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Topic

Evaluation of Oxidative Stress state in Elderly People in The Region of Tiaret

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Dedication

I dedicate this message to all those who have been a source of support, inspiration and encouragement throughout my academic journey.

To my dear father and mother,

Your unconditional love, patience and trust in me have been the cornerstones of my life. Thank you for your sacrifices, continued support and valuable advice. Thanks to you, I was able to complete this work.

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Your moral and emotional support helped me overcome challenges and persevere through difficult times. Thank you for your understanding, encouragement and presence by my side.

To my friends,

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I dedicate this work to you with gratitude, hoping that it will gain your trust and love.

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List of abbreviations

$^1O_2$:	Singlet oxygen
AD:	Alzheimer's disease
BMI	Body mass index
CAT:	Catalase
Cu:	Copper
CVD:	Cardiovascular diseases
FR:	Free radicals
GPxs:	Glutathione peroxidases
H_2O_2:	Hydrogen peroxide
HOCl:	Hypochlorous acid
$NO^{\bullet}$:	Nitric oxide
$O_2^{\bullet-}$:	Superoxide radical anion
$OH^{\bullet}$:	Hydroxyl radical
ONOO−/ONOOH:	Peroxynitrite
OS:	Oxidative stress
RNS:	Reactive nitrogen species
$RO^{\bullet}$:	Alkoxyl radical
$ROO^{\bullet}$:	Peroxyl radical
ROS:	Reactive oxygen species
SOD:	Superoxide dismutase's
Se	Selenium
Zn:	Zinc

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Abstract

Aging is characterized by a progressive decline in the efficiency of biochemical and physiological processes and an increased susceptibility to disease. Growing evidence indicates that aging and age-related diseases are associated with a state of oxidative stress (OS). This condition is marked by an imbalance between the production and elimination of reactive oxygen species (ROS) especially, and antioxidant reserve.

This study aims to highlight the presence of oxidative stress by measuring some markers (malondialdehyde (MDA), superoxide dismutase (SOD), catalase, and vitamin C) in older people over 60 compared to young controls.

Capillary blood samples from human volunteers were utilized in this observational study to evaluate markers of oxidative stress and biochemical parameters.

The data obtained broadly demonstrate that the majority of elderly people display an OS condition characterized by increased levels of MDA and a slight reduction in catalase.

Older adults are more likely to develop oxidative stress, making it crucial to monitor oxidative stress markers and, if needed, provide antioxidant supplementation alongside promoting a healthy lifestyle.

Key words: aging, oxidative stress, MDA, SOD, Catalase, young people.

Résumé

Le vieillissement se caractérise par un déclin progressif de l'efficacité des processus biochimiques et physiologiques, ainsi qu'une susceptibilité accrue aux maladies. De plus en plus de preuves indiquent que le vieillissement et les maladies liées à l'âge sont associés à un état de stress oxydatif (OS). Cette condition est marquée par un déséquilibre entre la production et l'élimination des espèces réactives de l'oxygène (ROS) en particulier et la réserve antioxydante.

Le but de cette étude est de mettre en évidence la présence de stress oxydatif en mesurant certains marqueurs (malondialdéhyde (MDA), superoxyde dismutase (SOD), catalase, vitamine C) chez les personnes âgées de plus de 60 ans par rapport à des témoins jeunes.

Des échantillons de sang capillaire de volontaires humains ont été utilisés dans cette étude observationnelle pour évaluer les marqueurs de stress oxydatif (OS) et les paramètres biochimiques.

Les données obtenues montrent largement que la majorité des personnes âgées présentent une condition de stress oxydatif caractérisée par des niveaux accrus de MDA et une légère réduction de la catalase.

Les adultes plus âgés sont plus susceptibles de développer un stress oxydatif, ce qui rend crucial le suivi des marqueurs de stress oxydatif et, si nécessaire, la fourniture de suppléments antioxydants ainsi que la promotion d'un mode de vie sain.

Mots clés : vieillissement, stress oxydant, MDA, SOD, Catalase, témoins jeunes.

ملخص

تتميز الشيخوخة بانخفاض تدريجي في كفاءة العمليات البيوكيميائية والفيزيولوجية وزيادة القابلية للإصابة بالأمراض. تشير الأدلة المتزايدة إلى أن الشيخوخة والأمراض المرتبطة بالعمر لها علاقة بحالة من الإجهاد التأكسدي. تتسم هذه الحالة بعدم التوازن بين إنتاج وإزالة أنواع الأكسجين التفاعلية خاصة واحتياطي مضادات الأكسدة.

الهدف من هذه الدراسة هو تسليط الضوء على وجود الإجهاد التأكسدي من خلال قياس بعض المؤشرات (فيتامين ج، كتلاز ، ديسموتاز فوق أكسيد، مالونديالدهيد) لدى الأشخاص الأكبر من 60 عامًا مقارنةً بالأفراد الشباب كعينات تحكم.

تم استخدام عينات دم شعيري من متطوعين بشريين في هذه الدراسة الرصدية لتقييم مؤشرات الإجهاد التأكسدي والمعايير البيوكيميائية.البيانات التي تم الحصول عليها تظهر بشكل عام أن غالبية الأشخاص المسنين يظهرون حالة من الإجهاد التأكسدي تميز بزيادة مستويات مالونديالدهيد وانخفاض طفيف في الكاتالاز. من المرجح أن يطور البالغون الأكبر سنًا حالة من الإجهاد التأكسدي، مما يجعل من الضروري مراقبة مؤشرات الإجهاد التأكسدي وتوفير مكملات مضادات للأكسدة إذا لزم الأمر، بالإضافة إلى تعزيز نمط حياة صحي.

الكلمات المفتاحية: الشيخوخة، الاجهاد التأكسدي، كتلاز،

Introduction

Introduction

Over the previous few decades, the average life expectancy has rapidly climbed, reaching an average of approximately 71.4 years worldwide, in 2015 (World Health Organization, 2014). The population over 60 is predicted to increase from 605 million to 2 billion individuals between 2000 and 2050 based on global population demographics (World Health Organization, 2018)**.**

Even if an increase in life expectancy is a sign of progress for humanity, a new problem is emerging. The biggest health risks of the twenty-first century are now age-related diseases. Aging is a natural, ubiquitous, complex, and progressive process that is degenerative in nature. It is marked by a gradual loss of function (Chang *et al*., 2017). In fact, aging is positively associated with biological and cognitive deterioration, including physical cognitive decline, psychological disability, and frailty (Jin *et al*., 2015). The free radical theory of aging is a well-established theory that explains the aging process. According to this idea, aging results from many defensive mechanisms' inability to counteract damage caused by reactive oxygen species (ROS) (Islam, 2017). Oxidative phosphorylation, which occurs in all eukaryotic species, is the primary source of ROS. Because their endogenous antioxidant systems are less effective as they age, older individuals are more vulnerable to oxidative stress (OS) (Kim et Sieburth, 2018).

Problem stateme Aging is a natural and complex process that affects all living organisms, including human beings. Over time, the accumulation of damage caused by free radicals can lead to a decline in cellular function, chronic inflammation, and alteration of metabolic processes. In this study, we aim to elucidate the link between oxidative stress and the aging process, as well as how it can be mitigated to promote healthy aging

Therefore, the objective of this study is focused on the determination of some oxidant/antioxidant markers in the elderly and controls groups of young people. The rest of this dissertation is organized in two parts as follows:

The first part presents the literature review in three chapters. Chapter I focuses on the relationship between aging and health. Chapter II covers the topic of oxidative stress, as well as the concept of reactive species and their various forms. It also studies the sources of reactive oxygen and the antioxidant defense system, which is made up of both enzymatic and non-

enzymatic systems. Chapter III explores the connection between ROS and the aging process, including age-related diseases, and how oxidative stress impacts various health conditions.

The second part, comprising of three chapters, details the experimental work. In the first chapter, details on the materials used and the methods used in this study are presented. Subsequently, the results obtained are presented and discussed in Chapter II and Chapter III, respectively. Finally, this document is closed with a conclusion.

Literature Review

Chapter I:

Aging and Health

I. Aging and health

I.1 Definition

The concept of aging has attracted the attention of many researchers, starting from the work entitled “the old age”, which focused on healthy aging (Rudnicka *et al*., 2020). It is said that the aging process is a change in the effectiveness of biochemical and physiological processes (Gorni *&* Finco, 2020) . In fact, the progressive loss of cell productivity brought on by structural and chemical alterations at the cellular and subcellular levels is known as aging. The build-up of toxins and unfolded proteins changed immunological responses and gene expression, and gradually reduced physiological activity. It is still up for debate what exactly causes these alterations at the cellular level despite the fact that they have been well-investigated and documented (Anik *et al*., 2022).Although aging is not a disease, it increases susceptibility to age-related diseases, like cancer, metabolic diseases, cardiovascular diseases, musculoskeletal diseases, as well as neurodegenerative diseases (Kennedy *et al*., 2014). Such diseases are known to be caused by OS.

OS is a condition marked by an imbalance between antioxidant defenses and pro-oxidant chemicals, such as reactive oxygen and nitrogen species. It is known that OS has significant effects on the health of the elderly(Verhaegen *et al*., 2022). Specifically, there is growing evidence that the excessive of reactive oxygen species (ROS) and reactive nitrogen species (RNS) intracellular generation and associated signaling pathways may contribute to cellular OS, which ultimately impairs cellular function and quickens the aging process (Sies, 2015; L. Zhang *et al*., 2019).

For these reasons, this chapter gives details on the concept of aging and its relation to oxidative stress.

I.2 Aging process

Harman's developed the "free radical theory of aging"(Harman, 1955). He classified this theory into two categories: programmed theories, and error theories.

(I) Programmed theories: define aging as the outcome of the ongoing management of the biological schedule.

(II) Error theories: primarily focus on environmental effects on organisms that lead to different levels of damage (Jin, 2010).

I.3 Hallmarks of aging

To better understand the mechanisms and effects of anti-aging therapies on age-related diseases, it is essential to first clarify the cellular and molecular markers of aging. From the study of many types of organisms, especially mammals, nine factors and corresponding candidate markers are usually considered to define the aging phenotype. The nine hallmarks of aging are genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication(López-Otín *et al*., 2013).

I.3.1 Genomic instability

The accumulation of genetic damage is one well-acknowledged factor in aging, as it may upset cell homeostasis and create genome instability (Kubben & Misteli, 2017). DNA damage can be exacerbated by somatic mutations, chromosomal aneuploidy, and copy mutations. Cell dysfunction results from flaws in the DNA repair mechanism that develop in older people. These flaws impact transcription pathways and the expression of vital genes(Tiwari & Wilson, 2019).

I.3.2 Cellular senescence

A condition of cell cycle arrest is called cellular senescence. The prevailing opinion is that a significant buildup of senescent cells in the tissue is what causes aging (Hernandez-Segura *et al*., 2018). The primary causes of senescence are DNA damage and the senescence-related secretory phenotype. Furthermore, a number of secreted phenotypic factors have weak or no transcriptional activation linked to mitochondrial dysfunction-associated senescence(Hernandez-Segura *et al*., 2017).

I.4 Physiological changes

I.4.1 Skeletal muscle

Skeletal muscle is the term for the muscular tissue that is connected to the bones and powers bodily movement. The human body's skeletal muscles are essential for regulating glucose homeostasis, posture, movement, and thermogenesis. Muscle mass and strength start to deteriorate with age, making daily chores more difficult to do and increasing reliance on others. This move toward reliance is frequently correlated with higher rates of hospitalizations, falls, disability, and mortality. Aging is frequently associated with sarcopenia, a degenerative illness that reduces skeletal muscle mass and function. Age-related muscle alterations include a decrease in skeletal muscle cross-sectional area, which is linked to a shift in fiber type (El Assar *et al*., 2022)**.**

I.4.2 Cardiovascular system

In older adults, cardiovascular disease is thought to be the main cause of death. cardiovascular events cause frailty, cognitive decline, and functional decline. Aging leads to structural and functional changes in the cardiovascular system. In addition to these changes, pathological conditions are often added (El Assar *et al*., 2022).

I.4.3 Renal function

Progressive, mostly cortical renal atrophy, is a sign of aging kidneys. From a histological perspective, the number of nephrons gradually decreases with age, peaking at roughly 40 (Zeitz *&* Smyth, 2023). The most notable alteration is a slow decline in renal blood flow that starts to happen around the age of 40 and continues for ten years, during which time there is a progressive loss of functional glomeruli. The age difference in creatinine clearance is halved between 20 and 80 (Jaeger, 2018).

I.4.4 Digestive system

Age-related change to the oral organs includes hypochlorhydria, decreased salivary flow, and decreased acid production from parietal cells. These modifications lead to a reduction in absorption, particularly of calcium and iron. Furthermore, bloating and constipation are encouraged by longer bowel transit times in older persons because of diminished peristalsis. These

conditions can worsen by dietary modifications and dehydration. Liver mass and flow are also known to decrease with age (Jaeger, 2018).

Chapter II:

Oxidative Stress

II. Oxidative stress

II.1 Definition

The concept of oxidative stress was developed by Sies and Cadenas, about four decades ago. It refers to the damage to healthy cells and organs(Ji & Yeo, 2021; Sies & Cadenas, 1985). It is defined as an imbalance between oxidants and antioxidants that favor the oxidants, which damages molecules by upsetting redox signaling and regulation (Verhaegen *et al*., 2022).

Oxidative stress is a pathological circumstance characterized by an overload of oxidant products, named unfastened radicals, that antioxidant mechanisms are ill-equipped to combat. Free radicals cause oxidative damage to a variety of bodily systems and organs(Perrone *et al*., 2023). ROS generation and removal are out of balance, which results in oxidative stress (Hussain *et al*., 2016) (see Figure 1).

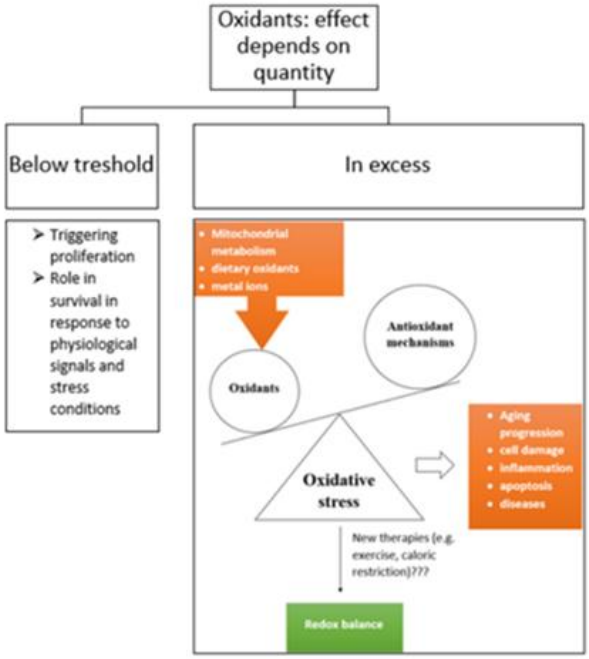

Figure 1. Oxidative stress caused by an imbalance in favor of oxidants over antioxidant processes (Childs *et al*., 2016)

II.2 Free radicals

Moses Gomberg made the discovery of free radicals (FR) at the start of the 20th century (1900). Leonor Michaelis postulated in the 1930s that FR most oxidation processes are mediated by organic molecules, if not all of them(Michaelis, 1939).

Free radicals are chemical entities that have unpaired electrons in the outermost shell of their electron orbital, which usually gives them high reactivity. Reactive oxygen species, or ROS, and reactive nitrogen species, or RNS, are the most common free radicals and reactive compounds found in biological systems. When electrons are lost or accepted during electron transfer processes, ROS or RNS are created(Halliwell *&* Gutteridge, 2015).

II.3 Reactive oxygen species

ROS are generally understood to be unstable active molecules centered around oxygen and having unpaired valence shell electrons(Bhattacharyya et al., 2014). Reactive oxygen species are generated primarily by mitochondria, during cells respiring (Skulachev, 2012). Through redox and electronic excitation processes, it is the pathway that leads to the generation of ROS(Sies *&* Jones, 2020). There are two ways that ROS can enter human cells: exogenously or endogenously (Wang *et al*., 2022).

II.3.1 Types of reactive oxygen species

Common ROS can be divided into two categories, radical ones and non-radical ones, depending on whether electrons are free:

II.3.1.1 Radical ROS

II.3.1.1.1 Nitric oxide (NO•)

When a set of enzymes converts L-arginine into L-citruline, the end product is called NO•. NO• can react with a broad range of compounds and RLs; for instance, it can create nitrite (NO^{2-}) or nitrate (NO^{3-}) following its reaction with H_2O_2(Bartesaghi *&* Radi, 2018) .

II.3.1.1.2 Superoxide radical anion ($O_2^{\bullet -}$)

As one of the main sources of H_2O_2, $O_2^{\bullet -}$ dismutates spontaneously or under the action of superoxide dismutases to produce H_2O_2 and O_2. Due to strong electrostatic attraction, $O_2^{\bullet -}$ oxidizes Fe–s clusters quickly, releasing iron, but because of its negative charge, it is less suitable for thiol-based redox signaling. As the protonated form of $O_2^{\bullet -}$, the perhydroxyl radical (HO_2) is uncharged, diffuses in lipids, and can generate a carbon-centered radical of polyunsaturated lipids (Valko *et al*., 2004).

II.3.1.2 Hydroxyl radical (OH•)

The most reactive ROS is $OH^{\cdot}$. It is an unspecific oxidant that oxidizes biomolecules at a diffusion-controlled rate. It is created by reducing H_2O_2 in a metal-catalyzed Fenton chemistry with the presence of free iron (Fe^{2+}) (Galaris *et al*., 2019). The position of Fe^{2+} establishes the site of $OH^{\cdot}$ toxicity because $OH^{\cdot}$ reacts directly with the closest neighbor at the site of its formation. Lipid peroxidation can be initiated by $OH^{\cdot}$(Koppenol *&* Hider, 2019).

II.3.1.3 Alkoxyl radical (RO•) and Peroxyl radical (ROO•)

Radicals associated with the peroxidation of fatty acids in lipids are known as alkoxyl and peroxyl radicals ($RO^{\bullet}$ and $ROO^{\bullet}$). Carbon-centric radicals react with oxygen to create $ROO^{\bullet}$. When lipoperoxides (ROOHs) are broken down by heat and UV light in the presence of transition metal ions, both types of organic radicals can occur (Zdenka Durackova, 2014).

$$ROOH + Fe^{3+} + ROO + Fe^{2+} + H^{+}$$

$$ROOH + Fe^{2+} + Ro + Fe^{3+} + OH^{-}$$

The following process can also be used to break down organic peroxides: superoxide protonates into peroxyl radical and hydrogen peroxide

$$HO_2 + ROOH \quad ROO \ + H_2O_2$$

II.3.2 Non-radical ROS

II.3.2.1 Hydrogen peroxide (H_2O_2)

H_2O_2 is a strong two-electron oxidant produced from O_2, mainly by Nicotinamide adenine dinucleotide phosphate oxidases in conjunction with superoxide dismutase, by the mitochondrial electron transport chain and numerous other enzymes(Dagnell *et al*., 2019).

II.3.2.2 Peroxynitrite (ONOO−/ONOOH)

The reaction between $O_2^{\bullet -}$ and $NO^{\bullet}$ produces $ONOO^-$, with a rate constant of approximately 6.7.109 L/mol/. s. Similar to $OH^{\cdot}$, the protonated version of the radical $ONOO^-$ (ONOOH) is a strong oxidant that can cause considerable damage (Jacques Delattre, 2005).

II.3.2.3 Hypochlorous acid (HOCl)

Hydrogen peroxide is used to create HOCI. Its potent oxidizing capability allows it to easily cross biological membranes and change the protein components of cells(Powers & Jackson, 2008). In the phagocytic vacuole of neutrophils, myeloperoxidase converts H_2O_2 to HOCl for pathogen defense(Winterbourn *et al*., 2016).

II.3.2.4 Singlet oxygen ($^1O_2$)

One type of O_2 that is electrically stimulated is $^1O_2$. The production of $^1O_2$ through photoexcitation confers particular significance on tissues exposed to light, such as the skin and the eyes. It is also created in darkening enzymatic reactions through chemiexcitation (Di Mascio *et al*., 2019; Mano *et al*., 2014).

II.3.3 Sources of ROS

ROS can enter human cells through two pathways: endogenous and external pathways. Both contribute significantly to the formation of ROS. Endogenous ROS sources are biological occurrences that have existed since the beginning of life, while exogenous ROS sources vary by culture and time period. Endogenous ROS production occurs primarily through oxidative

phosphorylation in mitochondrial complexes I and III (Kolodkin *et al*., 2020; Nolfi-Donegan *et al*., 2020). However, external sources and influences are inescapable (Figure 2).

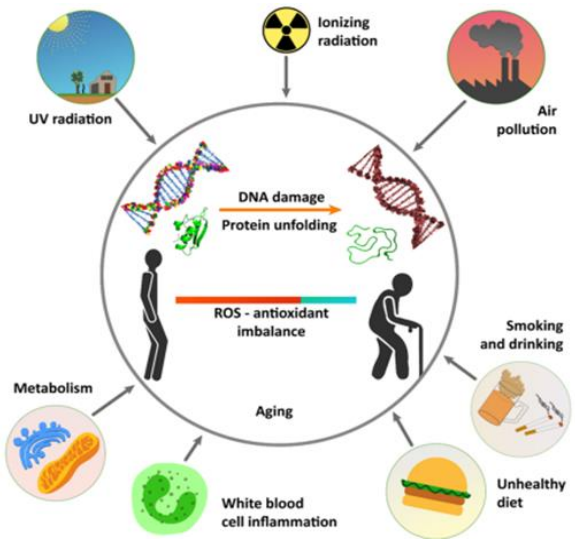

Figure 2. Various exogenous and endogenous sources of ROS and their effect on aging (Anik *et al*., 2022)

II.3.3.1 Sources of endogenous ROS

Sources of endogenous ROS include (Anik *et al*., 2022):

- Mitochondria;
- Genome Sequence of Mitochondria;
- Mutation of Mitochondrial-Encoded Complex I (NADH Dehydrogenase);
- Mutation of Mitochondrial-Encoded Complex III (Coenzyme Q-Cytochrome C Reductase).

II.3.3.2 Sources of exogenous ROS

ROS can also be caused by exogenous sources, such as (Anik *et al*., 2022):

- Cigarette Smoke;

- Alcohol Consumption;
- Food;
- Environmental Pollutants.

II.4 Mechanism of ROS

Mitochondria are the principal producer of ROS due to their function in ATP synthesis by oxidative phosphorylation, where molecular O_2 is reduced into H_2O in the electron transport chain (ETC). Mitochondrial superoxide generation is a major source of ROS in cells (Brand, 2010).

Accumulated mitochondrial ROS diffuses to the cytoplasm through mitochondrial membrane pores. Endogenous ROS can play a role in cellular signaling pathways and can be beneficial or damaging, depending on the reaction(Jaenen *et al*., 2021).

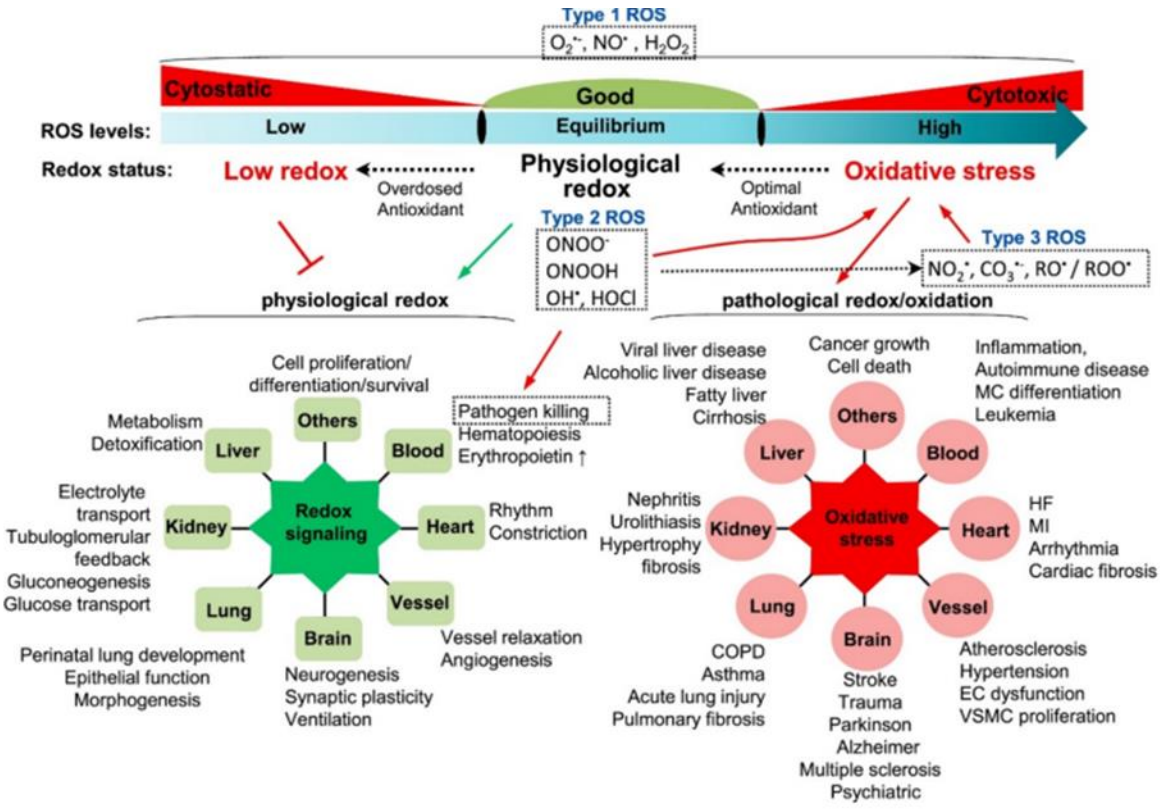

Figure 3. Homeostasis of ROS and its pathological implications (Anik *et al*., 2022)

Low to intermediate levels of ROS are necessary to maintain cell proliferation and the normal activities of healthy cells. On the other hand, an increased level of intracellular ROS may be harmful to the general health of the cell. These reactive species interact with biological macromolecules (Figure 3) like proteins, lipids, and nucleic acids, altering their structure and affecting normal tissue or organ functions (Schöneich, 2005).

II.5 Biomarkers of ROS-induced chemical modifications

This class includes biomarkers that arise from direct ROS/RNS assault on biomolecules, including protein modifications.

II.5.1 Protein carbonyls

Direct ROS assault results in irreversible modification of protein side chains. Following the oxidation of arginine, lysine, and threonine, protein carbonyls, which are aldehydes, form as side chain modifications in proteins. These modifications are chemically detectable and possess a stable structure (Stadtman, 2004).

II.5.2 Products of lipid peroxidation - malondialdehyde

The process of lipid peroxidation primarily targets polyunsaturated fatty acids. Lipid hydroperoxides react quickly with Fe^{2+} to create alkoxyl radicals (LO·), and they react much faster with Fe^{3+} to form lipid peroxyl radicals (LOO). Nevertheless, the latter response is delayed (Valko et al., 2006). Reactive aldehydes like malondialdehyde (MDA) are produced when peroxyl and alkoxyl radicals undergo further cyclization, depending on the kind of polyunsaturated fatty acid. MDA is a byproduct of biofilm lipid peroxidation and free radicals. Its composition can both directly and indirectly indicate the level of peroxidation of the tissue and the degree of cell damage brought on by oxygen-free radicals (Ramana *et al*., 2014).

II.6 Antioxidative mechanisms

Biological reductants convert 4-5% of the oxygen in the human body into ROS. In essence, ROS attack is constant in cells with higher partial pressures of oxygen, but it is countered by the coordinated activity of an advanced antioxidant defense mechanism (Bandyopadhyay *et al*., 1999).

The body has two types of natural defenses against free radicals: enzymatic and non-enzymatic. It is believed that enzymatic antioxidants are more effective. Superoxide dismutase's (SODs), catalase (CAT), glutathione peroxidase (GPx), and other enzymes are among the most significant antioxidants. The most significant low molecular weight antioxidants include glutathione, vitamin C, vitamin E, carotenoids, and flavonoids (Halliwell, 2006).

II.6.1 Antioxidant enzymes

A class of proteins called antioxidant enzymes, also known as metalloproteins, catalyze the conversion of ROS and/or their byproducts into more stable, generally less toxic species. Antioxidant enzymes are a crucial line of defense against oxidative stress brought on by reactive oxygen species, which damages every component of the cell (José, 1999). The three most significant antioxidant enzymes, which are essential for redox reactions, are superoxide dismutase, catalase, and glutathione peroxidases.

II.6.1.1 Superoxide dismutase (SOD)

Irwin Fridovich discovered SOD in 1967, where he founds that humans contain three primary types of superoxide dismutase: EC-SOD (SOD3), Mn-SOD (SOD2), and Cu, Zn-SOD (SOD1) (McCord & Fridovich, 1969).

The poisonous $O_2^{\bullet 2}$ is neutralized more quickly with SOD through catalyzing the subsequent reaction:

$$2O_2 + 2H+ \rightarrow H2O2 + O_2.$$

Aerobic organisms all include SOD. In comparison to the rate of synthesis without SOD, SOD catalyzes a 10.000X increase in the rate of hydrogen peroxide generation. Consequently, $O_2^{\bullet 2}$ is absent from the cell. The metal cofactors at the active site of SOD metalloenzymes that are engaged in the redox process are used to categorize them into several groups. Enzymes can incorporate different metals, although doing so renders them inactive (Alessio & Hagerman, 2006).

Mammals, for instance, include three different forms of SOD: extracellular SOD (SOD3), manganese containing SOD (SOD2), located in the mitochondrial matrix, and copper–zinc containing SOD (SOD1), which is found in the cytoplasm. Superoxide dismutase (MnSOD) functions as a major regulator of the $O_2^{\bullet -2}$ concentration within the mitochondrial matrix. Copper–zinc superoxide dismutase is located in the intermembrane (CuZnSOD) and reduces $O_2^{\bullet -2}$ to H_2O_2. The highest expression of SOD takes place in the renal tubules of healthy kidneys(Hitchler *&* Domann, 2014; Robb *et al*., 2014).

II.6.1.2 Catalase

One of the most significant antioxidant enzymes found in aerobic organisms is catalase. It comes from both prokaryotic and eukaryotic organisms that have been isolated. Two molecules of hydrogen peroxide are broken down by catalase into two molecules of water and one oxygen molecule (Nandi *et al*., 2019). In circumstances linked to oxidative stress, such as inflammation, mutagenesis, and the inhibition of apoptosis, catalase is essential

II.6.1.3 Glutathione peroxidase

A family of enzymes known as glutathione peroxidase has an active site including a selenocysteine residue and peroxidase activity. Lipid hydroperoxides are reduced to alcohols and hydrogen peroxide to water by GPx (Lubos *et al*., 2011).

The GPx family includes a number of isoenzymes, the most abundant of which is glutathione peroxidase-1 (GPx-1) with hydrogen peroxide as its substrate. In the intracellular environment, GPx-1 functions to sustain the physiological concentration of hydrogen peroxide, a critical component of mitochondrial activity, thiol redox homeostasis, and signal transduction(Brigelius-Flohé *&* Maiorino, 2013).

II.6.2 Antioxidant non-enzymes

An essential line of defense for cells against oxidants is represented by low molecular weight antioxidants. Antioxidants with low molecular weight stop the chain reaction of radicals. A

portion of the water-soluble low molecular weight antioxidants functions in the cytosol or cytoplasmic matrix. Another class of lipid-soluble antioxidants functions within the membranes.

II.6.2.1 Vitamin C

The primary antioxidant in plasma and cells is vitamin C, often known as ascorbic acid. The majority of mammalian species' livers can produce it from glucose, but humans are unable to do so, hence ingestion is necessary to prevent the potentially deadly scurvy. Fresh fruits and vegetables are a good source of vitamin C. Vitamin C stops other chemicals from oxidizing by giving them an electron (Kaźmierczak-Barańska *et al*., 2020).

II.6.2.2 Vitamin E

The molecule known as vitamin E is made up of eight different compounds, including α-tocopherol, which is vitamin E itself, β-tocopherol, γ-tocopherol, and δ-tocopherol. As a fat-soluble vitamin, vitamin E (α-Tocopherol) protects cellular membranes from ROS-induced lipid peroxidation. Vitamin E deficiency is linked to neurological issues (Joshi *&* Praticò, 2012).

II.6.2.3 Glutathione

An essential antioxidant found in plants, animals, and microbes is glutathione. Glutathione shields cells from ROS, such as heavy metals, lipid peroxides, free radicals, and peroxides. By enzymatic and non-enzymatic reactions glutathione can scavenge RO. The non-enzymatic antioxidant activity is contributed by the free thiol group of glutathione. Glutathione is essential for controlling the redox state of the cell, particularly through controlling the correct tertiary structure of proteins by thiol-disulfide exchange that occurs simultaneously with glutaredoxin and protein disulfide isomerases (Lushchak, 2012; Valko *et al*., 2006).

II.6.2.4 Carotenoids

Plants, microbes, and algae all contain red, orange, and yellow pigments called carotenoids (Car). Nature has about a thousand different types of carotenoids. Many of them show promise in preventing age-related muscle degeneration, cancer, atherosclerosis, macular degeneration, and other illnesses. A rotenoids' antioxidant qualities are associated with conjugated double bonds,

which have the capacity to accept unpaired electrons and widely delocalize them across the conjugated double bond system. Carotenoids have the ability to effectively scavenge radicals that include superoxide anion (O2·–), hydroxyl (·OH), alkoxyl (RO·), and peroxyl (ROO·). The primary antioxidant protective function of carotenoids is to shield membranes from damage brought on by ROS(Mortensen *et al*., 2001) .

II.6.2.5 Polyphenol

Polyphenols, which are often referred to as polyhydroxyphenols, are identified by the presence of several phenol structural units (Nascimento-Souza *et al*., 2018). The quantity and qualities of these phenol structures add to the special chemical, physical, and biological properties of polyphenol compounds. In summary, polyphenols are secondary metabolites that are made by plants and are commonly present in fruits and vegetables to shield themselves from UV rays (Zbikowska *et al*., 2016).

II.6.2.6 Flavonoids

Flavonoids' antioxidant qualities, which are demonstrated by their capacity to effectively chelate redox metal ions and end free radicals, are responsible for most of their health benefits in the prevention of chronic diseases. Furthermore, flavonoids have the ability to stimulate the production of antioxidant enzymes and regenerate certain vitamins, such as vitamin E, that have a higher capacity for electron reduction (Simunkova *et al*., 2019).

II.6.2.7 Melatonin

Melatonin is a hormone that is primarily produced in the pineal gland of mammals from serotonin. It is also present in the retina, lymphocytes, gastrointestinal system, and bone marrow (Mahmood, 2019). It is widely distributed and present in foods like yeast, oats, and other plants. It can easily pass through the blood-brain barrier and neutralize HO· and peroxyl radicals, $CO^{3\cdot}$, $\cdot NO_2$, $O^{2\cdot}$, and HOCl in both aqueous and lipid phases. Since melatonin is an antioxidant, it cannot be reduced after it has irreversibly oxidized. It is therefore called a terminal or suicidal antioxidant (Minich *et al*., 2022).

II.6.2.8 Trace elements

Trace elements, such as copper (Cu), zinc (Zn), and selenium (Se), function as cofactors of enzymes that are critical in the battle against free radicals.

II.6.2.8.1 Selenium

Natural sources of selenium (Se), a trace mineral, include a variety of foods and dietary supplements. Selenoproteins are a class of proteins that contain selenium as an important component (Zhang *et al*., 2020). Selenoproteins are necessary for the thyroid gland and reproductive systems to operate properly. Se also shields cells against infections and damage brought on by ROS. The antioxidant properties of seleniproteins are well-known, especially in relation to peroxides. People who consume a lot of selenium have a 30% lower risk of developing cancer. Selenoproteins are responsible for the good operation of the cardiovascular system because they shield cell membranes from damage caused by ROS and maintain the integrity of blood platelets. Studies using observational data showed that a higher risk of cardiovascular disease is linked to both high and low Se consumption (Vinceti *et al*., 2018).

II.6.2.8.2 Zinc and Copper

Zinc (Zn) and copper (Cu) are strong antioxidants engaged in cellular defense against oxygen-free radicals. The risk of deficiency in these two micro-nutrients seems likewise to increase in proportion to age. Specifically, an isoform of SOD has been discovered and demonstrated to have zinc and copper at its catalytic site. Consequently, Zn and Cu are regarded as necessary for the proper functioning of an organism. In addition to being a co-factor of SOD, Zn is necessary, as a functional component, in more than 200 enzymes and transcription factors. Moreover, it helps protect against vitamin E deficiency and stabilizes membranes. Structure and preservation of metallothionein's' tissue concentrations, a potent free radical scavenger (Sfar *et al*., 2009).

II.7 Consequences of oxidative stress

At high concentrations, the effects of ROS become deleterious to cells, tissues, and various physiological functions. Adverse effects of ROS are associated with disturbances in redox status.

II.7.1 Oxidative DNA damage and nucleic acid oxidation

The bases that form DNA, and particularly guanine, are sensitive to oxidation. The free radical attack can be direct and leads to the oxidation of the bases, generating a large number of modified bases: 8 oxo-guanine, 8 nitro-guanine. This can also attack the bond between the base and the deoxyribose, creating an abasic site, or attack the sugar itself, creating a single-strand chain break. Indirect damage can result from the attack of lipids whose peroxidation generates mutagenic aldehydes, forming adducts on the basis of DNA such as MDA guanine or Ethen derivatives. The radical attack of proteins, which are very numerous in contact with DNA to protect it (histones) or to read it (enzymes and factors of replication or transcription), leads to the bridging of proteins or adducts on lysinoguanine bases (Favier, 2003).

II.7.2 Oxidative damage of proteins

The proteins most susceptible to free radical attack are those with a sulfhydrile group. This is the case for many cellular enzymes and transport proteins, which are oxidized and inactivated.

Oxidized proteins also become highly hydrophobic, either by deletion of ionizable amine groups or by externalization of central hydrophobic zones. They will then form abnormal clumps in or around the cells. These clumps, together with lipids, form the lipofuschine deposits characteristic of the tissues of elderly subjects(Favier, 2003).

II.7.3 Oxidative damage to lipids

At the cellular level, ROS and NRS are capable of causing damage; either lipid peroxidation, protein oxidation, or DNA mutations. These alterations can lead to loss of function and integrity, or even cell death, particularly through apoptosis (programmed cell death). ROS also initiates apoptosis by activating the opening of the permeability transition pore. (Favier, 2003). During this reaction, lipids and mainly their polyunsaturated fatty acids are the preferred target of attack by ROS and RNS, which are able to tear hydrogen from the carbons located between two double bonds, to form a conjugated diene radical, oxidized to a peroxyl radical.

Chapter III:

Aging and Oxidative Stress

III. Aging and oxidative stress

III.1 ROS in aging and age-related diseases

Individual differences exist in the aging mechanisms that are influenced by genetics (25%) and environmental factors (75%). Aging and the deterioration of metabolic health are closely related to increased oxidative stress (Passarino *et al*., 2016). They include aging-related hearing loss, which primarily affects the elderly, among others (Tetsuya Oishi, 2020).

Moreover, studies have shown that the effective action of ROS plays an important role in the aging process by triggering many age-related diseases through oxidative stress (Figure 5). such as diabetes, neurological diseases, cardiovascular diseases (CVD), chronic obstructive pulmonary disease, chronic kidney disease, neurodegenerative diseases, and cancer (Bonomini *et al*., 2015).

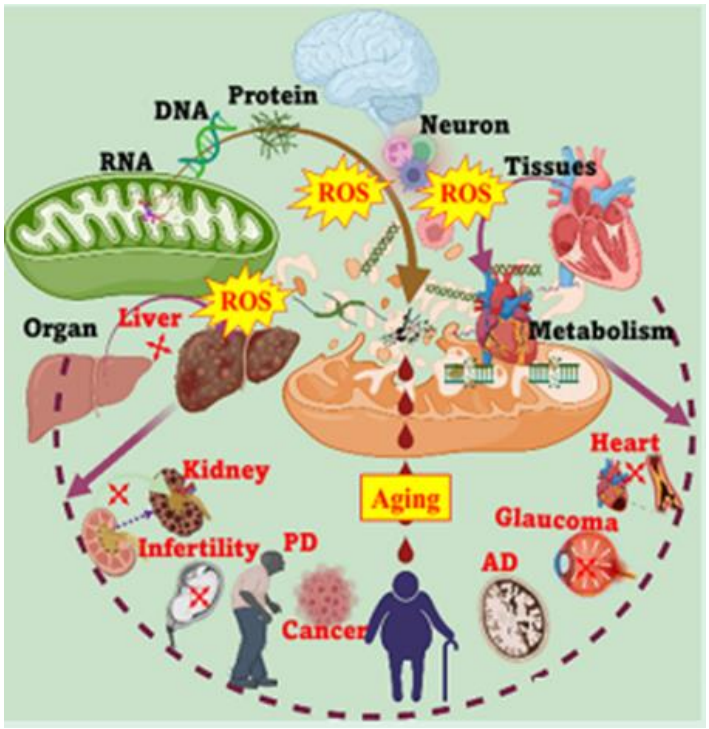

Figure 4. Relationship between ROS in aging and age-related diseases (Anik *et al*., 2022)

Thus, it seems logical to talk about these diseases and conditions associated with aging in light of ROS factors. These factors will be discussed specifically in this section.

III.2 Neurological diseases

The quality of life and longevity of aging populations are both impacted by neurodegenerative illnesses. The effects on senior citizens of all types of neurodegenerative disorders are varied. Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, and increasing memory loss are among the brain disorders that pose the greatest threat(Zhao & Zhao, 2013).

III.2.1 Alzheimer's Disease

Worldwide, Alzheimer's disease is regarded as one of the deadliest neurodegenerative diseases that affect people over the age of 60 (Birla *et al*., 2020). The primary causes of AD are a variety of genetic, environmental, and behavioral variables that influence the aging-related degeneration of neuron cells. Oxidative stress caused by ROS is involved in several pathophysiology's and is crucial in AD (Reichert et al., 2020). Ferroptosis is the word for the iron-dependent cell death caused by the buildup of lipid-based ROS. It has been proposed as the essential driver of AD neuronal cell death. The protein Δ133p53 prevents cell senescence by upregulating the production of antioxidant genes. The accumulation of amyloid-beta (Aβ) peptides in the mitochondria, which causes ROS generation to be boosted by metal ions like Fe^{2+} and Cu^{2+}, is one of the main signs of AD. Accordingly, oxidative stress may be brought on by lipid peroxidation, protein oxidation (DNA/RNA), carbohydrate modification, and the production of free radicals by Aβ alone (Zhao *et al*., 2021).

III.2.2 Parkinson's Disease

Parkinson's disease is another frequent neurological condition that causes inclusion bodies (Lewy bodies) of α-synuclein to accumulate in the substantia nigra and dopaminergic neurons (which are involved in learning, memory, and motor control) to degenerate. The redox imbalance and increased oxidative stress brought on by quinine production from changes in dopamine metabolism and synthesis are what cause oxidative damage to neurons (Dias *et al*., 2013; Rosik *et al*., 2020).

III.3 Diabetes Mellitus

Public health specialists are increasingly concerned about diabetes, a collection of metabolic disorders brought on by insulin or pancreatic failure. Diabetes can result in numerous life-threatening illnesses. Diabetes difficulties arise from oxidative stress, which causes malfunction of pancreatic β-cells and ATP generation that control insulin metabolism. Moreover, oxidative phosphorylation impairment accelerates β-cell failure by downregulating ATP generation, which is another way that inflammation in the pancreatic islets contributes to oxidative degradation (Wu *et al*., 2018).

III.4 Cardiovascular diseases

According to WHO, cardiovascular diseases are the leading cause of death worldwide and have multiple etiological factors (Benjamin *et al*., 2017). Atherosclerosis and blood vessel remodeling, which impede blood flow, are two of the main causes of CVD. Numerous additional illnesses are associated with cardiovascular disorders, with the primary ones being heart failure, vascular disorders, coronary artery diseases, hypertension, and other illnesses. Another risk factor is aging, as it raises the prevalence of cardiovascular illnesses mostly because of the build-up of oxidative damage (Dubois-deruy *et al*., 2020).

III.5 Hypertension

About one billion individuals globally, or more than 25% of the world's adult population, suffer from hypertension, a major cause of mortality (Nadar & Lip, 2021). Rising ROS production causes the production of ONOO, which depletes NO and causes endothelium-dependent vasodilation to decline and hypertension to occur within the vascular wall. ROS are created by vascular smooth muscle cells and endothelial cells (Mohamed *et al*., 2020; Varzideh *et al*., 2022).

Experimental Part

Chapter I:

Materials & Methods

I. Materials and methods

I.1 Recruitment and type of study

The main aim of this study was to gain insight into the relationship between oxidative stress and the process of aging. The research involved two distinct groups: the target group consisting of individuals aged 60 years and above, and the control group of younger people. The investigation took place at the Boubekeur Khaled Health Centre in Tiaret province. The experiments were conducted at the biochemistry laboratory within the Natural and Life Sciences Faculty, Ibn Khaldoun University, Tiaret. Each participant's age, weight, height, and body mass index (BMI) were documented, along with their medical history indicating the presence or absence of chronic illnesses and the usage of medicines.

I.2 Volunteer information

There was a total of 61 volunteers included in the study, consisting of 25 males and 36 females. The age range for males was 20 to 59, while for females it was 60 to 91.

The exclusion criteria for participation included individuals under the age of 20, recent history of trauma, and current or recent viral or bacterial infections.

I.3 Blood samples and sample preparation

The elbow vein was used to draw blood samples, which were then collected in EDTA and heparinized tubes. The plasma was collected by centrifugation at 3000 tr/min for 10 minutes and stored in Eppendorf's tubes labeled for the determination of plasma markers of oxidant/antioxidant status (Vitamin C, MDA, and dismutase superoxide). The remaining precipitate was washed 3 times with physiological water, then lysed with 2 volumes of ice-cold distilled water and incubated in the refrigerator (4°C) for 15 minutes. Cellular debris was subsequently removed by centrifugation at 4000 tr/min for 10 minutes. Finally, red blood cell lysates were obtained to study the parameters of the oxidative/antioxidant status of red blood cells (MDA and catalase activity).

I.4 Biochemical Analyses

I.4.1 Glycemia measurement

An enzyme approach based on colorimetry was used to measure plasma glucose **(DIAGNO PHARMA kits DIAGNO-Glu, Algeria)**. An enzyme known as glucose oxidase converts glucose to hydrogen peroxide and gluconic acid. When phenol and peroxidase are present, hydrogen peroxide oxidizes 4-aminoantipyrin, a colorless chromogen, to produce a red dye with a quinonimine structure. The produced product is tested for absorption at 505 nm, and the staining intensity is correlated with the glucose concentration.

I.4.2 Total cholesterol measurement

The determination of plasma total cholesterol was carried out by an enzymatic method **(DIAGNO PHARMA kits DIAGNO-Chol, Algeria)**. The reaction is to release cholesterol from the ester bond by cholesterol-esterase, and to oxidize free cholesterol not esterified by cholesterol-oxidase. The indicator is a quinonimine formed from hydrogen peroxide, 4-aminophenazone, under the catalytic action of peroxidase. The concentration of colored quinonimine was measured at 505 nm, it is proportional to the concentration of total cholesterol.

I.4.3 Triglycerides measurement

Plasma triglycerides were measured by an enzymatic method (**DIAGNO PHARMA kits DIAGNO-TG, Algeria**). Triglycerides are measured after enzymatic hydrolysis by lipases to glycerol and free fatty acids. The indicator is a quinonimine formed from hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol under the catalytic action of peroxidase. The triglyceride level was determined at a wavelength of 505 nm. The concentration of quinonimine is proportional to the total concentration of triglycerides

I.4.4 High-density and low-density lipoproteins assays

The determination of plasma high-density lipoproteins (HDL) was performed by enzymatic method after precipitation of very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) according to the protocol given by the DIAGNO PHARMA kits DIAGNO-HDL, Algeria. Plasma VLDL and LDL lipoproteins were precipitated by the phosphotungstate reagent in the presence of magnesium ions. After centrifugation, the supernatant contains HDL lipoproteins.

HDL cholesterol was determined at a wavelength of 505 nm. LDL cholesterol was calculated according to the following relationship:

$$\text{LDL} - \text{C} = \text{Total Cholesterol} - \text{HDl} - \text{C} - \left({}^{\text{TG}}/_{5}\right)$$

I.5 Markers of oxidant/antioxidant status

I.5.1 Oxidant status

I.5.1.1 Malondialdehyde assay

The Malondialdehyde assay was prepared according to the method described by Draper & Hadley (1990). MDA measurement was conducted under acidic conditions and at a temperature of 100°C. A biochemical method was employed to measure plasma and erythrocyte malondialdehyde. This method is widely utilized as a marker of peroxidation due to its straightforwardness and the sensitivity of the assay. Following treatment with hot acid, aldehydes interact with thiobarbituric acid (TBA) to generate a chromogenic condensation product consisting of two TBA molecules and one MDA molecule. This complex exhibits absorption at 530 nm.

The MDA concentration was calculated using the extinction coefficient of the MDA-TBA complex:

$$\epsilon = 1.56 \times 10^5 \text{ mol}^{-1}.\text{l}.\text{Cm}^{-1} \text{ at } 532 \text{ nm}$$

The concentration of MDA was calculated using the extinction coefficient, by the following equation: $\text{C} = {}^{\text{DO}}/_{\epsilon}$ (results are expressed in µmol/L)

I.5.2 Markers of antioxidant Status

I.5.2.1 Vitamin C assay

To determine the level of vitamin C in plasma, a combination of ascorbic acid within a specified range and Folin's reagent was employed. The process, based on the method of Jagota & Dani (1982), involved incubating the diluted Folin ciocalteau staining reagent with the remaining liquid after centrifugation and precipitation of plasma proteins using trichloroacetic acid (TCA). When vitamin C is present in the plasma, Folin's reagent changes color to yellow. The intensity of the staining, observed at a wavelength of 769 nm, directly corresponds to the quantity of vitamin

C in the sample. By utilizing an ascorbic acid solution as a reference, the concentration was determined using a standard curve.

I.5.2.2 Determination of catalase enzyme activity (EC: 1.11.1.6)

Catalase, a tetrameric enzyme containing a heme group, facilitates the breakdown of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). Catalase is present in most eukaryotic and prokaryotic organisms. The Claiborne (1985) method is employed to determine the activity of catalase in erythrocytes. This method involves observing the decrease in absorbance of an H_2O_2 solution at 25°C due to the presence of catalase in the enzymatic source. The process is monitored by continuously measuring the change in absorbance at 240 nm over a period of five minutes, with readings taken at the start (T0) and after five minutes (T5).

The catalase activity is calculated using the following formula:

$$Catalase\ activity\ (\mathrm{IU/mg}) = K/n$$

$$K = {}^{2.303}/_{T} \times logA_0/A_5$$

Where:

K: Reaction rate consent

T: Interval time (5 min)

A0: Absorbance at T0

A5: Absorbance at T5

n: mg of protein in mg present in the volume of the sample.

The catalase activity is expressed by IU/mg protein: μmol H_2O_2 consumed/min/mg protein.

I.5.2.3 Superoxide Dismutase Assay

The colorimetric method using the phenol reagent was employed to detect the presence of superoxide dismutase in both plasma and erythrocytes. This method, described byMarklund S (1974), relies on the ability of SOD to inhibit the autooxidation of phenol. Measurements were taken every minute for a duration of five minutes at a wavelength of 270 nm, denoted as DO0 to DO5. The concentration of SOD in erythrocytes and plasma was calculated using the following formula, expressed in mM/min/ml.

$$\mathrm{SOD} = \left(50 \times {}^{\mathrm{Y}}/_{5}\right) \times 10$$

Where:

$$Y = {}^{(50mM \times DO_5)}\Big/_{DO_0\ (mM)}$$

I.6 Statistical Analysis

The statistical comparison was performed using the student's t-test. The quantitative variables are presented as mean ± standard error. Values marked with asterisks indicate significant differences between elderly and young individuals as follows: Not significant: P>0.05, significant: P <0.05 (*), highly significant: P <0.01 (**), very highly significant: P< 0.001(***).

The qualitative variables are expressed as percentages (%).

Chapter II:

Results

II. Results

II.1 Study characteristics

This study involved the recruitment of 61 individuals from diverse areas of the city of Tiaret. The table below presents the investigated characteristics of this specific sample, including age, height, weight, gender, and BMI (Table 1).

Table 1. Characteristics of sample population

Characteristics	Elderly	Young
Age	69.13 ± 8.03***	38.10 ± 10.68
Gender (women/ men)	16/15	20/10
Height (m)	1.67 ± 0.05	1.71 ± 0.05
Weight (Kg)	69 ± 5.87	75.75 ± 11.67
BMI (Kg/m^2)	24.84 ± 3.18	26.14 ± 5.04

Values are expressed in the following format: mean ± standard deviation. Values marked with asterisks indicate significant differences between elderly and young individuals as follows: Not significant: P>0.05, significant: P <0.05 (*), highly significant: P <0.01 (**), very highly significant: P< 0.001(***).

The age values exhibited significant differences between the two groups, whereas the height, weight, and BMI values did not show any significant differences **(Table 1)**.

II.2 Biochemical parameters

The biochemical parameters obtained from the elderly and young groups are summarized in Table 2.

Table 2. Biochemical parameters in both groups

Parameters	Elderly	Controls	Reference values (g/l)
Glucose	1.03 ± 0.03	0.84 ± 0.01	0.70-1.10
Total Cholesterol (g/l)	1.51 ± 0.04	1.72 ± 0.04	1.5-2.5
Triglycerides (g/l)	0.91±0.04	0.85±0.03	0.40-1.60
High-density (HDL) (g/l)	0.48±0.007	0.49±0.010	> 0.30
Low-density (LDL) (g/l)	1.21±0.52	1.40±0.62	<1.30

Values are expressed in the following format: mean ± standard deviation. Values marked with asterisks indicate significant differences between elderly and young individuals as follows: Not significant: P>0.05, significant: P <0.05 (*), highly significant: P <0.01 (**), very highly significant: P< 0.001(***).

The results of the statistical analysis of the variations in the lipid profile of the two groups are presented in the table above. The study did not show any statistically significant differences. Cholesterol levels were found to be significantly higher in young people compared to older ones. Triglyceride (TG) levels were slightly higher in controls compared to cases.

II.3 Oxidant/antioxidant status in the elderly and young people

II.3.1 Markers of antioxidant status in elderly and young people

The mean vitamin C value in the elderly population was 22.88 ± 0.58 mg/ml, whereas the mean vitamin C value in the young population was 19.27 ± 0.31 mg/ml. There were no significant differences observed in the vitamin C values between the elderly and young populations (P = 0.28) **(Figure 5)**.

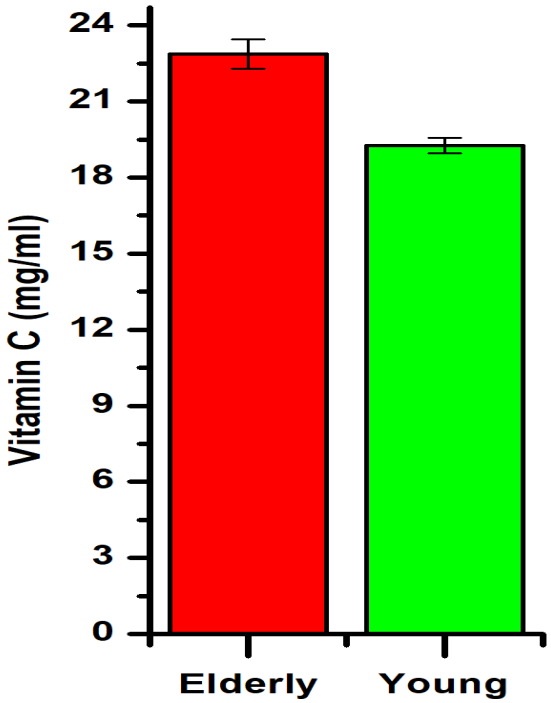

Figure 5. Plasma vitamin C levels in elderly people and young

Values are expressed in the following format: mean ± standard deviation. Values marked with asterisks indicate significant differences between elderly and young individuals as follows: Not significant: P>0.05, significant: P <0.05 (*), highly significant: P <0.01 (**), very highly significant: P< 0.001(***).

There was a significant decrease in catalase activity among elderly individuals compared to the young population **(Figure 6)**. However, both plasma and erythrocyte superoxide dismutase activities were similar in both groups **(Figure 7)**.

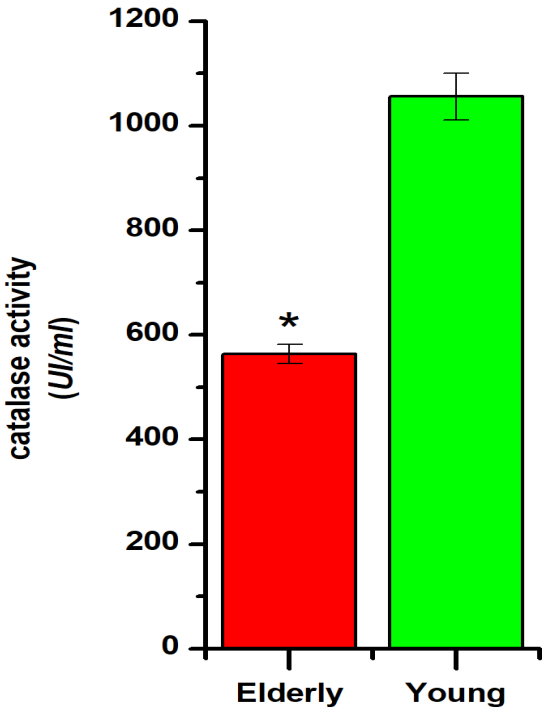

Figure II6. Erythrocyte catalase activity in elderly people and young

Values are expressed in the following format: mean ± standard deviation. Values marked with asterisks indicate significant differences between elderly and young individuals as follows: Not significant: P>0.05, significant: P <0.05 (*), highly significant: P <0.01 (**), very highly significant: P< 0.001(***).

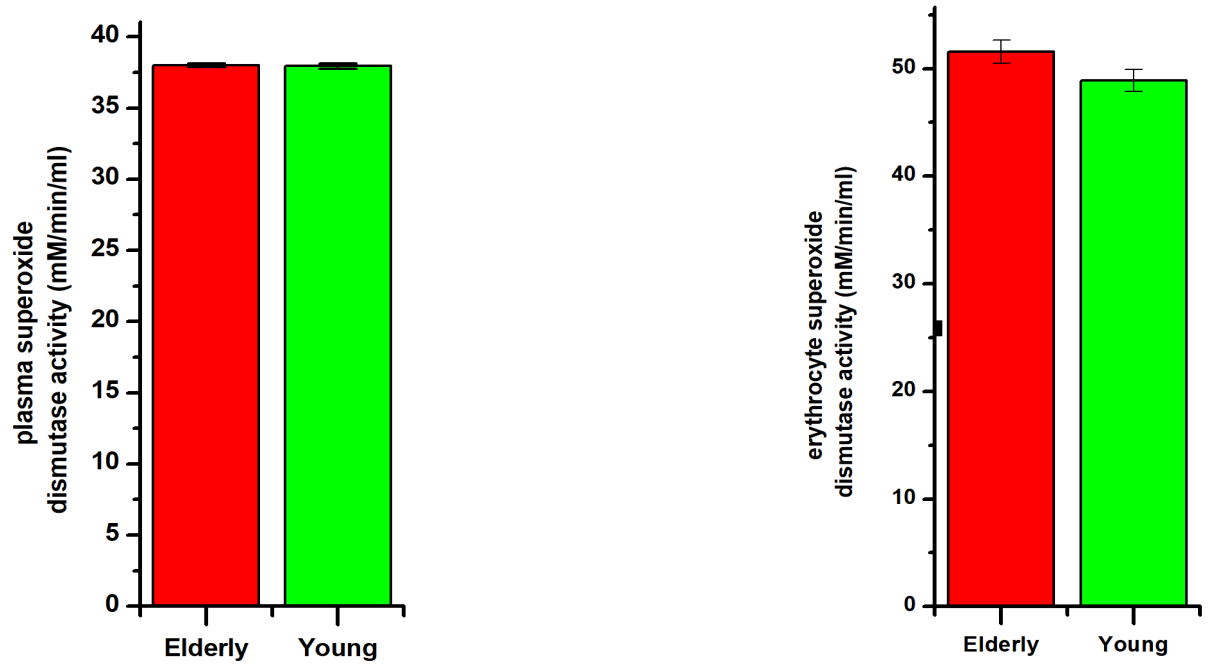

Figure 7. Erythrocyte and plasma superoxide dismutase activity in elderly people and young people

Values are expressed in the following format: mean ± standard deviation. Values marked with asterisks indicate significant differences between elderly and young individuals as follows: Not significant: P>0.05, significant: P <0.05 (*), highly significant: P <0.01 (**), very highly significant: P< 0.001(***).

II.3.2 Markers of oxidative status in elderly and young people

Our findings suggested that there were no significant differences observed in plasma MDA contents between the two groups **(Figure 8)**. However, there was a significant increase in erythrocyte MDA contents among elderly individuals compared to the young population **(Figure 9)**.

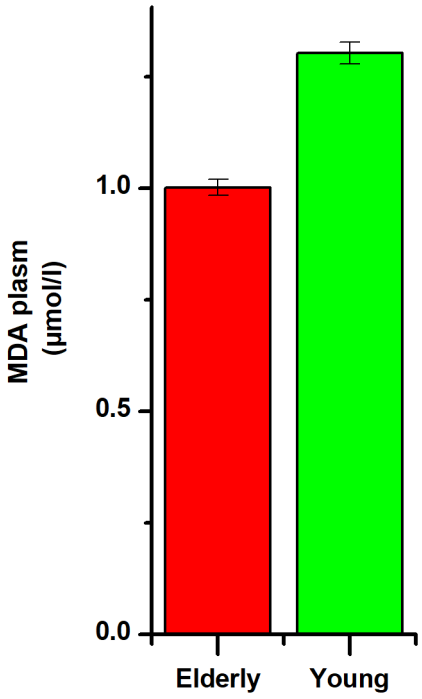

Figure 8. Plasma MDA level in elderly people and young people

Values are expressed in the following format: mean ± standard deviation. Values marked with asterisks indicate significant differences between elderly and young individuals as follows: Not significant: P>0.05, significant: P <0.05 (*), highly significant: P <0.01 (**), very highly significant: P< 0.001(***).

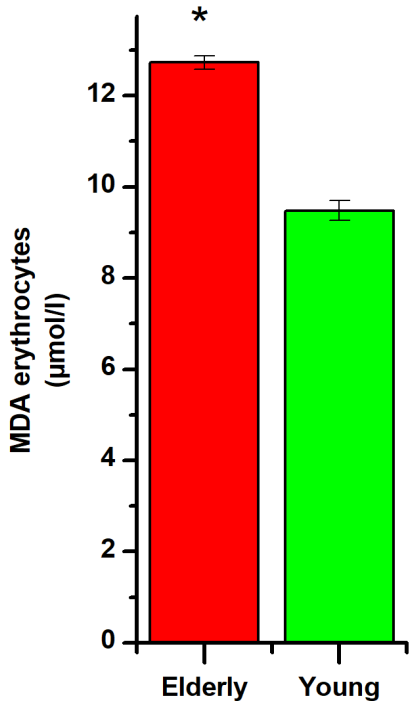

Figure 9. Erythrocyte MDA level in elderly people and young people

Values are expressed in the following format: mean ± standard deviation. Values marked with asterisks indicate significant differences between elderly and young individuals as follows: Not significant: P>0.05, significant: P <0.05 (*), highly significant: P <0.01 (**), very highly significant: P< 0.001(***).

Chapter III:

Discussion

III. Discussion

In the present study, the markers of oxidative stress were evaluated and assessed. The analysis included the assessment of oxidant and antioxidant status; vitamin C plasma levels, antioxidant enzymes activities such as catalase and superoxide dismutase, and oxidant products such as plasma and erythrocyte MDA contents. It is commonly recognized that a build-up of reactive oxygen species can play a role in the aging process and the etiology of various age-related illnesses, such as neurodegenerative diseases, chronic obstructive pulmonary disease, chronic kidney disease, and cardiovascular diseases (Liguori *et al*., 2018).

The two most significant low molecular weight antioxidants that the human body is unable to produce are ascorbic acid (vitamin C) and tocopherol (vitamin E)(Podda *et* Grundmann-Kollmann, 2001). Ascorbic acid functions are various; it plays a role as a cofactor in numerous enzyme-catalyzed processes, including those that preserve the integrity of connective and vascular tissue, improves collagen production and iron absorption, controls the activity of leukocytes and hematopoiesis, neuroprotection, and the hydroxylation of lysine and proline (Spector et Johanson, 2014). In this study, vitamin C levels were measured in elderly and young people. There were no significant differences observed in the vitamin C values between the elderly group and young group. The diet high in vitamin C among the elderly population could potentially be responsible for this result.

The most popular marker for evaluating lipid peroxidation is MDA. In this study, our findings indicated that there was a significant increase in MDA levels in erythrocytes among elderly individuals compared to the young population. Results are consistent with those obtainable by Boarescu *et al*. (2022) and Danilova *et al*. (2021) which showed an increase in MDA in elderly people with hypertension compared to controls. This increase may be explained by increased peroxide levels in tissue fat and decreased antioxidant capacity (Boarescu *et al*., 2022). Lipid peroxidation is characterized by several indicators. At 234 nanometers, conjugated dienes are easily quantified. The occurrence of lipid peroxidation can be inferred from their detection in particular. Without a question, the most well-known and frequently utilized is the MDA. It is the outcome of hydroperoxides breaking down produced when polyunsaturated fatty acids undergo

peroxidation. There is a noteworthy positive association found between the subjects' ages and MDA (Bonnefont-Rousselot, 2007).

Superoxide dismutase, or SOD, is an enzyme that is part of the first line of defense against an excess of free radicals. It transforms the superoxide anion radical into an oxygen molecule and hydrogen peroxide (Polak-Szabela *et al*., 2021). There are three different kinds of SOD: extracellular SOD3 (Cu-Zn), mitochondrial SOD2 (MnSOD), and red blood cells' SOD1 (CuZnSOD) (Zelko *et al*., 2002).

According to the results we found, the activities of plasma and erythrocyte superoxide dismutases are similar in both groups. However, another study conducted by **Huang *et al*. (2022)** have shown that the activity of superoxide dismutase is significantly low in older men and women compared to young people especially the elderly with hypertension.

A number of researches have been conducted to demonstrate the deficiency of antioxidant enzyme systems with age, specifically the enzyme superoxide dismutase. Moreover, it was observed that in cases of hypertension, SOD activity showed a clear increase and decrease (Bonnefont-Rousselot, 2007).

Catalase (E.C. 1.11.1.6) is a crucial enzyme that acts as an antioxidant. Almost all aerobic organisms include it. It is responsible of the decomposition of two molecules of hydrogen peroxide into one oxygen molecule by preventing such damage to the pancreatic β cells from hydrogen peroxide (Habib *et al*., 2010).Numerous illnesses, including diabetes mellitus, vitiligo, cardiovascular diseases, Wilson disease, hypertension, anemia, certain dermatological disorders, Alzheimer's disease, and others are linked to catalase shortage or malfunction(Nandi *et al.*, 2019).

In this study there was a significant decrease in catalase activity among elderly individuals compared to the young population. This is due to the presence of diseases in the elderly group (hypertension, diabetes, cardiovascular diseases and thyroid disease) compared to young people.

For older people, the normal blood sugar level was (1.03 ± 0.03) compared to controls (0.84 ± 0.01), signifying that the elderly of our study did suffer from diabetes. For the Total Cholesterol (g/l), Triglycerides (g/l), High-density (HDL) (g/l), all values were within the norms.

However, Low-density (LDL) (g/l) was found to be slightly high for the younger population. It appears that the group we studied did not have many diseases.

Conclusion

Conclusion

The research on aging and reactive oxygen species has made significant strides, clearly establishing a link between ROS and the aging process. Although aging is not classified as a disease, it contributes to age-related disorders and shares common causes with them, such as oxidative stress. Oxidative stress results from an imbalance between ROS and the body's antioxidant defense system, leading to irreversible cellular damage. ROS are highly reactive oxygen species that can cause significant cellular damage, ultimately contributing to aging.

Our findings highlight that oxidative stress is prevalent in older individuals, revealing metabolic and physiological changes associated with aging. This is characterized by elevated levels of oxidant markers, such as malondialdehyde in red blood cells, and a decrease in antioxidant defenses like catalase and superoxide dismutase in older adults with diseases, compared to younger controls. High levels of oxidants and a weakened defense system can accelerate aging and induce conditions like high blood pressure. Therefore, oxidative stress is a key factor in exacerbating severe aging and related diseases.

In conclusion, this research has significantly deepened our understanding of oxidative stress in the elderly. In order to take our findings to the next level, detailed in-vivo and in-vitro studies can be conducted to further investigate the oxidative stress in elderly. In-dept evaluation of the effect of diet on age-related diseases caused by oxidative stress in elderly.

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Appendices

Appendices

Appendix 1: Study questionnaire

Section 1: General details

Date: …… |__| |__| |_____|

Serial number: ……... |_____|

1. Volunteers details:

- First name: ……Family name: …… Sex: M=1, F=2 …|___|
- Addressee: …….
- Age (years): …… |____|
- Weight (kg): ……. |__|__|__|
- Height (cm): …… |__||__|__|
- Body mass index: ……. |____|

2. Particular habits

- Smoker: Yes (1), No (2) …… |___|
- Number of cigarettes per day: ……. |___|
- Do you drink alcohol? Yes (1), No (2) ..... |___|
- If yes, for how long: ……... |___|
- 3. Medical history:
- Do you suffer from chronic diseases? Yes (1), No (2)……...|____|
- If yes, what are they…….....|_____|
- Has anyone in your family suffered from chronic diseases: Yes (1), No (2)……...|____|
- If yes, please list them…….....|_____|

Section 2: knowledge of aging and oxidative stress

- To what extent do you consider yourself informed of the process of aging?
    a. not at all
    b. slightly informed
    c. moderately informed
    d. very informed
- Have you ever heard of oxidative stress? Yes (1), No (2)…… |___|
- If yes, can you briefly explain your understanding of the term "oxidative stress"?

Section 3: Personal experience of oxidative stress

- Have you ever been diagnosed with diseases associated with oxidative stress like cardiovascular diseases, neurodegenerative diseases, etc.)? Yes (1), No (2)…… |___|
- Do you have any family diseases linked to oxidative stress? Yes (1), No (2) …… |___|

Section 4: Life Habits

1. Food habits:

- Describe a typical meal for you……
- Do you have any food preferences or restrictions? ……
- Do you regularly have home-made meals or takeaways? ……
- How often do you take meals containing proteins, vegetables, fruits, and cereals……

2. Liquids consumption:

- How often do you drink water per day? ……
- Do you have drinks containing sugar or caffeine? ……
- Do you consume dairy products? If so, what are they, and how often? ……
- How often do you exercise?
    a. never
    b. rarely
    c. from time to time
    d. regularly

Section 4: evaluation of the well-being and lifestyle

- How do you evaluate your well-being?
    a. very bad
    b. bad
    c. Neutral
    d. good
    e. very good
- How do you find your lifestyle?
    a. very bad
    b. bad
    c. medium
    d. good
    e. very good

Appendix 2: Oxidant/antioxidant markers

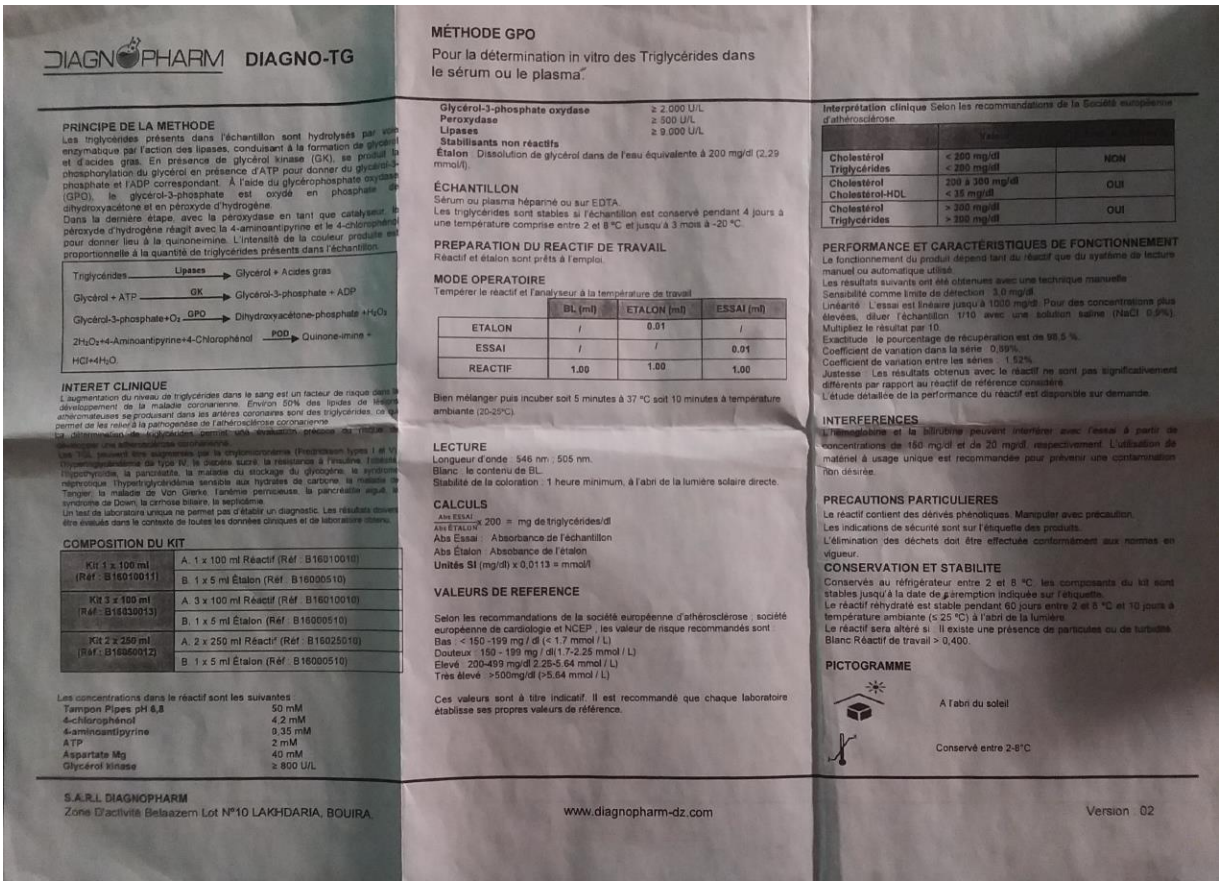
Table 3. Antioxidant markers in elderly and controls

Markers	Elderly	Controls	P-value
Vitamin C (µg/ml)	22.88 ± 0.58	19.27 ± 0.31	0.28
Plasma dismutase superoxide (mM/min/ml)	38.01 ± 0.13	37.94 ± 0.18	0.09
Erythrocyte dismutase superoxide (mM/min/ml)	51.58 ± 1.10	48.89 ± 1.01	0.72
Catalase (UI/mg)	563.72 ± 18.53*	1055.81 ± 44.36	0.05

Table 4. Oxidant markers in elderly and controls

Markers	Elderly	Controls	P-value
Plasma MDA (µmol/l)	1.00 ± 0.02	1.30 ± 0.02	0.084
Erythrocyte MDA (µmol/l)	12.73 ± 0.15*	9.48 ± 0.22	0.02

Appendix 3: Diagno pharm kit datasheets



DIAGNOPHARM **DIAGNO-TG**

PRINCIPE DE LA METHODE

Les triglycérides présents dans l'échantillon sont hydrolysés par voie enzymatique par l'action des lipases, conduisant à la formation de glycérol et d'acides gras. En présence de glycérol kinase (GK), se produit la phosphorylation du glycérol en présence d'ATP pour donner du glycérol-3-phosphate et l'ADP correspondant. À l'aide du glycérophosphate oxydase (GPO), le glycérol-3-phosphate est oxydé en phosphate de dihydroxyacétone et en péroxyde d'hydrogène. Dans la dernière étape, avec la péroxydase en tant que catalyseur, le péroxyde d'hydrogène réagit avec la 4-aminoantipyrine et le 4-chlorophénol pour donner lieu à la quinoneimine. L'intensité de la couleur produite est proportionnelle à la quantité de triglycérides présents dans l'échantillon.

Triglycérides —Lipases→ Glycérol + Acides gras

Glycérol + ATP —GK→ Glycérol-3-phosphate + ADP

Glycérol-3-phosphate+O_2 —GPO→ Dihydroxyacétone-phosphate +H_2O_2

$2H_2O_2$+4-Aminoantipyrine+4-Chlorophénol —POD→ Quinone-imine + HCl+$4H_2O$

INTERET CLINIQUE

L'augmentation du niveau de triglycérides dans le sang est un facteur de risque dans le développement de la maladie coronarienne. Environ 50% des lipides de plaques athéromateuses se produisant dans les artères coronaires sont des triglycérides, ce qui permet de les relier à la pathogenèse de l'athérosclérose coronarienne.
La détermination de triglycérides permet une évaluation précise du risque de développer une athérosclérose coronarienne.
Les TGL peuvent être augmentés par la chylomicronémie (Fredrickson types I et V), l'hypertriglycéridémie de type IV, le diabète sucré, la résistance à l'insuline, l'obésité, l'hypothyroïdie, la pancréatite, la maladie du stockage du glycogène, le syndrome néphrotique, l'hypertriglycéridémie sensible aux hydrates de carbone, la maladie de Tangier, la maladie de Von Gierke, l'anémie pernicieuse, la pancréatite aiguë, le syndrome de Down, la cirrhose biliaire, la septicémie.
Un test de laboratoire unique ne permet pas d'établir un diagnostic. Les résultats doivent être évalués dans le contexte de toutes les données cliniques et de laboratoire obtenu.

COMPOSITION DU KIT

Kit 1 x 100 ml (Réf : B16010011)	A. 1 x 100 ml Réactif (Réf : B16010010)
	B. 1 x 5 ml Étalon (Réf : B16000510)
Kit 3 x 100 ml (Réf : B16030013)	A. 3 x 100 ml Réactif (Réf : B16010010)
	B. 1 x 5 ml Étalon (Réf : B16000510)
Kit 2 x 250 ml (Réf : B16050012)	A. 2 x 250 ml Réactif (Réf : B16025010)
	B. 1 x 5 ml Étalon (Réf : B16000510)

Les concentrations dans le réactif sont les suivantes :

Tampon Pipes pH 6,8	50 mM
4-chlorophénol	4,2 mM
4-aminoantipyrine	0,35 mM
ATP	2 mM
Aspartate Mg	40 mM
Glycérol kinase	≥ 800 U/L

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MÉTHODE GPO

Pour la détermination in vitro des Triglycérides dans le sérum ou le plasma.

Glycérol-3-phosphate oxydase	≥ 2.000 U/L
Peroxydase	≥ 500 U/L
Lipases	≥ 9.000 U/L
Stabilisants non réactifs	

Étalon : Dissolution de glycérol dans de l'eau équivalente à 200 mg/dl (2,29 mmol/l).

ÉCHANTILLON

Sérum ou plasma hépariné ou sur EDTA.
Les triglycérides sont stables si l'échantillon est conservé pendant 4 jours à une température comprise entre 2 et 8 °C et jusqu'à 3 mois à -20 °C.

PREPARATION DU REACTIF DE TRAVAIL

Réactif et étalon sont prêts à l'emploi.

MODE OPERATOIRE

Tempérer le réactif et l'analyseur à la température de travail

	BL (ml)	ETALON (ml)	ESSAI (ml)
ETALON	/	0.01	/
ESSAI	/	/	0.01
REACTIF	1.00	1.00	1.00

Bien mélanger puis incuber soit 5 minutes à 37 °C soit 10 minutes à température ambiante (20-25°C).

LECTURE

Longueur d'onde : 546 nm ; 505 nm.
Blanc : le contenu de BL.
Stabilité de la coloration : 1 heure minimum, à l'abri de la lumière solaire directe.

CALCULS

$\frac{Abs\ ESSAI}{Abs\ ETALON} \times 200$ = mg de triglycérides/dl

Abs Essai : Absorbance de l'échantillon
Abs Étalon : Absorbance de l'étalon
Unités SI (mg/dl) x 0,0113 = mmol/l

VALEURS DE REFERENCE

Selon les recommandations de la société européenne d'athérosclérose ; société européenne de cardiologie et NCEP ; les valeur de risque recommandés sont :
Bas : < 150 -199 mg / dl (< 1.7 mmol / L)
Douteux : 150 - 199 mg / dl(1.7-2.25 mmol / L)
Elevé : 200-499 mg/dl 2.26-5.64 mmol / L)
Très élevé : >500mg/dl (>5,64 mmol / L)

Ces valeurs sont à titre indicatif. Il est recommandé que chaque laboratoire établisse ses propres valeurs de référence.

Interprétation clinique Selon les recommandations de la Société européenne d'athérosclérose

	Valeurs	
Cholestérol Triglycérides	< 200 mg/dl < 200 mg/dl	NON
Cholestérol Cholestérol-HDL	200 à 300 mg/dl < 35 mg/dl	OUI
Cholestérol Triglycérides	> 300 mg/dl > 300 mg/dl	OUI

PERFORMANCE ET CARACTÉRISTIQUES DE FONCTIONNEMENT

Le fonctionnement du produit dépend tant du réactif que du système de lecture manuel ou automatique utilisé.
Les résultats suivants ont été obtenues avec une technique manuelle
Sensibilité comme limite de détection : 3,0 mg/dl
Linéarité : L'essai est linéaire jusqu'à 1000 mg/dl. Pour des concentrations plus élevées, diluer l'échantillon 1/10 avec une solution saline (NaCl 0,9%). Multipliez le résultat par 10.
Exactitude : le pourcentage de récupération est de 98,5 %.
Coefficient de variation dans la série : 0,99%.
Coefficient de variation entre les séries : 1,52%.
Justesse : Les résultats obtenus avec le réactif ne sont pas significativement différents par rapport au réactif de référence considéré.
L'étude détaillée de la performance du réactif est disponible sur demande.

INTERFERENCES

L'hémoglobine et la bilirubine peuvent interférer avec l'essai à partir de concentrations de 150 mg/dl et de 20 mg/dl, respectivement. L'utilisation de matériel à usage unique est recommandée pour prévenir une contamination non désirée.

PRECAUTIONS PARTICULIERES

Le réactif contient des dérivés phénoliques. Manipuler avec précaution.
Les indications de sécurité sont sur l'étiquette des produits.
L'élimination des déchets doit être effectuée conformément aux normes en vigueur.

CONSERVATION ET STABILITE

Conservés au réfrigérateur entre 2 et 8 °C, les composants du kit sont stables jusqu'à la date de péremption indiquée sur l'étiquette.
Le réactif réhydraté est stable pendant 60 jours entre 2 et 8 °C et 10 jours à température ambiante (≤ 25 °C) à l'abri de la lumière.
Le réactif sera altéré si : Il existe une présence de particules ou de turbidité.
Blanc Réactif de travail > 0,400.

PICTOGRAMME

A l'abri du soleil

Conservé entre 2-8°C

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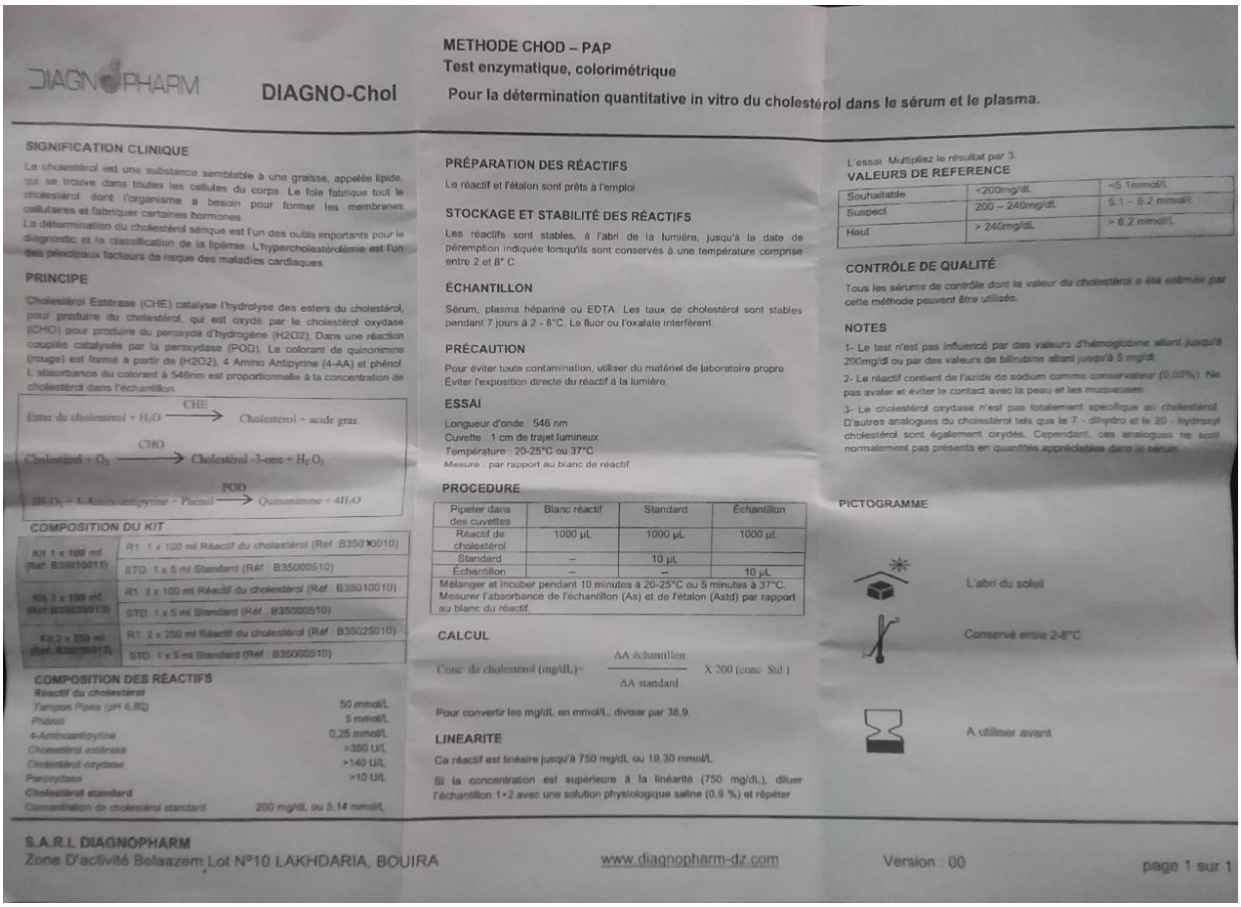
Version : 02



DIAGNOPHARM

DIAGNO-Chol

METHODE CHOD – PAP
Test enzymatique, colorimétrique
Pour la détermination quantitative in vitro du cholestérol dans le sérum et le plasma.

SIGNIFICATION CLINIQUE

Le cholestérol est une substance semblable à une graisse, appelée lipide, qui se trouve dans toutes les cellules du corps. Le foie fabrique tout le cholestérol dont l'organisme a besoin pour former les membranes cellulaires et fabriquer certaines hormones.
La détermination du cholestérol sérique est l'un des outils importants pour le diagnostic et la classification de la lipémie. L'hypercholestérolémie est l'un des principaux facteurs de risque des maladies cardiaques.

PRINCIPE

Cholestéryl Estérase (CHE) catalyse l'hydrolyse des esters du cholestérol, pour produire du cholestérol, qui est oxydé par le cholestérol oxydase (CHO) pour produire du peroxyde d'hydrogène (H_2O_2). Dans une réaction couplée catalysée par la peroxydase (POD), Le colorant de quinonimine (rouge) est formé à partir de (H_2O_2), 4 Amino Antipyrine (4-AA) et phénol. L'absorbance du colorant à 546nm est proportionnelle à la concentration de cholestérol dans l'échantillon.

$$\text{Esters de cholestérol} + H_2O \xrightarrow{\text{CHE}} \text{Cholestérol} + \text{acide gras}$$

$$\text{Cholestérol} + O_2 \xrightarrow{\text{CHO}} \text{Cholestérol-3-one} + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{Phénol} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

COMPOSITION DU KIT

Kit 1 x 100 ml (Réf: B35010011)	R1. 1 x 100 ml Réactif du cholestérol (Réf : B35010010)
	STD. 1 x 5 ml Standard (Réf : B35000510)
Kit 3 x 100 ml (Réf: B35030013)	R1. 3 x 100 ml Réactif du cholestérol (Réf : B35010010)
	STD. 1 x 5 ml Standard (Réf : B35000510)
Kit 2 x 250 ml (Réf: B35025013)	R1. 2 x 250 ml Réactif du cholestérol (Réf : B35025010)
	STD. 1 x 5 ml Standard (Réf : B35000510)

COMPOSITION DES RÉACTIFS

Réactif du cholestérol	
Tampon Pipes (pH 6,80)	50 mmol/L
Phénol	5 mmol/L
4-Aminoantipyrine	0,25 mmol/L
Cholestéryl estérase	>350 U/L
Cholestérol oxydase	>140 U/L
Peroxydase	>10 U/L
Cholestérol standard	
Concentration de cholestérol standard	200 mg/dL ou 5.14 mmol/L

PRÉPARATION DES RÉACTIFS

Le réactif et l'étalon sont prêts à l'emploi.

STOCKAGE ET STABILITÉ DES RÉACTIFS

Les réactifs sont stables, à l'abri de la lumière, jusqu'à la date de péremption indiquée lorsqu'ils sont conservés à une température comprise entre 2 et 8° C.

ÉCHANTILLON

Sérum, plasma hépariné ou EDTA. Les taux de cholestérol sont stables pendant 7 jours à 2 - 8°C. Le fluor ou l'oxalate interfèrent.

PRÉCAUTION

Pour éviter toute contamination, utiliser du matériel de laboratoire propre.
Éviter l'exposition directe du réactif à la lumière.

ESSAI

Longueur d'onde : 546 nm
Cuvette : 1 cm de trajet lumineux
Température : 20-25°C ou 37°C
Mesure : par rapport au blanc de réactif

PROCEDURE

Pipeter dans des cuvettes	Blanc réactif	Standard	Échantillon
Réactif de cholestérol	1000 µL	1000 µL	1000 µL
Standard	–	10 µL	
Échantillon	–	–	10 µL

Mélanger et incuber pendant 10 minutes à 20-25°C ou 5 minutes à 37°C. Mesurer l'absorbance de l'échantillon (As) et de l'étalon (Astd) par rapport au blanc du réactif.

CALCUL

$$\text{Conc. de cholestérol (mg/dL)} = \frac{\Delta A \text{ échantillon}}{\Delta A \text{ standard}} \times 200 \text{ (conc. Std.)}$$

Pour convertir les mg/dL en mmol/L, diviser par 38,9.

LINEARITE

Ce réactif est linéaire jusqu'à 750 mg/dL ou 19,30 mmol/L.

Si la concentration est supérieure à la linéarité (750 mg/dL), diluer l'échantillon 1+2 avec une solution physiologique saline (0,9 %) et répéter l'essai. Multipliez le résultat par 3.

VALEURS DE REFERENCE

Souhaitable	<200mg/dL	<5.1mmol/L
Suspect	200 – 240mg/dL	5.1 – 6.2 mmol/L
Haut	> 240mg/dL	> 6.2 mmol/L

CONTRÔLE DE QUALITÉ

Tous les sérums de contrôle dont la valeur du cholestérol a été estimée par cette méthode peuvent être utilisés.

NOTES

1- Le test n'est pas influencé par des valeurs d'hémoglobine allant jusqu'à 200mg/dl ou par des valeurs de bilirubine allant jusqu'à 5 mg/dl.

2- Le réactif contient de l'azide de sodium comme conservateur (0,05%). Ne pas avaler et éviter le contact avec la peau et les muqueuses.

3- Le cholestérol oxydase n'est pas totalement spécifique au cholestérol. D'autres analogues du cholestérol tels que le 7 - dihydro et le 20 - hydroxyl cholestérol sont également oxydés. Cependant, ces analogues ne sont normalement pas présents en quantités appréciables dans le sérum.

PICTOGRAMME

L'abri du soleil

Conservé entre 2-8°C

A utiliser avant

S.A.R.L DIAGNOPHARM
Zone D'activité Belaazem Lot N°10 LAKHDARIA, BOUIRA
www.diagnopharm-dz.com

Version : 00

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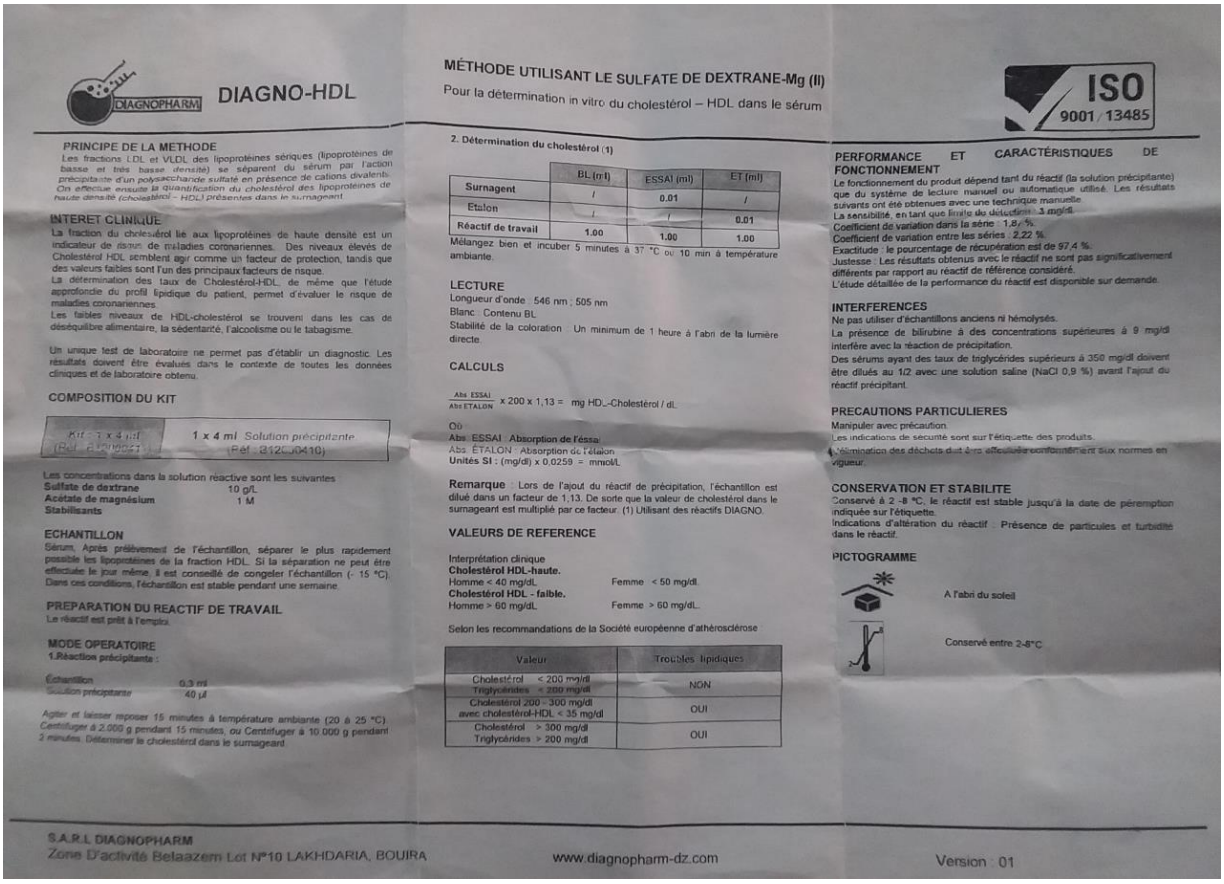

DIAGNOPHARM

DIAGNO-HDL

MÉTHODE UTILISANT LE SULFATE DE DEXTRANE-Mg (II)

Pour la détermination in vitro du cholestérol – HDL dans le sérum

ISO 9001 / 13485

PRINCIPE DE LA METHODE

Les fractions LDL et VLDL des lipoprotéines sériques (lipoprotéines de basse et très basse densité) se séparent du sérum par l'action précipitante d'un polysaccharide sulfaté en présence de cations divalents. On effectue ensuite le quantification du cholestérol des lipoprotéines de haute densité (cholestérol – HDL) présentes dans le surnageant.

INTERET CLINIQUE

La fraction du cholestérol lié aux lipoprotéines de haute densité est un indicateur de risque de maladies coronariennes. Des niveaux élevés de Cholestérol HDL semblent agir comme un facteur de protection, tandis que des valeurs faibles sont l'un des principaux facteurs de risque.
La détermination des taux de Cholestérol-HDL, de même que l'étude approfondie du profil lipidique du patient, permet d'évaluer le risque de maladies coronariennes.
Les faibles niveaux de HDL-cholestérol se trouvent dans les cas de déséquilibre alimentaire, la sédentarité, l'alcoolisme ou le tabagisme.

Un unique test de laboratoire ne permet pas d'établir un diagnostic. Les résultats doivent être évalués dans le contexte de toutes les données cliniques et de laboratoire obtenu.

COMPOSITION DU KIT

Kit : 1 x 4 ml (Réf : 312C0410)	**1 x 4 ml** Solution précipitante (Réf : 312C0410)

Les concentrations dans la solution réactive sont les suivantes

Sulfate de dextrane	10 g/L
Acétate de magnésium	1 M
Stabilisants	

ECHANTILLON

Sérum. Après prélèvement de l'échantillon, séparer le plus rapidement possible les lipoprotéines de la fraction HDL. Si la séparation ne peut être effectuée le jour même, il est conseillé de congeler l'échantillon (- 15 °C). Dans ces conditions, l'échantillon est stable pendant une semaine.

PREPARATION DU REACTIF DE TRAVAIL

Le réactif est prêt à l'emploi.

MODE OPERATOIRE

1.Réaction précipitante :

Échantillon	0.3 ml
Solution précipitante	40 µl

Agiter et laisser reposer 15 minutes à température ambiante (20 à 25 °C). Centrifuger à 2.000 g pendant 15 minutes, ou Centrifuger à 10.000 g pendant 2 minutes. Déterminer le cholestérol dans le surnageant.

2. Détermination du cholestérol (1)

	BL (ml)	ESSAI (ml)	ET (ml)
Surnagent	/	0.01	/
Etalon	/	/	0.01
Réactif de travail	1.00	1.00	1.00

Mélangez bien et incuber 5 minutes à 37 °C ou 10 min à température ambiante.

LECTURE

Longueur d'onde : 546 nm ; 505 nm
Blanc : Contenu BL
Stabilité de la coloration : Un minimum de 1 heure à l'abri de la lumière directe.

CALCULS

$$\frac{Abs\ ESSAI}{Abs\ ETALON} \times 200 \times 1{,}13 = \text{mg HDL-Cholestérol / dL}$$

Où
Abs. ESSAI : Absorption de l'éssai
Abs. ÉTALON : Absorption de l'étalon
Unités SI : (mg/dl) x 0,0259 = mmol/L

Remarque : Lors de l'ajout du réactif de précipitation, l'échantillon est dilué dans un facteur de 1:13. De sorte que la valeur de cholestérol dans le surnageant est multiplié par ce facteur. (1) Utilisant des réactifs DIAGNO.

VALEURS DE REFERENCE

Interprétation clinique
Cholestérol HDL-haute.
Homme < 40 mg/dL Femme < 50 mg/dl.
Cholestérol HDL - faible.
Homme > 60 mg/dL Femme > 60 mg/dL.

Selon les recommandations de la Société européenne d'athéroscérose

Valeur	Troubles lipidiques
Cholestérol < 200 mg/dl Triglycérides < 200 mg/dl	NON
Cholestérol 200 - 300 mg/dl avec cholestérol-HDL < 35 mg/dl	OUI
Cholestérol > 300 mg/dl Triglycérides > 200 mg/dl	OUI

PERFORMANCE ET CARACTÉRISTIQUES DE FONCTIONNEMENT

Le fonctionnement du produit dépend tant du réactif (la solution précipitante) que du système de lecture manuel ou automatique utilisé. Les résultats suivants ont été obtenues avec une technique manuelle.
La sensibilité, en tant que limite de détection : 3 mg/dl.
Coefficient de variation dans la série : 1,8 / %.
Coefficient de variation entre les séries : 2,22 %.
Exactitude : le pourcentage de récupération est de 97,4 %.
Justesse : Les résultats obtenus avec le réactif ne sont pas significativement différents par rapport au réactif de référence considéré.
L'étude détaillée de la performance du réactif est disponible sur demande.

INTERFERENCES

Ne pas utiliser d'échantillons anciens ni hémolysés.
La présence de bilirubine à des concentrations supérieures à 9 mg/dl interfère avec la réaction de précipitation.
Des sérums ayant des taux de triglycérides supérieurs à 350 mg/dl doivent être dilués au 1/2 avec une solution saline (NaCl 0,9 %) avant l'ajout du réactif précipitant.

PRECAUTIONS PARTICULIERES

Manipuler avec précaution.
Les indications de sécurité sont sur l'étiquette des produits.
L'élimination des déchets doit être effectuée conformément aux normes en vigueur.

CONSERVATION ET STABILITE

Conservé à 2 -8 °C, le réactif est stable jusqu'à la date de péremption indiquée sur l'étiquette.
Indications d'altération du réactif : Présence de particules et turbidité dans le réactif.

PICTOGRAMME

A l'abri du soleil

Conservé entre 2-8°C

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