# Free Radicals and Antioxidants in Ankylosing Spondylitis

**AUREL NECHITA[1], CIPRIAN DINU[1], ALEXANDRU BOGDAN CIUBARA[1]*, GHEORGHE RAFTU[2]*, CODRINA ANCUTA[3]**

[1]Dunarea de Jos University of Galati, Medicine and Pharmacy Faculty, 47 Domneasca Str., 800008, Galati, Romania

[2]Ovidius University of Constanta, Faculty of Dental Medicine, 7 IlarieVoronca Str., 900684 Constanta, Romania

[3]Grigore T. Popa University of Medicine and Pharmacy Iasi, Rheumatology Department, 16 Universitatii Str., 700115, Iasi, Romania

*Free radicals are widely recognized as overloaded atoms, molecules or compounds that become unstable when lacking an electron;they steal an electron from various macromolecules (e.g. DNA, RNA, proteins) to chemically stabilize, while preferred targets remains polyunsaturated fatty acids in their membranes. When electron theft produces a chain reaction, normal cell processes turn into a real chaos that ultimately degrades the normal functioning of the cell. Variance of free radicals existing or formed in nature as a result of many processes (ultraviolet radiation, gamma, action specific particles, etc.) makes extremely difficult their classification. A partof the oxygen molecules ($O_2$) that have entered the body through breathing is divided and oxygen atoms become reactive (free radicals) damaging the cell wall by oxidation. Oxidative stress, a term widely used to characterise inflammatory disorders caused by destructive oxygen molecules called free radicals, may exacerbate inflammation and impair immune system response due to free radicals. Oxidative stress is defined as the imbalance between oxidants and antioxidants, in favour of oxidants, with destructive and pathogenic potential. Depending on intensity, oxidative stress can occur inside or outside the cell. Intracellular stress can lead to cell necrosis or a more or less marked disruption of the cell, and may be catastrophic in the case of a non-reproducible cell; the extracellular oxidative stress is cytotoxic. Although considered in the pathobiology of several inflammatory immune-mediated rheumatic conditions, the exact role of oxidative stress in ankylosing spondylitis is still debatable.*

*Keywords: free radicals, oxidative stress, oxidation process, inflammation, ankylosing spondylitis*

.

Free radicals are molecules that have lost an electron; electrons are in pairs, but forces that can *break* an electron out of the molecule. Superoxide - the anion and hydrogen peroxide are free radicals. Free radicals mean atoms, ions or molecules that do not have electrons in orbit, have many forms, such as oxygen radicals and hydroxyl radicals. This rupture causes the molecule to search for and *steal* electrons from other molecules. And incomplete molecules work wrong or do not work at all.

All this abnormal activity is called the oxidation process, which causes the appearance of more free radicals and stimulates inflammation. The oxidation process is limited by the action of antioxidants (ie, vitamins A, C and E) that donate electrons to free radicals, slowing tissue degradation. The functioning of the human body is the result of a chemical process, it is carried out in a normal way as long as the natural balance of these processes are maintained.

Free radicals are substances derived from incomplete oxidized compounds that have undergone partial combustion, having in their structure oxygen groups capable of initiating aggressive oxidation reactions on the surface of cellular membranes or even within cells; they come from processes that occur in the body (phagocytosis, incomplete catabolism, energy production) and from the outside environment. The most active free radicals are ions: superoxide ($O_2$-), peroxide ($O_2$-), hydroxide (OH-), nitric oxide (NO-). Peroxides and superoxides, the anions that exert a vigorous oxidative action come from hydrogen peroxide ($H_2O_2$) as well as from other sources or processes. Free radicals come from two major sources: *endogenous* (free radicals that form in the body during metabolic or physiological phenomena); *exogenous* (free radicals penetrate the body from the external environment).The exposure of organisms to very varied chemicals or to radiation can cause oxidation of biological compounds.[1-17]

Free radicals are formed by photolysis of chemical bonds as a result of the absorption of a photon, and the passage of the molecule into an excited state of singlet or triplet. If the cleaved chemical bond was one> C-O-, then the new radicals will be located on the C and O atoms,> C • and, respectively, -O •. Free radicals are highly reactive and can interact with either functional groups containing double bonds> C = C <by addition, or they can attract an H atom, creating instead a new radical. The unpaired electron can be located at both carbon atom (C) and other atoms (such as oxygen, O). In a chemical reaction in solution the radicals have a very short life, ranging between nanoseconds and microseconds.

In an environment that greatly slows diffusion, such as a polymer film, radicals can have a long life of seconds and even minutes. At very low temperatures, radicals can be studied with a special technique called electron resonance spectroscopy.

Free radicals in the human body have both beneficial and harmful effects and the main source of free radicals in the body is mitochondria. The most important are: oxygen free radicals (singlet oxygen - $^1O_2$, superoxide - $O_2$ -, hydroxyl - OH, alkoxyl - RO, phenoxyl - ArO, peroxyl - ROO) and nitrogen (NO, $NO_2$, $NO_3$). Free radicals produced in the body interfere with various physiological processes such as defense of the organism against microbial attack (with bactericidal action during the phagocytosis process), stimulation of cell growth and proliferation, induction of cell apoptosis. The oxidase acts on the amino acids and hydroxyacids according to the reaction: D (L) amino acids $\rightarrow$ketoacids + $H_2O_2$ L-hydroxyacids $\rightarrow$ ketoacids + $H_2O_2$. Phagocytes are activated and recruited into the inflammatory response. Polinuclears are the first to reach the site of inflammation, followed by macrophages. Phagocytes adhere to the bacteria and ingest them. The main bactericidal mechanism is an oxygen-dependent mechanism that accompanies increased oxygen

* *email :abciubara@yahoo.com; gheorgheraftu@yahoo.com*

consumption with NADPH-oxidase activation. Cytochrome p450 is a group of enzymes present in every cell of the body except for erythrocytes and skeletal muscle cells. It is important in the metabolism of certain substances in the body, such as steroids, liposoluble vitamins, fatty acids, prostaglandins and alkaloids [1-17].

Also, the enzymes in this group detoxify drugs and a large number of pollutants from the environment. Oxygen not only underpins energy metabolism and repress but is also involved in many degenerative diseases and other diseases (L. Marx 1985). These include partially reduced oxygen forms, called reactive oxygen species, which are relatively small, organic or inorganic molecules: singlet oxygen, superoxide anion, hydrogen peroxide, hydroxyl radical, alkoxyl and peroxyl radicals; these molecules are highly reactive due to unpaired electrons and play under physiological conditions important roles in normal metabolic processes (mobilization of ion transport systems, lesion scarring, blood homeostasis, immune processes). Under abnormal environmental conditions (excessive exposure to heat, ultraviolet radiation, pollutants), the level of oxygen species increases dramatically, resulting in serious cellular damage. The reactive oxygen species are formed under the action of ionizing radiation. Oxidative stress accumulates its effects with the nitrous stress due to the formation of reactive nitrogen species. Nitrogen reactive species are a family of antimicrobial molecules derived from nitric oxide and superoxide, produced by the $NOS_2$ and NADPH-oxidase enzymes, resulting in peroxynitrite. $NOS_2$ is expressed primarily in macrophages, and the peroxynitrite is highly reactive and can react with various cellular components. The reactions of reactive species with organic substrates are complex. The nature of oxidative lesions that can cause cell death is not always obvious. Oxidative stress lesions can be classified into: DNA lesions, polyunsaturated fatty acid oxidation from lipids, and protein amino acid oxidation [1-17].

Oxygen alone is a stronger, more reactive oxidant than the hydroxyl radical, reacting with many organic compounds, such as polyunsaturated fatty acids. Oxidation is a chemical reaction that occurs in the presence of oxygen, during which electrons are transferred by a substance to an oxidizing element. This type of reaction can produce free radicals, which are chemical agents, oxidized derivatives of the electron deficient molecule (are unstable). Free radicals attack the cells, DNA, certain molecules (proteins, fatty acids, etc.), causing dysfunctions and chain reactions that can even lead to cell death. Oxidative stress refers to the imbalance that favours pro-oxidants to antioxidants. An increased oxidative stress can lead to extensive lesions of lipids, proteins and DNA. The process has been involved in rheumatoid arthritis and muscular dystrophy. The production of uncontrolled free radicals in during physical effort induces muscle damage and fatigue [1-17].

The role of antioxidants begins with the introduction of free radicals into the body, hindering the chain reactions they have and functioning as a system of protection against reactive oxygen species and the harmful effects of the environment.

Free radicals and antioxidants are in a certain natural ratio; if defense systems are weakened, free radicals can attack cell membranes, proteins, fats and deoxyribonucleic acid, with an essential role in the hereditary mechanism. In the long run, this oxidative stress leads to degeneration or premature death of cells; many studies have highlighted the fact that a deficiency of antioxidants increases the risk of atherosclerosis [1-17].

That's why some people and scientists are of the opinion that the attack of free oxygen radicals on fats, especially cholesterol, is the first step in atherosclerosis. Other scientific research indicates a link between the low level of antioxidants in the blood and the risk of cancer. Researchers believe that free radicals are also involved in some cellular signalling processes, known as redox signalling. Primary oxygen radicals are superoxide and hydroxyl radicals. This is derived from molecular oxygen under conditions of chemical reduction. These free radicals can participate in undesirable side effects, with cellular lesions. Other diseases, in which the free radicals are thought to make a significant contribution, are: chronic inflammation; neurological diseases such as stroke, senile dementia or Parkinson's disease; neuropathies occurring as a complication of diabetes; and so on[1-17].

Inflammation is a consequence of wounds or a reaction of the body to the unfavourable action of external factors such as bacterial or fungal infections and chemicals. The inflammatory reaction is characterized by four symptoms: redness, fever, pain and swelling. Chronic systemic inflammation is the root of many diseases. Inflammation is a very complex process, consisting of a sequence of only partially known biochemical, biological and hematological reactions. The most eloquent evidence of involvement of free radicals in the inflammatory process is anti-inflammatory drugs. They do not have related structures and act through several mechanisms involving very varied processes and reactions; they act on the synthesis or properties of PG or LT[1-17].

Superoxide produced by neutrophils and other phagocytes is another component of their bactericidal weapon even if the same action can contribute to tissue damage associated with inflammation. The production of superoxide radical appears as a result of the activation of NADPH or NADH-membrane oxidase according to the formula:

$$NADPH + 2O_2 \xrightarrow{NADH-oxidaza} NADP^+ + 2O_2^- + H^+$$

The genetic incapacity to produce superoxide threatens life. Neutrophils have impaired ability to kill microorganisms that have been ingested, leading to multiple recurrent local infections and often sepsis. The result of over production of superoxide is dangerous for the body as well as low production of superoxide. It is worth mentioning the involvement of RLO in the pathogenesis of SLE. On the one hand, the accumulation of RLO contributes to the increase in photosensitivity and, on the other hand, the monocytes of these patients have been found to inhibit the generation of superoxide radical in normal phagocytosis. DNA fragments resulting from the RLO action on DNA strands represent the preferred antigen for human anti-DNA antibodies. The nature of inflammation is the same, regardless of where the inflammatory process develops. However, acute inflammation such as microcrystalline inflammation is widely defined by the markers such as redness and rising joint temperatures; these signs are related to biologically active substances produced by the body contribute to the expansion of blood vessels and increased blood flow to the inflammation site; in this case, the amount of leukocytes in the outbreak increases in such a way that it contributes to increasing the volume of the affected area (swelling)[1-17].

Consequently, there is a mechanical irritation of the nerve endings, which causes the sensations of pain; toxic substances that are released in the areas where confusion of infection agents occurs with the body's protective systems contribute to the appearance of pain. In the

inflammatory process, essential fatty acids, which are the sources of prostaglandin transport, play a more important role than any other food component. Generally, *good* prostaglandins attenuate inflammation and pain sensations, and the *bad* ones stimulate them; the additional consumption of substances that stimulate prostaglandin formation will improve the situation. It is necessary to balance the quantities of basic macro-elements in the diet. Vitamin B12 helps to alleviate inflammation and pain sensations. The need for vitamin C increases in case of trauma or infections, which usually result in an inflammatory process; vitamin C is a powerful antioxidant, minimizes the destruction of tissues by free radicals formed by these toxins. Bioflavonoids favour the action of vitamin C, preventing swelling during inflammation and prevent the production of *bad* prostaglandins. Recent studies have shown that antioxidants are able to reduce inflammation in the body. These are organic compounds found in food, especially in live vegetables coloured antioxidants are the coenzyme Q9, coenzyme Q10, alpha-tocopherol, vitamins A, C and E, lycopene and selenium microelements [1-17].

Antioxidants have also been called *sacrificial substances* because they neutralize free radicals and protect human body cells from oxidative stress with their own destruction; blocks the oxidation process, neutralizing free radicals, but sometimes it happens that antioxidants get oxidized. Because of this, we must constantly refresh our antioxidant reserves.

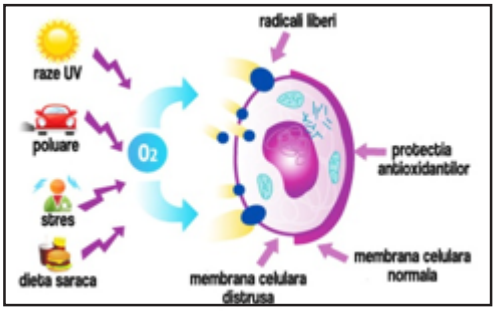


Fig. 1. Natural antioxidants

Antioxidants neutralize free radicals and, in order not to turn themselves into free radicals, the body produces a *primary antioxidant* - glutathione. Glutathione - a small protein, present in almost all the cells of our body, has a strong antioxidant and toxin elimination role; the low level of glutathione predisposes the body to severe chronic conditions such as liver or diabetes. When the patient has a rheumatic illness with inflammation in general, he should consider increasing the number of antioxidants in the body to eliminate free toxins and free radicals in a very large number. Also a diet rich in antioxidants, improves immunity, reduces the risk of cancer and infections. These are found in abundance in vegetables and fruits, in whole grains, medicinal and oil plants[1-17].

Inflammation is largely manifested by oxidative stress, in which aggressive unstable molecules (free radicals) end up by destroying other molecules. But the human body has a complex system that maintains the balance between free radicals and endogenous antioxidants[1-17].

*Free Radicals and Oxidative Stress in Chronic Inflammatory Rheumatic Conditions and Their Pathobiological Significance-Focus on Ankylosing Spondylitis*

On the other hand, recent data endorse the role of oxidative stress in the pathobiology of a number of chronic inflammatory immune-mediated rheumatic disorders including rheumatoid arthritis, lupus, ankylosing spondylitis, as well as their key involvement in early accelerated atherosclerosis [18-20].

The excess of pro-inflammatory cytokines, chiefly tumour necrosis factor alpha (TNFα), interleukin 6 (IL-6), IL-1β and IL-17, is widely associated with increased oxidative stress and reactive oxygen species synthesis promoting tissue damage, but also linked to insufficient antioxidant defence, such as the loss of thiol/disulphide homeostasis [18, 19]. As an important plasma antioxidant, thiols, mostly low molecular weight species as cysteine, homocysteine, glutathione and albumin, are known to remove reactive oxygen molecules by forming disulphide bonds, regulating the signalling pathways and apoptosis, resulting in a dynamic thiol/disulphide balance [19]. Furthermore, abnormal thiol/disulphide concentrations are reported in different inflammatory conditions [19].

Ankylosing spondylitis (AS) belongs to the spondylo-arthropathies (SpA), a heterogenous group of disorders that occur in a genetic predisposed background (HLA-B27 positivity) and shareseveral clinical (articular and systemic features), imagistic (sacroiliitis, spondylitis on X-rays or magnetic resonance imaging) and therapeutic (prompt response with non-steroidiananti-inflammatory drugs and biologics) characteristics [21,22].AS remains the leading entity of this group, irrespective of the disease concept or the diagnostic or classification criteria used [21].

Recent advances in understanding the pathobiology of SpA and AS revealed the key role of inflammatory cytokines (TNFα and the IL-17/IL-23 axis) and signalling molecules in driving both local (synovial, entheseal) and systemic inflammation (ocular, skin, gastro-intestinal), its interconnection with osteoproliferativetissue damage (sacroiliac joint, spine) accountable for radiographic progression and disease disability [21, 22].

The link between skin, enthesis and joints as well as the link between gut inflammation with intestinal epithelial damage, dysbiosis and microbial translocation and joint inflammation in SpA are currently recognized and seem to play critical role in inducting immune system activation [21, 22].

The treatment landscape in AS andSpA has also dramatically changed in the last decade, offering successful outcomes according to the new recommendations for treat to target [21, 22]. Clinical perspectives as well as blood analysis in selecting the anti-TNF agent or IL-17 inhibitor, effective switching in patients with suboptimal disease control, ideal therapeutic strategy for those patients achieving stable remission represent the main challenges for experts in routine practice [21,22,25,26].

Despite all these achievements in AS, there is still lacking data concerning the complex interplay between oxidative stress, anti-oxidative capacity and disease activity and the impact of biologic drugs in AS [18-20]. Only few studies assumed that oxidative stress might be involved in disease onset and progression in AS, emphasizing the oxidative dysbalance and related factors in different AS settings [18-20, 23, 24] including AS associated with traditional cardiovascular risk factors [20].

It seems that the measurement of thiol/disulphide homeostasis is critical for elucidating the oxidative stress and AS activity [19, 24]. Thus, thiol levels are significantly lower in patients with active disease (negative correlation between plasma thiol concentration and disease activity scores) [19, 24], while normal in inactive AS [19], suggesting the potential use of serum thiol level as a biomarker of disease activity and response to specific medication. However, discordant data about the disulphide

plasma levels and, subsequent, thiol/disulphide ratio are reported; Dogru et al, 2016 demonstrated low disulphide levels, irrespective of AS activity, with lower disulphide/native thiol and disulphide/total thiol ratio compared to controls suggesting potential oxidative stress independent of disulphide levels or insufficient thiol/disulphide homeostasis in AS [19].

As total antioxidant status (TAS) and total oxidant status (TOS) are suitably to evaluate the oxidative stress, Solmaz et el, 2016 reported significantly higher TOS levels in active AS, while TAS were similar irrespective of AS activity; [18] moreover, both TAS and TOS were not influenced by biologics compared to conventional agents [18]. As a marker of oxidative stress in AS, TOS levels correlated with TAS, disease activity scores and metrology index, as well as with high sensitivity C-reactive protein supporting the role of oxidative stress defined an imbalance between oxidative-anti-oxidative status in AS [18]. Although, oxidative stress could be impaired by TNF inhibitors, data on this topic is still inconclusive for AS patients [18].

Finally, high oxidative stress parameters and systemic inflammation (erythrocyte sedimentation rate and C reactive protein) were described by Staneck et al, 2017, as well as abnormal pro-atherogenic lipid profile meaning decreased lipids and HDL-cholesterol fraction associated with significant increase in total cholesterol, LDL-cholesterol fraction, triglycerides, triglycerides/HDL ratio and HDL/LDL ratio [20]. Increased lipid peroxidation, as a result of insufficient antioxidant capacity and highly active reactive oxygen species, was also demonstrated. Taken together, all these data support increased oxidative stress, aberrant lipid pattern and systemic inflammation closely related to atherosclerosis in AS patients [20].

## Conclusions

Antioxidants have the role of protecting the body from oxidative stress (the imbalance between the level of oxidation products and the antioxidant capacity of the body); endogenous enzyme systems with antioxidant role, most commonly known as superoxide dismutase (SOD), catalase, glutathione peroxidase. When free radical action exceeds that of antioxidants, there are phenomena of wear out and premature cellular aging, which can lead to the installation of some degenerative diseases including cancer.

The balance between the oxidative action of free radicals and the level of antioxidants in the body is the essence of life and characterizes the body's resistance capacity.

Oxidative stress or imbalance between pro-oxidant agents and antioxidants typically result in tissue damage, particularly in the synovial microenvironment, but also systemically. Further clarifications are still required in order to define the pathobiological implications of free radical and oxidative stress, not only in ankylosing spondylitis, but also for cardiovascular comorbidity in patients with ankylosing spondylitis.

## References


1. TURRO, NJ, RAMAMURTHY, V., SCAIANO, J.C., Principles of Molecular Photochemistry - An Introduction, University Science Books, Sausalito, 2009.

2. NORMAN, A. S., Photochemistry and Photophysics of Polymer Materials, Wiley, 2010.

3. IVAN, M.G., SCAIANO, J.C., Photochem. Photobiol., 2003, 78, 4, p. 416.

4. MACOVEI, L.A., DEBITA, M., ILIE, M., MOISEI, M., CHISCOP, I., CARDONEANU, A., ARBUNE, M, REZUS, C., Rev. Chim.(Bucharest),**66**, no.10, 2015, p. 1645-1650.

5. MACOVEI,L.A., MATEI, M.N., NICHITA, A., LEATA, R., CHISCOP, I., ILIE, M., ARBUNE, M., REZUS, C., Chim.(Bucharest),**66**, no. 9, 2015,p. 1516-1520.

6.SITI HN, KAMISAH Y, KAMSIAH J. The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review).VasculPharmacol. 2015; 71: 40-56.

7. DINU, C., BRUJBU, I., CERGHIZAN, D., BULIMAR, V., MACOVEI, L.A., BOTEZATU, D., Rev. Chim.(Bucharest),**67**, no.6, 2016, p. 1203-1206.

8. NAITO A, MIDORIKAWA T, YOSHINO T, OHDERA M. Lipid peroxides induces early onset of catagen phase in murine hair cycles. Int J Mol Med. 2008; 22 (6): 725-729.

9. GAETKE LM, CHOW CK. Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology. 2003; 189 (1-2): 147-163.

10. BALAN, G., PELIN, A.M., MACOVEI, L.A., et al., Rev. Chim.(Bucharest),**68**, no. 3, 2017, p. 608

11. HULSMANS, M., HOLVOET, P., J. Cell. Mol. Med., 14, no. 1-2, 2010, p. 70

12. VINCENT, A.M., RUSSELL, J.W., LOW, P., FELDMAN, E.L., Endocr Rev., 25, no. 4, 2004, p. 612

13. BROOKS, S.V., VASILAKI, A., LARKIN L., MCARDLE A., JACKSON M.J., Physiol, 2008, Volume 586, Number 16, 3979-3990. J. Physiol., 586, no. 16, 2008, p. 3979

14. IONESCU, R., Essential in Rheumatology, Amaltea Medical Publishing House, Bucharest, 2006.

15. BOJINCÃ, M. Genetic Aspects in Rheumatic Diseases. In: Essential in Rheumatology. Amaltea Publishing House, 2007.

16. MARIA GREABU, ALEXANDRA TOTAN, MARIA MOHORA, ANICA DRICU, ALINA ELENA PÂRVU, LILIANA FOIA, MARILENA MOTOC, Medical Biochemistry Guide, Ed. CurteaVeche, Bucharest, 2014.

17. CIUREA P Rheumatology, Medical University Publishing House Craiova, 2007.

18. SOLMAZ, D., KOZACI, D. SARI, I., TAYLAN, A., ONEN, F., AKKOC, N. AKAR, S. European Journal of Rheumatology 2016; 3:20-4.

19. DOGRU, A., BALKARLY, A., CETIN, Y.G., NESELIOGLU, S., EREL, O., TUNC, E.S., SAHIN, M. Bosnian Journal of Medical Sciences 2016; 16(3):187-92.

20. STANECK, A., CHOLEWKA, A., WIELKOSZYNSKI, T., ROMUK, E., SIERON, K, SIERON, A. Hindawi Oxidative Medicine and Cellular Longevity 2017; article ID 9712536, 8 pg.

21. PROFT, F., PODDUBNYY, D., TherAdvMusculoskel dis 2018, 10 (5-6): 129-39.

22. DUBASH, S., MCGONAGLE, D., MARZO-ORTEGA, H. TherAdv Chronic Dis 2018; 9 (3):77-87.

23. KARAKOK, M., ALTINDAG, O., KELES, H., SORAN, N., SELEK, S. RheumatolInt 2007; 27 (12):1131-4

24.YAZICI, C., KOSE, K., CALIS, M., KUZUGUDEN, S., KIRNAP, M. Rheumatology (Oxford) 2004; 43 (10): 1235-9

25.SALARU,D.L., Mertens,P.R., BARTSCH, P, INTERNATIONAL UROLOGY AND NEPHROLOGY,45(5), 2013, PG.248-250

26 .SALARU,D.L., MACOVEI, L., STATESCU, C., et al., REVISTA ROMANA DE MEDICINA DE LABORATOR, 21(4),2013, PG.407-414


Manuscript received:11.08.2018