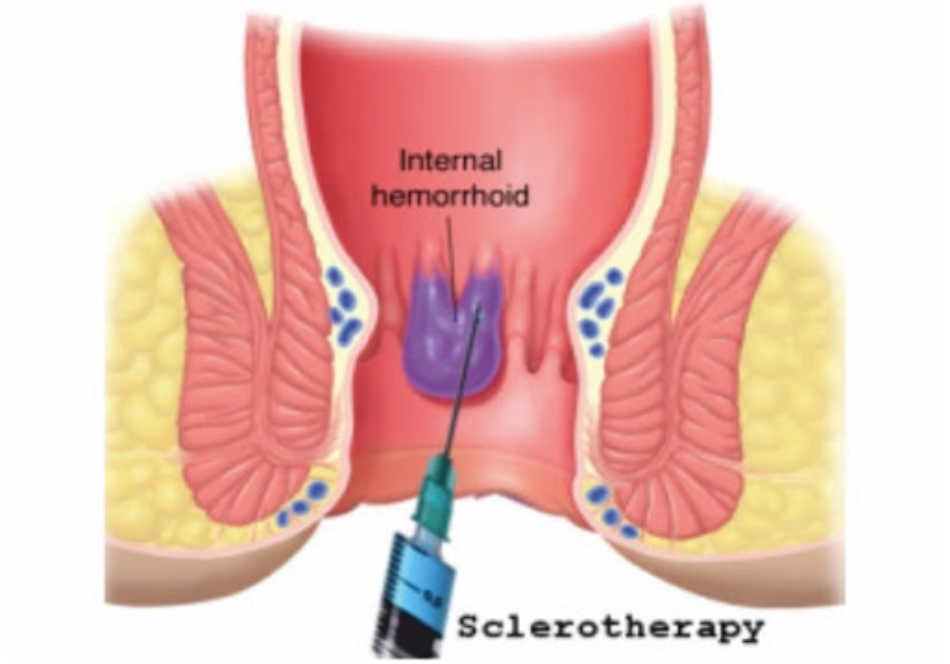


Photo 3: Sclerotherapy injection

Injection is tus administered in the immediate neighborhood of the anorectal junction. The injection should be painless. Approximately 2 to 5 ml of sclerosing compound is injected at the injection site. The needle is left in place for about ten seconds in order to avoid any reflux of the compound into the anal canal.

The entire hemorrhoidal ring is sclerosed in one single session of four sclerosing injections.

Repeated injections involving multiple sessions are no longer justified.

Important points:

- an injection which is too superficial leads to the immediate formation of a submucosal bubble or whitening of the mucosa. The injection must be stopped immediately because of the risk of necrosis and bleeding.

- too deep an injection, into muscle, is painful. The injection must be stopped if pain occurs.

Sclerosing injections are sometimes complicated by hematuria, hematospermia and prostitis or submucosal abscesses.

Commissural injections should be avoided;

- injection of too large an amount of the compound; is associated with the risk of necrosis.

Complications and management:

-intolerance manifestations (faintness, headache, vertigo) can sometimes be avoided if prior efforts are made to relax the patient. Ifthey occur injection must be stopped. A subcutaneous injection of atropine may be necessary in rare instances.

- discomfort is possible during the days which follow 1/3 of patients. Analgesics may be necessary.

-bleeding is possible:

. either just after injection, indicatingtaht a submucosal vessel has been damaged. A dilute 1:1000 adrenaline solution may be applied.

.or during subsequent 2 days. This requires nothing to be done other than warning the patient of the possibility

. or 5 to 10 days later. This most often results from ulseration at b the injection site. Local treatment is necessary only in rare instances: application adrenalin or suturing.

- external hemorrhoidal thrombosis is possible.

Indications and Contraindications of Sclerotherapy

Sclerotherapy is indicated in grade I and II hemorrhoids with rectal bleeding that have not responded to conservative measures [3,2,12]. With the introduction of ALTA, the indication has been extended to grade III hemorrhoids and, in selected cases, to grade IV hemorrhoids[13,14].

The contraindications for the use of sclerotherapy [13,14,15,16] are:

-Inflammation in the perianal region, abscess, fissures, fistulas, external hemorrhoids, and other proctological conditions

-Hemorrhoidal thrombosis and acute hemorrhoidal prolapse

-Previous anal surgery and previous sclerotherapy are relative contraindications

-Cardiac, hepatic, renal, or hematologic diseases

-Pregnancy and lactation

-Allergic asthma

-Inflammatory bowel disease

-Coagulopathy

Preventive Treatment

The only preventive treatment is that of regularization of intestinal function, essentially the elimination of constipation. This may notbe sufficient, and thromboses can recur in some patients.

If thromboses are very frequent, painful, and hence incapacitating, this, may indicate the need for excision of the external hemorrhoidal plexus by classical surgical hemorroidectomy.

Outcomes

Published series have shown good results. Reduction of bleeding has been reported in 100% of patients with grade I-III hemorrhoids, but complete resolution was achieved in only 69% (88% with grade I and 52% with grade III hemorrhoids). Prolapse resolution was also present in 90%–100% of cases. The rate of bleeding recurrence was around 29% and the prolapse recurrence rate was 16% [12,13,16]. Some groups, as Tomiki et al, recently reported the greater efficacy of ALTA compared with other sclerosing agents, especially in grade III hemorrhoids; however, more clinical trials with longer follow-up are needed [13,14].

b. Rubber band ligation

Indications and Contraindications of Rubber Band Ligation

The main indications for RBL are symptomatic and Grade II and III hemorrhoids. However, RBL can be used in selected Grade IV cases (such as in patients with high surgical risk because of comorbidity). The RBL technique is also useful to treat non-excised internal hemorrhoids during hemorrhoidectomy, and it has also been employed to remove rectal polyps [12].

The most frequent exclusion criteria for RBL [17,18]

1. First- and fourth-degree hemorrhoids (but this must be individualized in each patient).
2. Thrombosed hemorrhoids.
3. Anorectal pathologies (fissures, fistulas, and abscess).
4. Colitis.
5. Colorectal malignancies.
6. Pregnancy.
7. Coagulation disorders: unless it appears to be safe to stop antiplatelet and anticoagulant therapy before the procedure.

Principle:

The procedure consists of placing a rubber band at the base of the clump of hemorrhoidal tisuue. Necrosis of apical hemorrhoidal tissue occurs within a few hours, enabling partial entrapment of the vessel supplying the hemorrhoid, thus reducing the size of hemorrhoid, inducing fibrosis which will attach the plexus.

Technique:

Equipment necessary: a ligator, a proctoscope.

Procedure: the instrument is positioned at the summit of the mucosa.

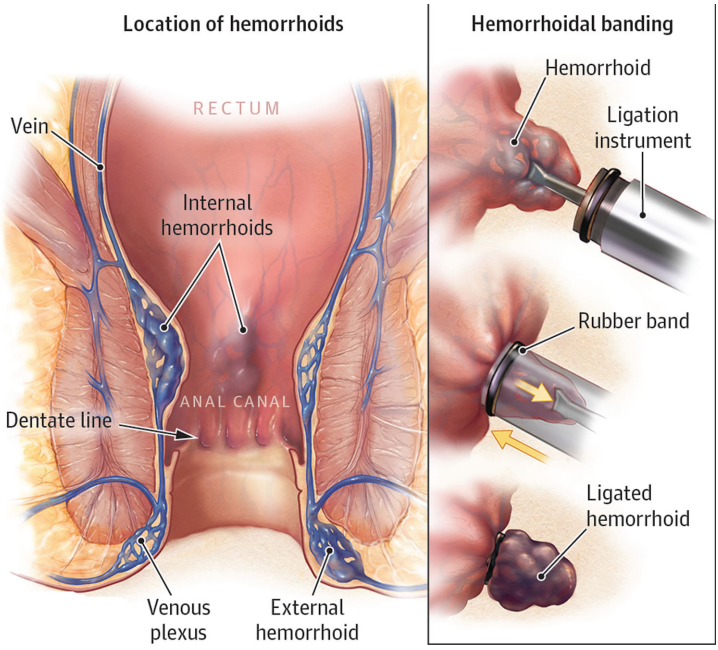


Diagram 8: Rubber band ligation

The mucosa is pulled into a cylinder either by suction or traction. A mobile ring is used to slide a rubber band from the outside of this cylinder onto the stalk oh the mucosa which, under traction, has become pediculizedwithin the cylinder of ligator. The mucosa is strangled, deprived of blood supply and will necrose.

Photo 4: Rubber Band Ligator

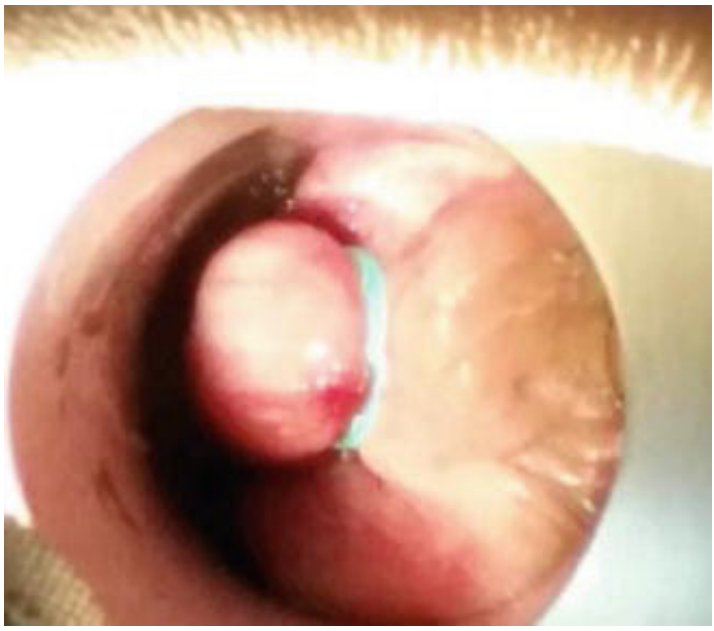


Photo 5: After Rubber Band Ligaton

Errors to be avoided:

traction of mucosa should be gentle and totally painless. If the ligature has benn appiled too near the pectinate line, pain will be very severe because in areas of anal canal sensitive to pain.

Complications and management methods of rubber band ligating:

- Immediate dropping of rubber band:

the tisuue mass is too small to retain the rubber band or too large, leading to the application of excessive tension to the elastic band. Dropping off is favored by early defecation.

- Pain:

. immediate pain: teh rubber band should be removed

. subsequent days: pain is common and the patient should be warned about this. In almost 20 % of cases, pain is frank and requires analgesics. In 10 % of cases pain is very severe and may render the patient unable to work.

Non steroidal anti-inflammatory agents are effective, but have been accused favoring the onset of extensive gangrene. In case of any doubt especially in diabetic patients prescribing metronidazole relieves the patient.

. pain of late onset: It may indicate the onset of infection. This is a rare possibility except in the case of immunodeficiency.

- Bleeding:

It occurs 2 to 6 % of cases. Hospital follow up or even surgical hemostasis may be required in certain cases.

Number of sessions: maximum of two ligation should be done in one session. The risk of pain is increased by the number of ligations. Treatment involves 1 to 3 sessions at intervals of 5 weeks.

A liquid nitrogen cryode is sometimes applied to freeze the ligated tisuue to decrease the risk of pain and bleeding.

Outcomes

The reported long-term success rate of RBL (with long-term defined as 6 months minimum) is approximately 90% in patients with grade II-III hemorrhoidal disease [19]. If more than four banding sessions are required for symptom control a conventional hemorrhoidectomy may be required [12,20]. The incidence of postoperative pain ranges from 8% to 80% in different studies and the incidence of postoperative bleeding ranges from 3,5% to 50%. Overall, recurrence of bleeding and prolapse at follow-up occur in 10%–18% and 2,2% of patients, respectively. However, some series report higher percentages of recurrence (46% for bleeding and 34% for prolapse)[12,29]. The results of many studies have confirmed the superiority of RBL over sclerotherapy, cryotherapy, and anal dilatation, and similar efficacy to infrared coagulation, with lower recurrence rates than either sclerotherapy or infrared coagulation

c. Ultroid (Direct Current Probe)

The direct current probe (Ultroid; Ulterior Technologies, Tampa, FL, USA) is a dispositive device that uses low-voltage monopolar current and generates sodium hydroxide on its negative electrode, producing coagulation of hemorrhoidal tissue. Unlike other techniques, it does not produce tissue destruction by heat. Treatment of hemorrhoids using Ultroid technology is limited by the long time required to treat the involved tissue (up to 14 min per site), post-procedural pain (in up to 20% of patients), and poor results in correcting prolapse [21,22].

d. Cryotherapy

Other than freezing during rubber band ligation, some authors suggest application of a cryode to the summit of the hemorrhoids in order to attach the mucosa as a result of scarring of the area of necrosis produced.

Cryosurgery was popularized in the 1970s to 1980s, although currently it is in disuse. Liquid nitrogen is applied into the hemorrhoidal tissue, causing a necrotizing effect, with a permanent result. The main disadvantage is the long time required for each session; also, the technique can cause secondary bleeding [3].

e. Infrared Coagulation

. Principle:

This is identical to that of injection sclerotherapy. Sclerosis is obtained by the healing of a controlled area of thermal coagulation produced by an infrared probe. Rays appiled to the summit of the hemorrhoids are converted to heat in hemorrhoidal tisuue.

. Technique:

Equipment necessary: infrared coagulator.

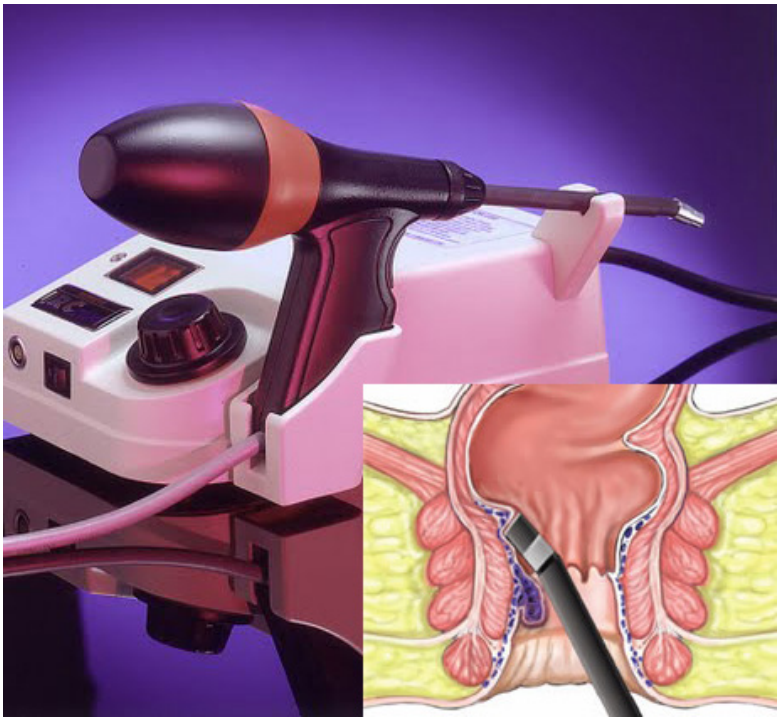


Photo 6: Infrared coagulator

Procedure: the tip of the pistol-shaped probe is applied to the tissue, in the neighborhood of the anorectal junction.

Duration of exposure is authomatic and can be adjusted between 0,5 and 2 seconds. Three to four plaques of coagulation, each measuring 3 mm in diameter, should be obtained at each session.

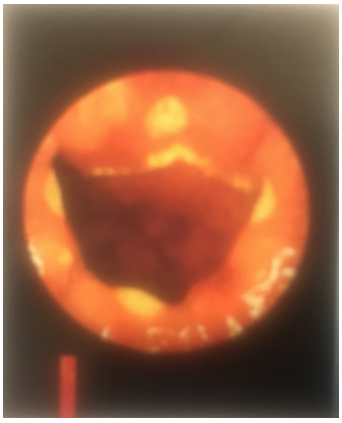


Photo 7: After infrared coagulation

Errors to be avoided:

Too high or too low application will be ineffective or painful.

Complications are rare . Pain may occur in less than 5 % of cases. Bleeding is possible usually without any particular consequences.

The results, in terms of resolution of bleeding and prolapse, are similar to those for RBL (improvement in 81–93% of patients) [19]. However, several studies have demonstrated a higher recurrence rate, especially in grade III hemorrhoids, and the need for more retreatments than for RBL. In addition, the cost of the equipment is higher than that for RBL, so this method is used less often [2,3,12].

Comparison of rubber band ligation (RBL), sclerotherapy, and infrared coagulation

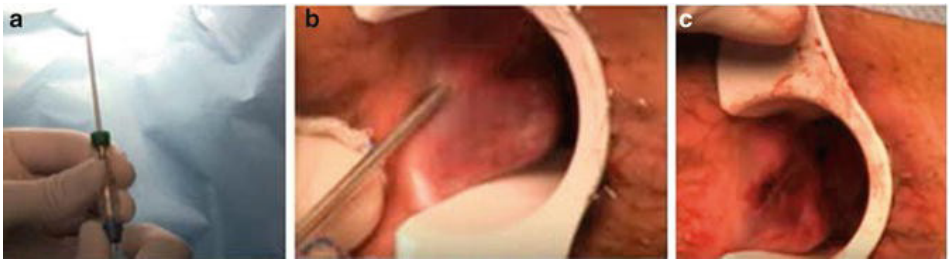
Technique	Indications	Contraindications	Outcomes	Adverse events
Rubber band ligation	Grade III hemorrhoids if conservative treatment has failed	Hemorrhoidal thrombosis Other anorectal disorders Colitis Coagulopathy Pregnancy	Success rate: Bleeding: 90% Prolapse: Second-degree 93%–100% Third-degree 78%–83,8% Recurrence rates: Bleeding:10%–18% Prolapse: 2,2%	Pain Bleeding Thrombosis Urinary retention Pelvic sepsis
Sclerotherapy	Grade I and II Hemorrhoids if conservative treatment has failed Hemorrhoids third-degree with new sclerosing agents (e.g., ALTA)	Hemorrhoidal thrombosis Other anorectal disorders Coagulopathy Pregnancy and lactation Inflammatory bowel disease Allergic asthma	Success rate: Bleeding: 69%–88% Prolapse: Second-degree 90%–100% Recurrence rate: Bleeding: 1.5%–29% Prolapse: 16%	Pain Bleeding Thrombosis Urinary retention Impotence Hematuria Hemospermia Epididymitis Urethral stricture
Infrared coagulation	Grade I and II Hemorrhoids if conservative treatment has failed	Hemorrhoidal thrombosis Other anorectal disorders Colitis Coagulopathy Pregnancy Renal, cardiac, and pulmonary diseases	Success rate: 62%–93% Recurrence rate: Bleeding: 13%	Pain Bleeding Thrombosis Urinary retention

Table 7: Comparison of RBL, Sclerotherapy and Infrared coagulation

OTHER METHODS

a. Laser coagulation

CO2 or Nd-YAG and diode lasers have been used to treat hemorrhoids. The laser beam is applied to the submucosal layer and causes shrinkage and degeneration of hemorrhoidal tissue at different depths, depending on the laser power (irradiance) and the duration of laser light application



. Photo 8: Laser coagulation of hemorrhoids. (a) Laser probe. (b) Laser sclerotherapy of hemorrhoids. (c) Final result of laser hemorrhoid coagulation

The Nd-YAG laser has an output of 10–20 W. It uses a 0,2 to 0,4 mm probe for excision and a 0,4- to 0,6-mm probe for coagulation. The advantages of this method are minimal bleeding during the procedure, less pain after the procedure, and short treatment time [13,15].

The use of the diode laser in the treatment of hemorrhoids was first described in 2007. It has a low penetration depth (up to 2 mm); therefore it can be applied in submucosal tissue without causing anal sphincter injury. However, reports in the literature are limited and the role of the diode laser is not well established [23,24].

b.Bipolar Diathermy and Probe Coagulation

Heater probe and bipolar diathermy devices generate heat, causing coagulation of the hemorrhoidal tissue, resulting in a fibrotic reaction at the treatment site, with fixation of the treated tissue. These methods are indicated for grade I-III hemorrhoids after failure of conservative measures [2,3,25].

Success rates range from 88% to 100%. The two technologies provided similar efficacy for the treatment of bleeding, with a recurrence rate of 6,2% per year, but the heater probe controlled bleeding more quickly (76,5 vs. 120,5 days), although it caused more pain. However, the results regarding the alleviation of prolapse are poor for these two technologies [25,26].

Complications include pain, bleeding, fissure, or spasm of the internal

sphincter. Compared with RBL, these technologies require more treatment sessions and have more treatment failures [3,4,27].

c. Doppler-guided Hemorrhoidal Artery Ligation (DGHAL)

This technique involves use of Doppler ultrasound to identify and ligate the hemorrhoidal arteries [28]. This is also referred to as transanal hemorrhoidal dearterialization (THD). Different platforms with different associated nomenclatures exist for this technique, but the principles include the use of a Doppler probe to identify the six main feeding arteries within the anal canal, ligation of these arteries with absorbable suture and a specialized anoscope, and then plication of redundant hemorrhoidal mucosa. The plication is often referred to as recto-anal-repair, mucopexy, or hemorrhoidopexy. Proposed benefits of this procedure are similar to stapled hemorrhoidopexy, with less associated pain due to the suturing being above the dentate line.

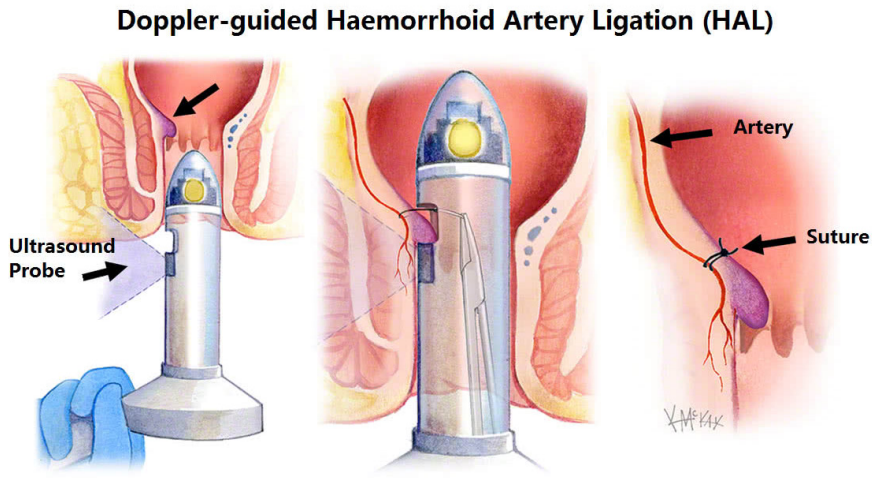


Diagram 9: Doppler-guided Hemorrhoidal Artery Ligation (DGHAL)

Early results of Doppler-guided hemorrhoidal artery ligation (DGHAL)/THD were promising, with lower pain scores than hemorrhoidectomy, and relief of bleeding and tissue prolapse in over 90% of patients [29]. Since then, several randomized clinical trials have been performed with mixed results [30,31,32]. Currently, DGHAL/THD remains a viable approach to multicolumn internal hemorrhoids. However, the short-term benefits regarding postoperative pain have recently not been as remarkable as in the earlier studies.

SURGICAL TREATMENT

The aim of the surgery is to remove or destroy hemorrhoidal tissue. Surgery removes not only the internal hemorrhoidal plexus but also the external hemorrhoidal plexus.

Surgical hemorrhoidectomy:

-Methods: There are three excision techniques:

a. Milligan Morgan hemorrhoidectomy or open hemorrhoidectomy

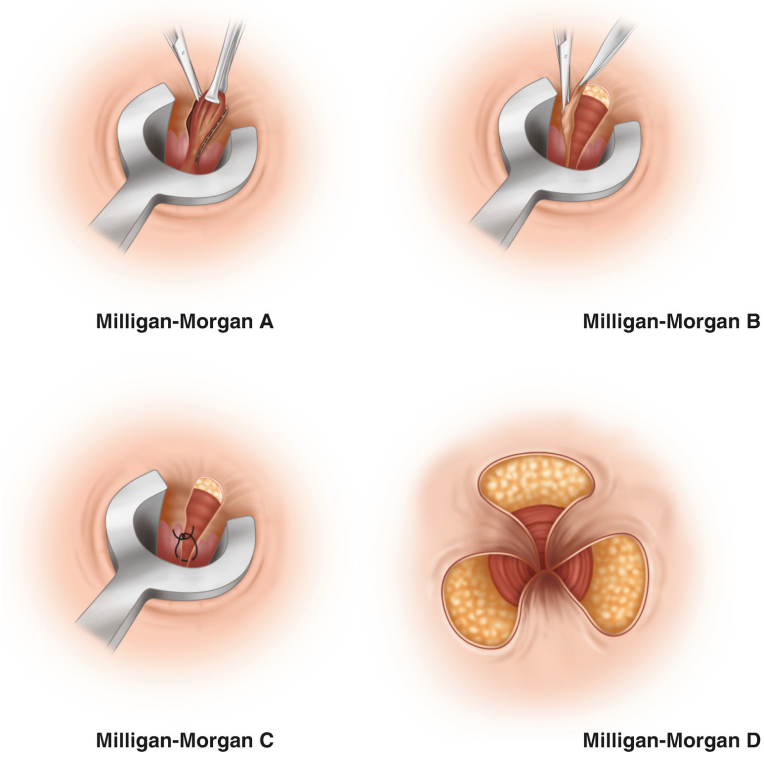


Diagram 10: Milligan Morgan Hemorrhoidectomy

This technique involves excision of the 3 or 4 hemorrhoidal clumps starting from the anal verge. The hemorrhoid is ligated in the upper part of the canal before its excision.

A cutanemucosal bridge, freed of submucosa and subcutaneous vessels, is left in place between each surgical wound.

Certain authors routinely perform sphincterotomy in the left posterior wound.

Others carry out hemorrhoişdectomy by posterior sphincterotomy with bringing down of the rectal mucosa onto the sphincter wound. There is a concomitant fissure or a posterior hemorrhoid.

b. Submucosal hemorrhoidectomy (Parks):

Hemorrhoids are dissected by opening the mucosa, which is closedafter ligation and ablation of hemorrhoidal tissue.

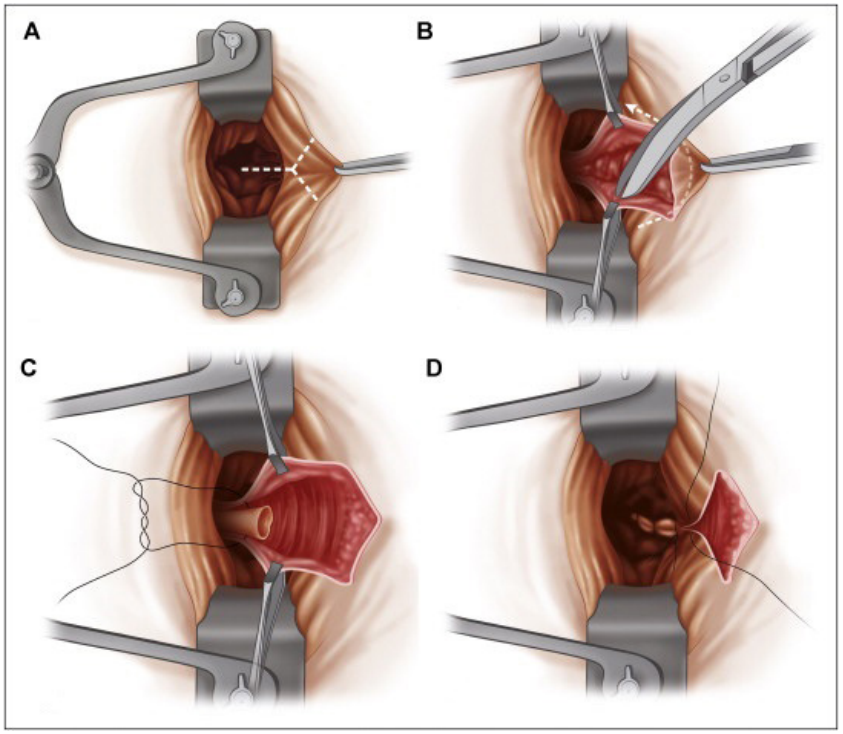


Diagram 11: Submucosal hemorrhoidectomy (Parks)

c. Ferguson's closed hemorrhoidectomy:
Excision wounds are closed by a running suture in absorbable suture material.

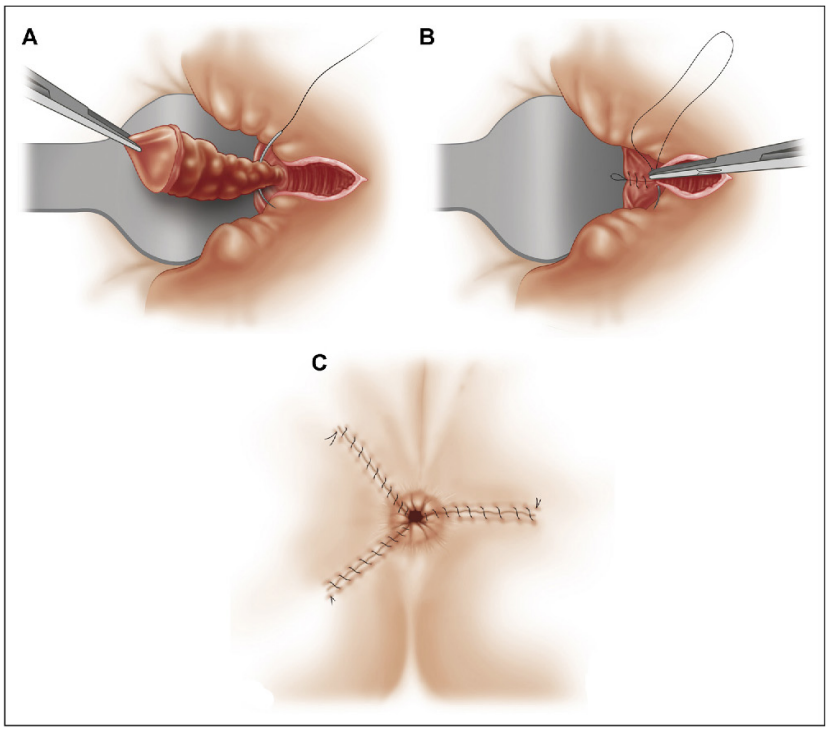


Diagram 12: Ferguson hemorrhoidectomy

d. Whitehead hemorrhoidectomy appears to be abandoned at present due to its severe complications.

e. Stapled hemorrhoidopexy (PPH)

An alternative to operative hemorrhoidectomy is stapled hemorrhoidopexy, in which a stapling device is used to resect and fixate the internal hemorrhoid tissues to the rectal wall. Since the staple line is above the dentate line, patients typically experience less pain than those who undergo hemorrhoidectomy. To perform this procedure, a circular stapler is introduced into the anus and prolapsing tissue is brought into the stapler. The most critical component of stapled hemorrhoidopexy is the placement of a circumferential, purse-string, nonabsorbable suture in the submucosa far enough away to avoid any sphincter muscle involvement—usually at ~4 cm from the dentate line. Additionally, before engaging the stapler, an examination of the posterior vaginal wall should be conducted. Finally, the staple line should be evaluated for any bleeding that would require additional suture ligation.

Photo 9 : PPH stapler

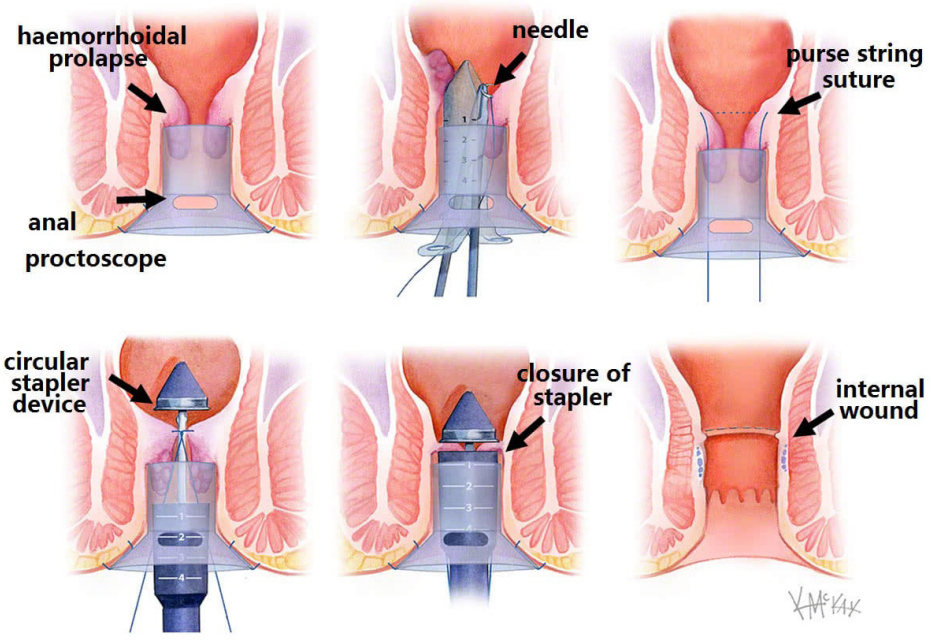


Diagram 13: Stapled hemorrhoidopexy

This minimally invasive maneuver occlude the blood supply of the superior hemorrhoidal artery above the hemorrhoidal tissue and thus piles is cured as well as prolapsed mucosa is retracted up.

Indications of stapler hemorrhoidopexy:

Grade III hemorrhoids, with uncomplicated grade IV hemorrhoids that are reducible at surgery or after manipulation in the operating room. In the surgery of MIPH (irreducible prolapsed hemorrhodis) hemorrhoidal tissue is not excised during the procedure, and in those who failed other treatment modalities.

Contraindications of stapler hemorrhoidopexy:

Active sepsis, anal stenosis, and full-thickness rectal prolapse are the contraindications; because these conditions are not adequately treated by PPH.

Complications from stapled hemorrhoidopexy include bleeding from the staple line, incontinence for injury of the sphincter muscles, and stenosis from incorporation of excess rectal tissue. Moreover, there is a risk of recto-vaginal fistula in women due to incorporation of vaginal tissue into the purse-string.

Three systematic reviews concluded that stapled hemorrhoidopexy was less effective than conventional hemorrhoidectomy[33,34,35]. Stapled hemorrhoidopexy was associated with a higher long-term risk of hemorrhoid recurrence. Due to need for additional operations, the incidence of prolapse and tenesmus was also higher after stapled hemorrhoidopexy as compared with hemorrhoidectomy. Conversely, the stapled approach was associated with significantly less pain, shorter operative time, and shorter time to resumption of normal activity. In a 2010 European multicenter randomized trial of stapled hemorrhoidopexy versus hemorrhoidectomy, both options were shown to be equally effective in preventing recurrence after 1 year. Patients undergoing hemorrhoidectomy were more likely to have symptomatic relief from the hemorrhoids (69 vs. 44%), but had significantly greater postoperative pain [36].

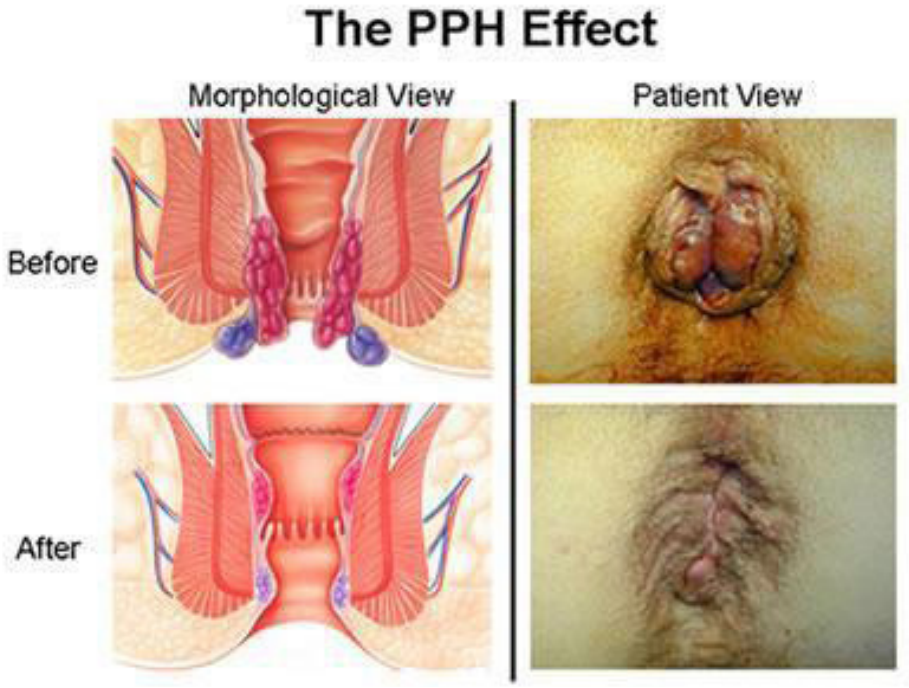


Diagram 14: The effect of the stapled hemorrhoidopexy (PPH)

Overall, stapled hemorrhoidopexy remains a viable alternative to hemorrhoidectomy, and is especially attractive for patients without much external disease. However, while the published complication rates are low, they can be quite severe, and the surgeon must have appropriate training and proceed with great caution, when performing this procedure.

POST OPERATIVE CARE

The laxatives are used to encourage defecation, which is all the less painful when it is early and made up of soft stools.

Analgesics are essential.

Antibiotics are given before, during, and after surgery (metronidazole, 3rd-generation cephalosporins).

Postoperative care involves antiseptic baths which are possible after removal of the compressive dressing.

COMPLICATIONS

-Immediate complications

. Postoperative bleeding:

This may result from a coagulation disturbance or unusually heavy mucosal bleeding.

Compression of the anal verge for a few minutes followed by application of a compressive dressing is sufficient in most instances.

. Retension of urine:

This is possible as with all proctological surgical procedures, being favored by spinal anesthesia or prostatic hypertrophy. Reflex constipation or a fecal impaction are possible.

Seconadry complications

. Delaed bleeding:

The elimination of necrotic tisuue between 7 and 15 days may cause bleeding, about which the patient should be warned.

. Abscess:

.Delayed healing:

This is possible in certain underlying conditions; diabetes, AIDS. It may occur if local care is inadequate, leading to indolent or hypertrophic wounds.

Application of silver nitrate to wounds may be necessary.

Late complications

. Anal fissure

This is usually a posterior fissure. It should be treated medically, and in case of failure of the latter by internal lateral sphincterotomy.

. Post operative stenosis

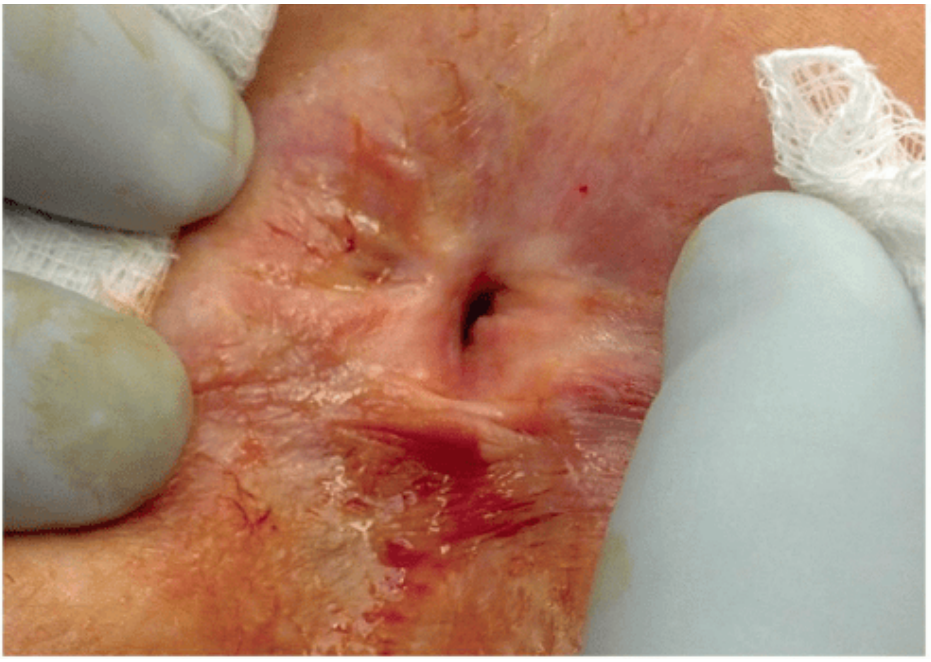


Photo 9: Anal canal stenosis after hemorrhoidectomy

This is prevented by post operative rectal examination and by normalization of intestinal function.

Boguie dilatation may be necessary at the very beginning. Surgery by anoplasty may prove necessaryif the stenosis is symptomatic.

.Impaired fecal continence

It should be possible to avoid this by excluding tecniques which destroy the sensitive mucosa of the anal canal (Whitehead), by preoperative detection of disturbances of perineal function, and by avoiding routine leiomyotomy.

.Recurrence

This possible but rare, and can usually then be dealt with by instrumental methods.

. Anal tags

Excess cutaneous tissue should be removed per-operatively.

RESULTS AND INDICATIONS

Criteria of therapeutic choice

The treatment of internal hemorrhoidal disease is based upon the following criteria:

- symptomatic hemorrhoid disease,
- confirmation of the hemorrhoidal origin of symptoms,
- nature and efficacy of previous treatment,
- coexistence of symptomatic external disease,
-the grade of disease,
- its predominant symptoms.

In all cases;

. Medical treatment is invariably indicated.

It may be sufficient in itself. It is dominated by regularisation of intestinal function. This treatment is based upon the use of appropriate combination for each individual case of general and dietary advice with laxatives.

. Oral treatment is dominated by flavonoid derivatives.

These are well tolerated and rare indicated at high doses for the acute symptoms of internal hemorrhoids.

Topical agents can be prescribed in the form of creams. It is essential to resist the demands which may be made by the patients, in particular because of the risk of contact dermatitis. Suppositories are often prescribed but this presentation is illogical since the ingridients are released essentially in the rectum.

. It is essential to routinely try such medical treatment before any decision regarding surgery.

This is very often followed by the suprise of a notable lessening of symptoms and in size of hemorrhoids during an acute symptomatic episode. This regression sometimes makes it possible to avoid surgery by rendering hemorrhoidal disease accesible to medical treatment.

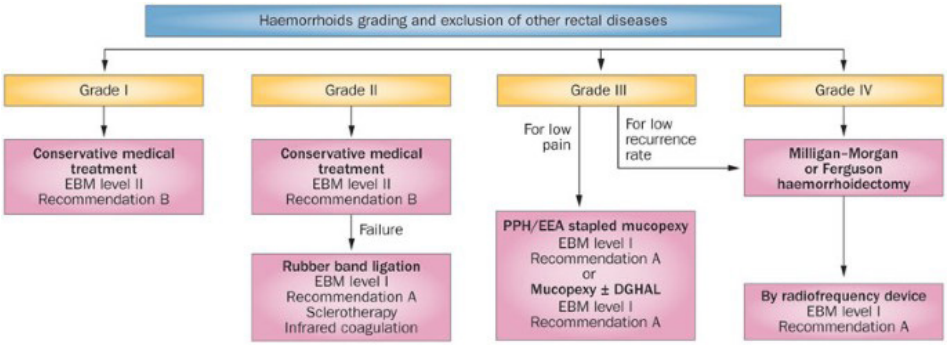


Table 8: Indications for instrumental or surgical treatment of hemorrhoidal disease [11]

Data from the literature show wide differences. Furthermore, bleeding disappears spontaneously in more than one case out of two.

Hemorrhoidectomy is the method which gives the best long term result, but atthe expense of a surgical procedure, while 90 to 95 % of hemorrhoids can be controlled by the combination of medical treatment + instrumental treatment.

There is a little difference in terms of efficacy regarding bleeding between ligations, sclerosis procedures, and infrared coagulation. However, ligation is the most effective method for the control of prolapse.

In cases of grade 2 hemorrhoids, ligation is preferred if hemorrhoids are particularly mobile.

In grade 3, ligation is preferred in the elderly with a less tonic sphincter, while surgeryis more appropriate for younger people. It should be possible to use instrumental methods in all cases, enabling the application to each individual of the method which will result in the proper control of symptoms.

Less than 5 to 10 % of all hemorrhoids finally require excision surgery.

Special Considerations:

a. Crohn Disease

Hemorrhoids should be distinguished from hypertrophic skin tags that are associated with Crohn disease. Skin tags in Crohn disease are often tender and associated with ulceration of the anal canal. For patients with Crohn disease and active anorectal inflammation, treatment of hemorrhoids should be kept as conservative as possible, with every attempt made to avoid surgery, as these patients can have significant issues with wound healing after hemorrhoidectomy, and surgery

may actually exacerbate their disease and worsen symptoms. Hemorrhoidectomy can be performed in a highly selective basis when disease is quiescent, but it is generally discouraged [37].

b. Immunosuppression

Immunosuppressed patients such as those with acquired immunodeficiency syndrome (AIDS) or those on chronic immunosuppressive medications are at greater risk of sepsis and poor wound healing[38,39,40]. Conservative treatments should be exhausted before performing any invasive procedures; however, less-invasive approaches can be undertaken. In a small series of 22 AIDS patients that underwent sclerotherapy injection of their hemorrhoids, all demonstrated improvement after 6 weeks. Four patients with 4-year follow-up showed improvement lasting 18 months but subsequently required repeat injections for recurrence symptoms [39].

c. Cirrhosis and Portal Hypertension

Contrary to previous teachings, the incidence of hemorrhoid disease in patients with portal hypertension is not different from the general population [41]. Rectal varices, the result of porto-systemic communication via the hemorrhoid veins, occur commonly in patients with portal hypertension. However, bleeding from rectal varices is rare, accounting for <1% of massive bleeding in portal hypertension. When it does occur, it should typically be treated with portal decompression [42].

Hemorrhoid disease is a common but complex disease. Patients who present with signs and symptoms of hemorrhoids should be carefully evaluated to exclude other masquerading entities. There are a multitude of options for the management of hemorrhoid disease and specific treatment choice should be based on individual patient and clinical factors.

d. Thrombosed hemorrhoidal prolapse

. Medical treatment

This combines;

- warm sitz baths,
- application of gauze squares soaked with anti-inflammatory substances, notably hydrocortisone,
- application corticosteroid ointments,
- analgesics, acetaminophen, dextropropoxiphen,
- oral NSAIDs,

- laxatives.

. Instrumental treatment:

All instrumental treatment is contraindicated. Replacement of the prolapse can be attempted after anesthesia of the sphincter using xylocaine or bupivacaine.

. Surgical treatment:

Certain authors have suggested internal lateral sphincterotomy. If not, emergency hemorrhoidectomy is possible. Severer edema results in the risk of excessive removal of mucosa and skin, with the resultant potential complication of stenosis.

. Indications:

Medical treatment is simple, effective, and does not interfere with future management. After the thrombosed prolapse is resolved, it is very common to finf only minimal abnormalities, for which instrumental treatment enables totally adequate control of disease.

Hemorrhoidectomy is useful when pain is very severe.

DISCUSSION

The recent epidemiological investigation was conducted to determine the prevalence of hemorrhoids in adults and to define associated risk factor. Overall prevalence of 39% for grades I to IV hemorrhoids classified according to the international classification of hemorrhoids in the current adult population. Only 17% of patients complained about symptoms related to hemorrhoids, whereas 22% of patients reported not to have any problems [43]. The majority of patients in the asymptomatic group represented grade I hemorrhoids, thus merely a bulge in the anal canal caused by hemorrhoidal tissue was visible. To the author's knowledge, there are no data available whether primary asymptomatic grade I hemorrhoids will become symptomatic in the future. It might also be possible that these hemorrhoids represent normal endoscopic findings and will never lead to typical hemorrhoidal complaints. However, further longitudinal studies need to clarify that. Epidemiological data has great importance as they reflect the burden of a disease. There is a considerable paucity of studies that intended to investigate the prevalence of hemorrhoids. Haas et al. assessed the prevalence of hemorrhoids by reviewing patients seen in a colon and rectal surgical clinic [44]. Patients underwent rectal examination and anoscopy. The overall prevalence rate of hemorrhoids was found to be 86%. In contrast to our results, 102 patients with grade I hemorrhoids reported symptoms compared to 42 patients without [43]. However, it should be taken into consideration that the study population consisted of a highly selected group of patients; thus, selection bias cannot be ruled out.

CONCLUSION

Hemorrhoidal disease is common, probably resulting in a large number of medical consultations, investigations and treatment. As a result, it raises a public helth problem. The percentage of patients with hemorrhoidal symptoms and presenting for this reason is nevertheless unknown.

The concept of an acute hemorrhoid attack also raises problems since certain authors deny its existence. Patients spontaneously describe fluctuating symptomatology punctuated by paroxysmal episodes of worsening. There is no definite dividing line between the attack itself and an asymptomatic basal state. Clinical and therapeutic trials require a precise definition of patient groups, the use of placebos and of double-blind methods.

A number of studies have shown that the disappearance of symptoms could involve as many as 50% of cases in placebo groups. This also applies to the assesment of medical instrumental techniques.

Certain unknowns thus persist in hemorrhoidal disease, because of failure to comply with the rules of good clinical practice. When these rules are obeyed, oral medications with vascular effects, e.g. flavonoid derivatives, while they are not affect the essential cause of the disease, provide symptomatic relief, certainly fulfilling the principal demands of the patient.

The mechanism of action of these medications remains controversial. They can not be a substitute for the regulation of intestinal function.

If the presenting symptom is rectal bleeding: it is necessary to convince the patient, very often anxious, that the condition is benign, never progresses to malignancy and is never associated with any life-threatining risks.

From therapeutic standpoint, the practioner has acces to a wide range of treatment methods. Medical treatment has the advantages of being applicable to all patients, well tolerated, free of any notable side effects and nothing interfering with instrumental techniques.

A number of types of the latter exist. They should be used only if the patient has benn adequately informed of their side-effects. The multiplicity of methods is proof in itself that none is greatly superior to the others.

Certain tecniques require only simple instruments(sclerothreapy), while the financial investment in other situations (cryotherapy) may be considerable. If such instrumental tecniques are unavailable, there is the risk of treatment of hemorrhoids being limited to an alternative between medical and surgical treatment.

This situation is far from ideal since surgery is painful, interferes wtih normal anatomy and is accompanied by morbidity which certainly exists. Hence difficult to accept for a benign disease [1]. All this implies that surgery should be considered

only as a last choice, restricted to failures of medical treatment despite persistent attempts.

The current treatment trend is out-patient application, without hospitalisation, of excision tecniques. Hemorrhoid surgery is then performed in day hospitals.

REFERENCES

1. Philippe Godeberge. Atlas of Hemorrhoids. 1992:5-82.
2. V. Lohsiriwat.World J Gastroenterol. Aug 21, 2015; 21(31): 9245-9252.
3. 3.Trompetto M, Clerico G et al (2015) Evaluation and management of hemorrhoids: Italian Society of Colorectal Surgery (SICCR) consensus statement. Tech Coloproctol 19(10):567–575
4. MacRae and McLeod 1997; MacRae HM, McLeod RS (1997) Comparison of hemorrhoidal treatments: a metaanalysis. Can J Surg 40:14–17
5. Seok-Gyu S, Soung-Ho K (2011) Optimal treatment of symptomatic hemorrhoids. J Korean Soc Coloproctol 27(6):277–281
6. Thomson WH. The nature and cause of haemorrhoids. Proc R Soc Med. 1975;68:574–575
7. Goenka M K, Kochhar R, Nagi B, Mehta S K. Rectosigmoid varices and other mucosal changes in patients with portal hypertension. Am J Gastroenterol. 1991;86(9):1185–1189.
8. Morgado P J, Suárez J A, Gómez L G, Morgado P J Jr. Histoclinical basis for a new classification of hemorrhoidal disease. Dis Colon Rectum. 1988;31(6):474–480.
9. Arantxa Muñoz-Duyos Chapter Rubber Band Ligation, Sclerotherapy, Infrared Coagulation and Other Techniques in the Treatment of Hemorrhoids January 2018.Hemorrhoids (pp.1-12)
10. Alexandra K. Macmillan M.B.Ch.B., The Prevalence of Fecal Incontinence in Community-Dwelling Adults: A Systematic Review of the Literature. Diseases of the Colon & Rectum 47, 1341–1349(2004).
11. T. Higuero, L. Abramowitz, A.Castinel, N.Fathallahe, P.Hemery, C.Laclotte Duhoux, F.Pigot, H. Pillant-Le MoulteA.Senéjoux, L. Siproudhis, G. Staumontk, M. Suduca, B.Vinson-Bonnet. Guidelines for the treatment of hemorrhoids. Journal of Visceral Surgery. Volume 153, Issue 3, June 2016, Pages 213-218
12. Cocorullo G, Tutino R et al (2017) The non-surgical management for hemorrhoidal disease. A systematic review. G Chir 38(1):5–14
13. Yang HK (2014) Hemorrhoids. Chapter 6. In: Nonsurgical treatment of hemorrhoids. Springer, Berlin/Heidelberg
14. Tomiki Y, Ono S et al (2015) Treatment of internal hemorrhoids by endoscopic sclerotherapy with aluminum potassium sulfate and tannic acid. Diagn Ther Endosc 2015:517690
15. Akindiose C, Alatise OI et al (2016) Evaluation of two injection sclerosants in the treatment of symptomatic haemorrhoids in Nigerians. Niger

Postgrad Med J 23(3):110–115;
16. Miyamoto H, Hada T et al (2016) Aluminum potassium sulfate and tannic acid sclerotherapy for Goligher grades II and III hemorrhoids: results from a multicenter study. World J Hepatol 8(20):844–849.
17. Iyer VS, Shrier I, Gordon PH (2004) Long-term outcome of rubber band ligation for symptomatic primary and recurrent internal hemorrhoids. Dis Colon Rectum 47:1364–1370
18. Jacobs D (2014) Hemorrhoids. N Engl J Med 371:944–95
19. Marques CF, Nahas SC et al (2006) Early results of the treatment of internal hemorrhoid disease by infrared coagulation and infrared coagulation and elastic banding: a prospective randomized cross-over trial. Tech Coloproctol 10(4):312–317
20. Chaundhry V, Abscarian H (2016) Hemorrhoids. Large bowel. Elsevier, Philadelphia, pp 256–261
21. Zinberg SS, Stern DH, Furman DS, Wittles JM (1989) A personal experience in comparing three nonoperative techniques for treating internal hemorrhoids. Am J Gastroenterol 84(5):488–492;
22. Azizi R, Rabani-Karizi B, Taghipour MA (2010) Comparison between Ultroid and rubber band ligation in treatment of internal hemorrhoids. Acta Med Iran 48(6):389–399
23. Crea N, Pata G, Lippa M et al (2014) Hemorrhoidal laser procedure: short- and long-term results from a prospective study. Am J Surg 208:21–25
24. Naderan M, Shoar S et al (2017) A randomized controlled trial comparing laser intra-hemorrhoidal coagulation and Milligan-Morgan hemorrhoidectomy. J Invest Surg 30(5):325-331
25. Hinton CP, Morris DL (1990) A randomized trial comparing direct current therapy and bipolar diathermy in the outpatient treatment of third-degree hemorrhoids. Dis Colon Rectum 33(11):931–932).
26. Dennison A, Whiston RJ et al (1990) A randomized comparison of infrared photocoagulation with bipolar diathermy for the outpatient treatment of hemorrhoids. Dis Colon Rectum 33(1):32–34
27. Serventi A, Rassu PC et al (2011) Haemorrhoidal disease: role of conservative outpatient treatments. Ann Ital Chir 82(5):341–347
28. Morinaga K, Hasuda K, Ikeda T. A novel therapy for internal hemorrhoids: ligation of the hemorrhoidal artery with a newly devised instrument (Moricorn) in conjunction with a Doppler flowmeter. Am J Gastroenterol. 1995;90(4):610–613.
29. Giordano P, Overton J, Madeddu F, Zaman S, Gravante G. Transanal

hemorrhoidal dearterialization: a systematic review. Dis Colon Rectum. 2009;52(9):1665–1671.
30. De Nardi P, Capretti G, Corsaro A, Staudacher C. A prospective, randomized trial comparing the short- and long-term results of Doppler-guided transanal hemorrhoid dearterialization with mucopexy versus excision hemorrhoidectomy for grade III hemorrhoids. Dis Colon Rectum. 2014;57(3):348–353.
31. Denoya P I, Fakhoury M, Chang K, Fakhoury J, Bergamaschi R. Dearterialization with mucopexy versus haemorrhoidectomy for grade III or IV haemorrhoids: short-term results of a double-blind randomized controlled trial. Colorectal Dis. 2013;15(10):1281–1288.
32. Elmér S E, Nygren J O, Lenander C E. A randomized trial of transanal hemorrhoidal dearterialization with anopexy compared with open hemorrhoidectomy in the treatment of hemorrhoids. Dis Colon Rectum. 2013;56(4):484–490.
33. Giordano P, Gravante G, Sorge R, Ovens L, Nastro P. Long-term outcomes of stapled hemorrhoidopexy vs conventional hemorrhoidectomy: a meta-analysis of randomized controlled trials. Arch Surg. 2009;144(3):266–272.
34. Jayaraman S, Colquhoun P H, Malthaner R A. Stapled versus conventional surgery for hemorrhoids. Cochrane Database Syst Rev. 2006;(4):CD005393.
35. Nisar P J, Acheson A G, Neal K R, Scholefield J H. Stapled hemorrhoidopexy compared with conventional hemorrhoidectomy: systematic review of randomized, controlled trials. Dis Colon Rectum. 2004;47(11):1837–1845.
36. Nyström P O Qvist N Raahave D Lindsey I Mortensen N; Stapled or Open Pile Procedure (STOPP) trial study group. Randomized clinical trial of symptom control after stapled anopexy or diathermy excision for haemorrhoid prolapse Br J Surg 2010972167–176.
37. Wolkomir A F, Luchtefeld M A. Surgery for symptomatic hemorrhoids and anal fissures in Crohn's disease. Dis Colon Rectum. 1993;36(6):545–547.
38. Morandi E, Merlini D, Salvaggio A, Foschi D, Trabucchi E. Prospective study of healing time after hemorrhoidectomy: influence of HIV infection, acquired immunodeficiency syndrome, and anal wound infection. Dis Colon Rectum. 1999;42(9):1140–1144.
39. Scaglia M, Delaini G G, Destefano I, Hultén L. Injection treatment of

hemorrhoids in patients with acquired immunodeficiency syndrome. Dis Colon Rectum. 2001;44(3):401–404.
40. Wexner S D, Smithy W B, Milsom J W, Dailey T H. The surgical management of anorectal diseases in AIDS and pre-AIDS patients. Dis Colon Rectum. 1986;29(11):719–723.
41. Hosking S W, Smart H L, Johnson A G, Triger D R. Anorectal varices, haemorrhoids, and portal hypertension. Lancet. 1989;1(8634):349–352.
42. Johansen K, Bardin J, Orloff M J. Massive bleeding from hemorrhoidal varices in portal hypertension. JAMA. 1980;244(18):2084–2085.
43. Stefan Riss, Friedrich Anton Weiser, Katrin Schwameis, Thomas Riss, Martina Mittlböck, Gottfried Steiner, Anton Stift. The prevalence of hemorrhoids in adults.International Journal of Colorectal Disease volume 27, pages215–220(2012)
44. Haas PA, Haas GP, Schmaltz S, Fox TA Jr (1983) The prevalence of hemorrhoids. Dis Colon Rectum 26:435–439.