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Memoire DE FIN D'ETUDES POUR
L'OBTENTION DU DIPLOME DE DOCTEUR EN PHARMACIE

Smoking and vitamin c

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Dedication

I dedicate this modest work first to the memory of my beloved Grand mother رحمة الله عليها

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HAMOUA

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LIST OF ACRONYMS:

AA:ascorbic acid

AOx :antioxidant

ASC :ascorbat

BMI:body mass index

c/d:cigarette /day

CS :Cigarette smoke

DCIP :Dichlorophenolindophenol

DHA: dehydroascorbate

DTH:Delayed-Type Hypersensitivity

DHAA :dehydro-L-ascorbic acid

FTND:Fagerstrom Test for Nicotine Dependence

MDHAA:monodehydro-L-ascorbic acid

MMP:matrix metalloproteinase

NHANES :National Health and Nutrition Examination Survey

NMR : Nuclear Magnetic Resonance

RDA:recomanded daily allowance

ppd: pack per day

TAA: total ascorbic acid

VIT C:Vitamin C

INTRODUCTION

Cigarette smoke (CS) contains a range of xenobiotics, including oxidants and free radicals that can increase lipid peroxidation. One estimate suggests that cigarette smoke contains in the order of 1014 free radicals per inhalation (1)

The active smokers are exposed to reactive free radicals, which are capable of directly and indirectly inducing oxidative stress in the body. The direct exposures from cigarette smoke represent only a portion of the total oxidative stress eventually experienced by the smoker (1, 2)
A CS also contributes to additional endogenous oxidant formation through effects on the inflammatory-immune response.(2) Because free radicals cause oxidative damage to macromolecules such as lipids, proteins, and DNA, they are believed to be involved in the pathogenesis of cardiovascular diseases and cancer.(3)

The free radicals in CS deplete some plasma antioxidants in vitro, and several studies found lower plasma concentrations of these antioxidants. The requirement for antioxidant nutrients depends on a person's exposure to endogenous and exogenous reactive oxygen species. Since cigarette smoking results in an increased cumulative exposure to reactive oxygen species from both sources, it would seem that cigarette smokers might have an increased requirement for antioxidant nutrients.(1, 3)

Vitamin C (ascorbic acid) is an indispensable cofactor in the hydroxylation of proline and lysine, and it is essential to collagen synthesis and connective tissue integrity. Vitamin C (VIT C) functions as a reducing agent in hydroxylation reactions catalyzed by dopamine b-monooxygenase and peptidyl glycine a-amidating monooxygenase. It plays an important role in increasing the content of endothelial cell tetrahydrobiopterin and thereby increasing the activity of nitric oxide synthase. It is involved in the biosynthesis of carnitine, histamine, and several adrenal steroids; it promotes iron absorption and mobilization; and it functions in tyrosine, folate, and xenobiotic metabolism when intake of vitamin C is below a critical amount (10 mg/d) for prolonged periods, failure of wounds to heal, petechial hemorrhages, follicular hyperkeratosis, bleeding gums, and related abnormalities ensue in a condition known as scurvy.(4)

Since ascorbic acid is a critical component of the body's antioxidant defense mechanisms, it has been investigated assessing a range of biomarkers of oxidative stress in smokers. Interventions consisting of ascorbic acid supplementation have also been investigated in smokers assessing other functional biomarkers, including DNA damage, endothelial function, monocyte adhesion, and sperm quality.(1) it was demonstrated that heavy smoking in males is associated with a 20-40% decrease in serum ascorbic acid levels.(5)

The recommended dietary allowance of ascorbic acid for smokers was increased from 60 to 100mg to reduce the risk of low serum ascorbic acid concentrations.(6)

In the pursuit of attempting to look for the effect of smoking and vitamin c status we have measured serum ascorbic acid levels into two groups (smokers and non-smokers).

bibliographic review

I. THE ASCORBIC ACID

I.1.Generalities

The vitamin c, acid L-ascorbic is an organic hydrosoluble compound ubiquitous in the living world, the ascorbic acid (AA) had been discovered for the first time in the 18° century during a sea voyage , the crew of the ship was severely ill due to a disease called (scurvy) characterized by gingival hemorrhage ,anemia,osteodynia .Since the human body is unable to produce the AA ,all of our daily intakes comes from nutrition specially vegetables and fruits .(7)

The recommended daily intake of vitamin C for adults is 75 mg for women and 90 mg for men, person who smokes and those indulged in rigorous training requires higher amount of vitamin C because of increased production of free radical.(8)

I.2 structure:

Vitamin C is a redox-system comprising L-ascorbic acid (AA), the free radical monodehydro-L-ascorbic acid (MDHAA) and dehydro-L-ascorbic acid (DHAA) (Figure 1)The three substances are interconvertible but each has distinct individual physicochemical properties (9)

The structure of the vitamin C system is essentially planar. Each of the three components has, in its molecule, a lactone ring one keto-group (C=O) in C-l, and two hydroxyl-groups (OH) in C-5 and C-6. The carbon atom in C-5 is asymmetric, the space orientation of its Hand OH groups and the ring structure of the molecule are responsible for the positive optical rotation of AA and DHAA, having a molecular formula of $C_6H_8O_6$, makes it belong to the group of hexoses, vit c could be extracted from nature or synthesized by the D-Glucose pathway.(10)

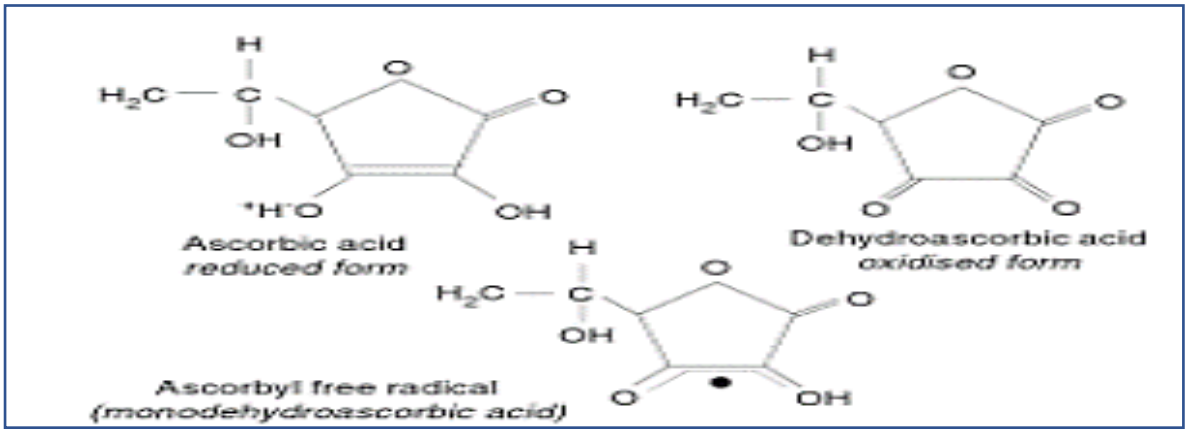


Figure 1: Structures of L-ascorbic acid, monodehydroascorbate (MDHA) and dehydroascorbate (DHA)

The majority of the vertebrae are capable of synthesizing the vitamin c from Glucose, on the contrary of primates who are in need of outside supplies to cover up their needs.The AA is hydrosoluble matter which cannot be stocked in the human body, unlike the liposoluble vitamins (A,D,K,E) .(11)

I.3 Physicochemical properties:

The AA is a white crystallin substance, soluble in water and alcohol, the molecule balance between the two forms (ox/red) in the presence of oxygen, this process is catalyzed by traces of metals such as iron and copper.(12) (figure 2)

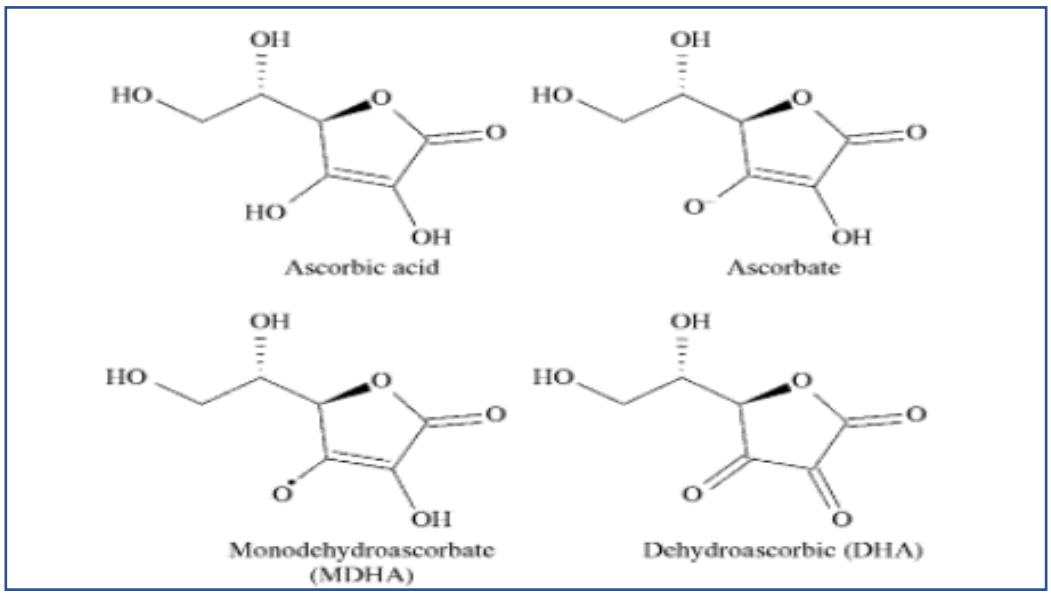


Figure 2: Ascorbic acid and its oxidation products

Table I: Physicochemical properties of ascorbic acid

Property Name	Property Value
Molecular Weight	176.12
XLogP3	-1.6
Hydrogen Bond Donor Count	4
Hydrogen Bond Acceptor Count	6
Rotatable Bond Count	2
Exact Mass	176.03208797
Monoisotopic Mass	176.03208797
Topological Polar Surface Area	107 $Å^2$
Heavy Atom Count	12
Formal Charge	0
Complexity	232
Isotope Atom Count	0
Defined Atom Stereocenter Count	2
Undefined Atom Stereocenter Count	0
Defined Bond Stereocenter Count	0
Undefined Bond Stereocenter Count	0
Covalently-Bonded Unit Count	1
Compound Is Canonicalized	Yes

Table II: Experimental Properties of ascorbic acid(13)

II.Metabolism and biosynthesis:

Odor	Odorless
Taste	Pleasant, sharp, acidic taste
Melting Point	374 to 378 °F (decomposes) (NTP, 1992) 191 dec °C
Solubility	greater than or equal to 100 mg/mL at 73° F (NTP, 1992) 400000 mg/L (at 40 °C)
Vapor Pressure	9.28X10-11 mm Hg at 25 °C (est)
LogP	-1.85
Stability/Shelf Life	Stable to air when dry; impure preparation and in many natural products vitamins oxidizes on exposure to air and light. Aqueous solutions are rapidly oxidized by air, accelerated by alkalies, iron, copper
Optical Rotation	Specific optical rotation (c = 1 in water): +20.5 to +21.5 deg at 25 °C/D; (c = 1 in methanol): +48 deg at 23 °C/D
Autoignition Temperature	380 °C
Decomposition	When heated to decomposition it emits acrid smoke and irritating fumes.
Heat of Vaporization	The heat of vaporization is 1.487X10+8 J/kmol at 465.15 deg K.
pH	Between 2,4 and 2,8 (2 % aqueous solution)

II.1 Pharmacokinetics of vitamin c:

II.1.1 Oral route of administration

a. Absorption:

Regard to vitamin c three potential modes of membrane transport exist: passive diffusion, facilitated diffusion, and active transport (14)

VitC exists primarily in two forms in vivo, ASC (ascorbate (reduced form) and DHA (dehydroascorbate) (oxidized form), of which the former is by far the predominant. For most low molecular weight drugs, simple diffusion is the primary means of membrane transport, but vitC is predominantly represented by its anionic form. As such, it will only be able to diffuse across the plasma membrane at a relatively slow rate even in the presence of a considerable concentration gradient. The amount of unionized ascorbic acid increases to 99.9% and 15% in the stomach (pH 1) and small intestine (pH 5) respectively(15)

And under these local conditions, passive diffusion could perhaps play a more substantial role in vitC absorption.(16)

Facilitated membrane diffusion is facilitated by carrier proteins, yet it, like passive diffusion, is dependent on an electrochemical gradient. DHA has been demonstrated to compete with glucose for transport via a variety of glucose transporters.(17)

Finally, vitC absorption is influenced by concentration gradient-independent active transport. The bioavailability of ASC has been found to be substantially dose-dependent since the 1970s.Several publications found that intestinal ASC absorption is subject to saturable active transport after increasing oral dosages resulted in decreased absorption fractions.(18) (figure3)

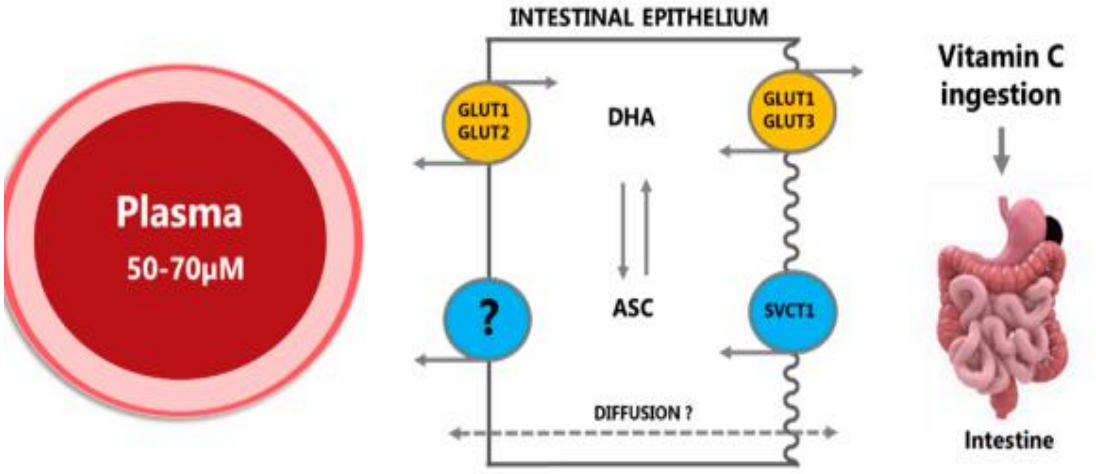


Figure 3: Absorption of vitamin c.

b. Distribution:

Vitamin C distribution is highly compartmentalized (figure 4). Simple diffusion is unlikely to play a significant role in vitC transport through membranes, at least in terms of its dissemination beyond the bloodstream. According to a dissociation-determined equilibrium,

ASC plasma steady state concentrations would be 2.5-fold greater than tissue concentrations. According to a dissociation-determined equilibrium, intracellular concentrations of ASC range from 0.5 to 10mM compared to the mere 50-80 µm in the plasma of healthy individuals.(19)

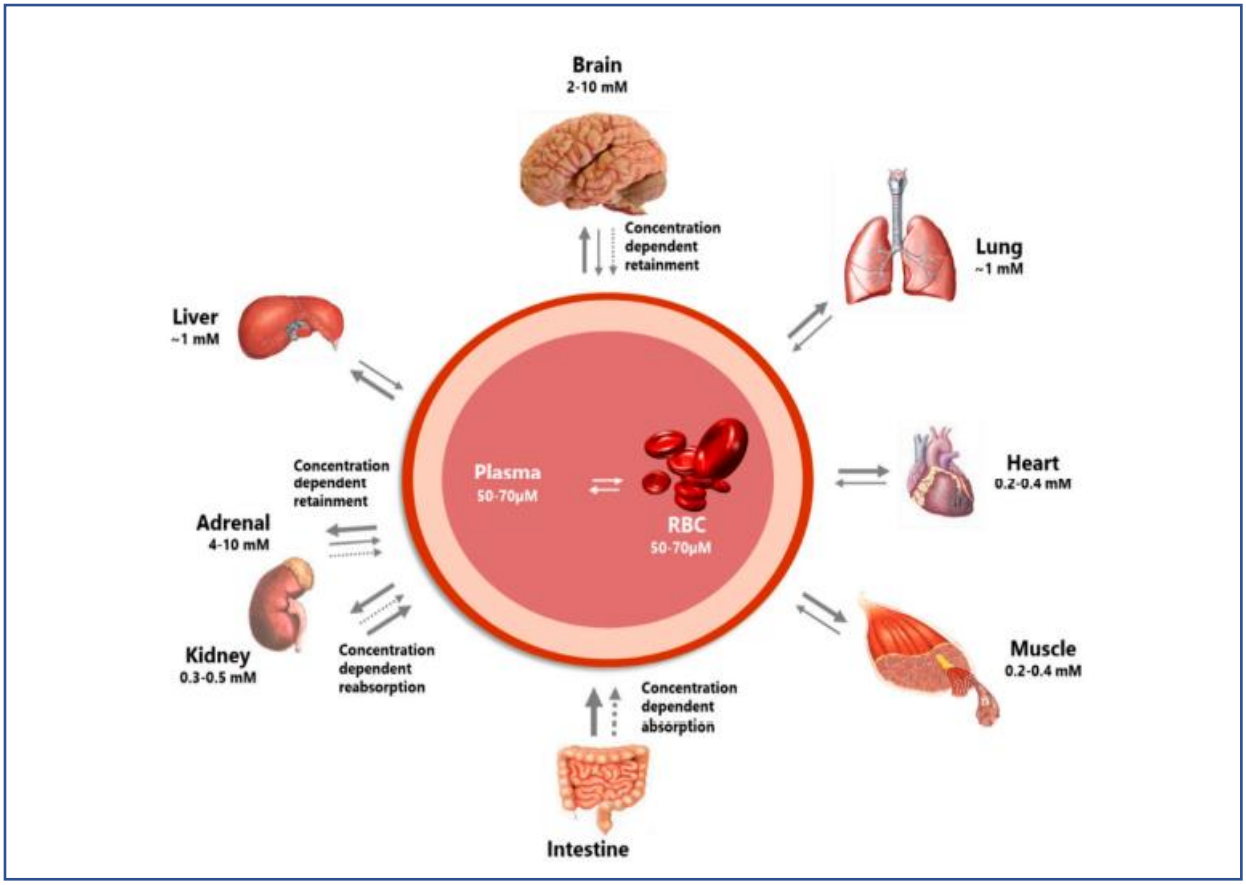


Figure 4: Distribution of vitamin C in the body.

c. Metabolism:

In contrast to plants, which have a variety of ASC derivatives and analogues, including numerous glucosides, mammals exclusively have ASC.(20)

The antioxidant action of ASC is strongly tied to its metabolism. ASC functions as an effective electron donor in biological reactions according to its enediol structure, which is strongly resonance stabilized and controlled by the acidity of the molecule. When ASC is used as a cofactor or a free radical quencher, it is oxidized to the comparably stable radical intermediate ascorbyl free radical, which can be disproportionate to one molecule of ASC and one molecule of DHA at normal ph. (21)

As previously stated, a variety of cell types successfully reduce DHA intracellularly, conserving the ASC pool. VitC (vitamin c) turnover is thus closely connected to DHA catabolism, which happens via hydrolysis to 2,3-diketogulonic acid and decarboxylation to l-xylonate and l-lyxonate, all of which can be degraded via the pentose phosphate pathway.(22)

d. Excretion and reuptake

ASC would be expected to be eliminated efficiently by the kidneys as a very hydrophilic low molecular weight molecule. Indeed, ASC is quantitatively filtered through the glomerulus by a hydrostatic pressure gradient, and then concentrated in the pre-urine after water resorption (Figure5). When the pH dips to around five, the percentage of unionized ascorbic acid to ASC increases. The ascorbic acid concentration gradient of 1500:1 from 0.01% in plasma to around 15% in pre-urine would normally result in significant passive reabsorption for most molecules, but this does not appear to be the case with ascorbic acid, owing to its low lipid solubility. Saturable active transport regulates the reuptake of ASC in the proximal renal tubules.(23, 24)

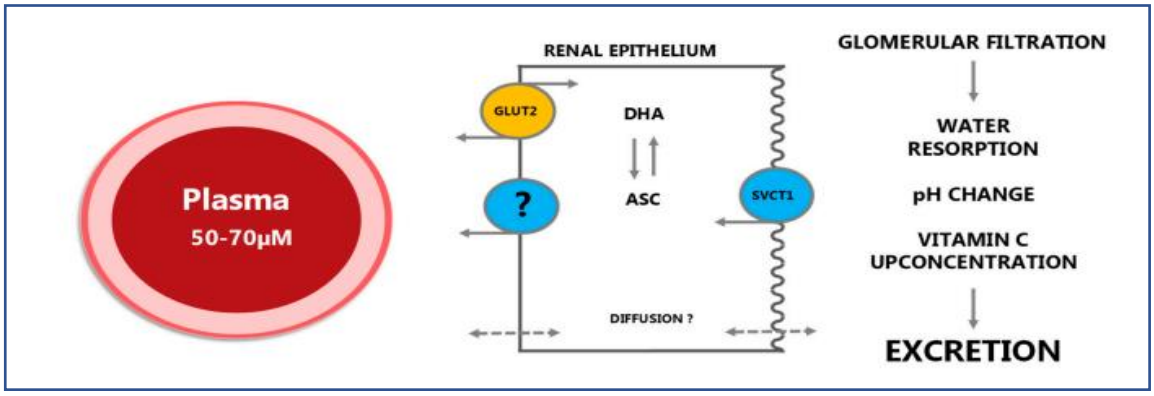


Figure 5: Excretion and reuptake of ascorbic acid

II.1.2. Intravenous route of administration

The administration of medications intravenously usually produces a predictable plasma concentration, resulting in 100% bioavailability. For vitC, in particular, intravenously administration bypasses saturable absorption mechanisms, virtually eliminating the upper limit of the maximum achievable plasma concentration. Parenteral vitC administration is usually treated by intravenous infusion. This approach provides a predictable stable plasma concentration.(24) (figure6).

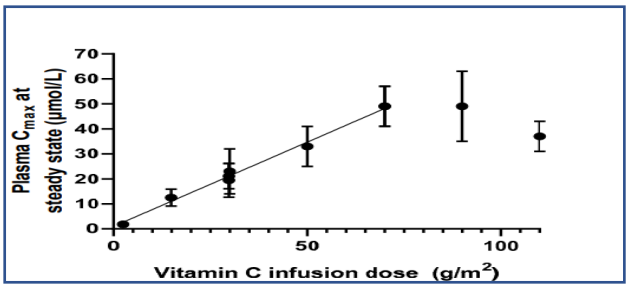


Figure 6: Relationship between infusion dose of vitC and plasma Cmax .

a. Distribution:

The distribution of vit C following infusion is dependent, at least initially, on the vascularization of the various tissues, as it is for all substances in circulation. While millimolar plasma concentrations do not appear to affect normal tissue distribution beyond

saturation, poorly vascularized tumors have piqued researchers' interest because ASC has been shown to be cytotoxic to cancer cells but not normal cells at high concentrations in in vitro and in vivo studies, possibly due to the fact that it is cytotoxic to cancer cells but not normal cells.(25)

b. Metabolism and excretion:

In normal tissue, metabolism of ASC has not been shown to deviate from the general pattern However, in poorly vascularized tumor tissues, high-dose vitC combined with the hypoxic tumor environment has been proposed to promote the formation of cytotoxic levels of hydrogen peroxide, thus providing a putative mode of action and a potential role of ASC in cancer treatment.(26, 27)

VitC is fast eliminated by glomerular filtration without significant recapture. This makes the half-life constant and first order elimination kinetics.(28)

II.2 Biosynthesis

The main features of metabolism of ascorbate, particularly in vertebrates, are briefly summarized on figure 7.

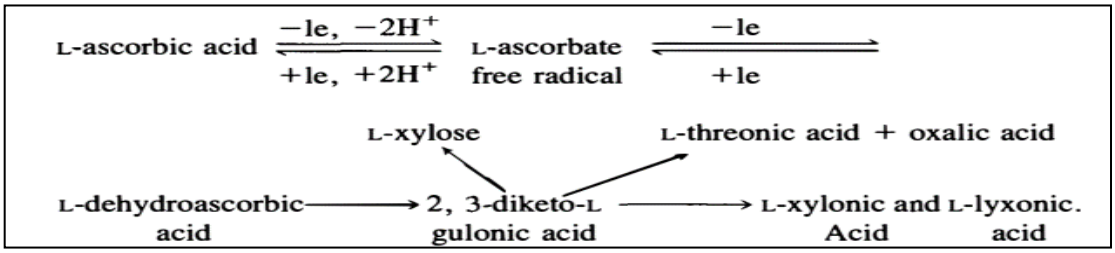


Figure 7: Metabolism of ascorbate

Ascorbate can be converted to L-dehydroascorbate by removing two electrons and two protons. Evidence suggests that this occurs in two stages, with a free radical formed intermediately by removal of one electron and two protons. Semi-dehydroascorbate can then give up a second electron to yield L-dehydroascorbate. The free radical can also undergo disproportionation, in which two molecules form one of ascorbate and one of dehydroascorbate .

In some plants, ascorbate is converted to L-dehydroascorbate irreversibly by an ascorbic acid oxidase that is a copper-requiring enzyme. In many cells of different species, including mammals, the conversion is reversible to some degree, and is catalyzed by a different enzyme, dehydroascorbate reductase. That enzyme uses reduced glutathione as a cosubstrate, and the products are ascorbate and oxidized glutathione. Should the reaction operate in reverse, oxidized glutathione would be reduced. In some of these cells another enzyme, glutathione reductase, utilizes NADPH for reduction of oxidized glutathione; one can then construct a cycle in which glutathione and NADPH are used in the overall reconversion of

dehydroascorbate to ascorbate, allowing the last two to exist in equilibrium. If NADPH is then syphoned off for use in other reductions (for instance, in the reduction of folate to tetrahydrofolate or biopterin to tetrahydrobiopterin), one can visualize how ascorbate, can be used as a source of electrons and protons. The existence of the intermediate L-ascorbate free radical is incorporated into mechanisms proposed for certain hydroxylation reactions that appear to depend on ascorbate; for instance, such a mechanism has been proposed for the hydroxylation of dopamine to norepinephrine. Other aspects of the above scheme apply to' species that are able to open the lactone ring of L-dehydroascorbate to form 2,3-diketo-L-gulonate. This compound then can go through several possible degradation pathways, as shown above. The enzyme involved is a lactonase. If a species were to lack that enzyme, dehydroascorbate could either be reconverted to ascorbate, if mechanisms exist to perform that reaction, or be excreted in the urine. Species that apparently lack the lactonase are humans, other primates, and fishes. Significantly, the lactonase is present in the livers of guinea pigs. In theory, the presence or absence of lactonase in species such as the human and guinea pig, both of which cannot synthesize ascorbate, could influence ‘the dietary requirement for the vitamin. In considering the metabolism of ascorbate, one must recognize that it must be transported into cells and subcellular compartments where it performs its functions. Under physiological conditions ascorbate exists as a monoanion that cannot traverse most membranes readily. However, the subject of transport of ascorbate is only beginning to be studied. We refer to this matter further under our discussion of chromaffin cells of the adrenal glands in synthesis of norepinephrine.(29)

III.Role of ascorbic acid:

III.1 Antioxidant functions in cells, tissues and organs:

a. Neutrophil activity:

Neutrophils play an important role in the body's defense system against bacterial infections by phagocytizing and killing the bacteria. When neutrophils are activated, there is a rapid consumption of oxygen and the consequent production of several reactive oxygen species. This activation process has been referred Antioxidant Role of Vitamin C to as the respiratory burst. Reactive species may include superoxide and hydroxyl free radicals as well as hydrogen peroxide, singlet oxygen and hypochlorous acid. These reactive species are responsible for the killing of pathogenic organisms. During responses to pathogens, irritants or allergens, the protecting cells (neutrophils, mast cells, macrophages) can also be damaged by the reactive species they may release. High levels of ascorbic acid in these cells have been proposed as a protective mechanism against self-destruction (30, 31)

b. Lung protection:

Cigarette Smoke. Along with many other known harmful chemicals found in cigarette smoke, the generation of free radicals, such as nitrogen dioxide, has also been observed. 70-75 As an adaptive response to this increase in free radical burden from cigarette smoke, the level of antioxidants present in the lung may increaseè. However, as a consequence of free radical quenching, the overall result may be lower serum antioxidant levels. 76-84 Serum vitamin C levels were significantly decreased when rats were exposed to cigarette smoke, even though

rats can synthesize vitamin C. 76 Reports from the NHANES II (National Health and Nutrition Examination Survey (1976-1980) survey 77 of the American population showed that serum ascorbic acid levels were consistently. lower in smokers than non-smokers. 78 Smokers needed to consume approximately twice the RDA (**Recommended Dietary Allowances)** for vitamin C daily (60 mg vitamin C/day for adults) as non-smokers to have similar concentrations of serum vitamin C. The reduction of serum vitamin C in smokers may be the result of a shift in balance between the levels of natural antioxidants and the free radical burden imposed on the system. The lung of the healthy nonsmoker contains very few neutrophils. However, there are increased numbers of neutrophils in the lungs of smokers. 80 Cigarette smoke can activate neutrophils in the lung to generate reactive oxygen-containing species. These reactive species can then interact with the alpha-l-proteinase inhibitor and inactivate this protective enzyme. Vitamin C has been shown to protect human alpha-l-proteinase inhibitor from direct deactivation by cigarette smoke 83 as well as deactivation by free radicals generated by activated neutrophils. Within the lung, alveolar macrophages, along with neutrophils, protect lung tissue from pathogens as well as free radical attack. Alveolar macrophages have a high concentration of ascorbic acid. The macrophages from smokers' lungs have twice the concentration of ascorbic acid as those from nonsmokers. The higher concentration of ascorbic acid in these cells is thought to reflect a "protective utilization of ascorbic acid under conditions of increased oxidant stress" found in the lungs of smokers. 84 One might speculate that ascorbic acid may protect smokers against some of the damage caused by free radicals present in and generated by the inhaled smoke.(32, 33)

III.2 Immune enhancing activity:

The high cellular concentration of vitamin C and its rapid decline in plasma and leukocytes during stress and infection suggest a role in the process of immune response. Vitamin C was found to be a stimulant of leukocyte functions, especially of neutrophil and monocyte movement. Supplementation of healthy adults (1–3 g/ day) and children (20 mg/kg/day) enhanced neutrophil chemotaxis in vivo, but bactericidal activity was not enhanced. Lymphocyte proliferation was not impaired in vitamin C deficiency in humans and the number of circulating CD4+ or CD8+ cells was not altered. Vitamin C in doses of 1–5 g/day for 3 days and over several weeks increased human T lymphocyte proliferation and neutrophil motility towards lipopolysaccharide-activated autologous serum. In vitro treatment of peritoneal macrophages with antioxidant vitamins, including vitamin C, was shown to stimulate the entire process of phagocytosis. The observed decrease of vitamin C in plasma and leukocytes during infective periods suggests that the increased generation of oxidizing agents is counteracted by reaction with vitamin C, and herewith the host is protected against any harmful oxidative action. In studies in healthy subjects, administration of vitamin C resulted in improvement of several components of human immune parameters, such as antimicrobial and natural killer cell activities, lymphocyte proliferation, chemotaxis, and DTH(Delayed-Type Hypersensitivity). The effect of vitamin C intake with the diet on immune function was studied in young, healthy nonsmokers. The volunteers consumed a vitamin-C-deficient diet and then increased their vitamin C intake from 5 to 250 mg/day. Upon ingestion of the vitamin-C-deficient diet, plasma and leukocyte vitamin C concentrations were decreased by about 50%, and DTH response to several antigens was decreased as well. With higher doses (60 and 250 mg/day) the DTH response was normalized, but lymphocyte proliferation was not affected. In older people, known to have reduced vitamin C plasma and leukocyte concentrations even if they are not institutionalized, vitamin C supplementation resulted in enhanced cell-mediated immunity. Intracellular concentrations of vitamin C in human leukocytes have been shown to decline

with increasing age, accompanied by neutrophil function impairment. Oral administration of vitamin C resulted in improved neutrophil functions and serum immunoglobulin levels. An earlier investigation showed that administration of 1 g of vitamin C together with 200 mg of vitamin E for 16 weeks to healthy elderly women enhanced neutrophil chemotaxis and phagocytosis, and decreased concentrations in serum lipid peroxides, which is indicative of improved resistance to oxidative stress. Other studies, however, did not show any alterations in indices of immune function following vitamin C administration, which may be due to the fact that individuals participating in these studies had already adequate vitamin C baseline concentrations. Vitamin C stimulated interferon production in vitro when incubated with cultured mouse cells and in vivo when administered to mice. There is evidence that ascorbic acid may also have antiviral activity in vivo. Topical application of ascorbic acid in patients with herpes simplex virus infections decreased the duration of the lesions and viral shedding .(34)

III.3 Role of vitamin c in atherogenesis and vascular dysfunction:

Since oxidative stress inhibits the biological activity of NO, antioxidants may be protective. Numerous recent studies have reported beneficial effects of vitamin C, administered either orally or by intra-arterial infusion, on vasodilation in various patient groups. We and others have found that vasodilation in patients with cardiovascular disease is significantly improved following treatment with vitamin C, and is comparable to vasodilation seen in healthy control subjects. Similar beneficial effects of vitamin C with normalization of the vasodilatory response were observed in patients with coronary spastic angina, chronic heart failure, hypercholesterolemia, or hypertension, as well as in healthy volunteers following methionine loading to cause hyperhomocysteinemia observed significantly improved vasodilation in smokers given vitamin C infusions. In addition, patients with noninsulin-dependent and insulin-dependent diabetes mellitus demonstrated increased blood flow after infusion of vitamin C. Finally, healthy individuals given an oral dose of 1000 mg of vitamin C in combination with 800 IU of vitamin E exhibited normal vasodilation several hours after a single high fat meal, whereas control subjects not given the antioxidant combination showed impaired vasoreactivity. Vitamin E alone also has been shown to improve endothelium-dependent vasodilation in various patients, although a recent study found that vitamin E supplementation does not improve vasodilation in older adults.(35)

III.4 Vitamin c in the function of the vascular endothelium

Asc effects on endothelial cell proliferation and apoptosis Ascorbate has several effects on endothelial function and survival. It causes endothelial cells to proliferate and to form capillary-like structures in cell in culture. It is likely that the effect of ascorbate to stimulate endothelial cell proliferation is due to its ability to increase the synthesis of type IV collagen, since in the absence of ascorbate there is little generation of mature type IV collagen by cultured endothelial cells or of type IV collagen mRNA relative to that of elastin in blood vessels. Further, it has been shown that type IV collagen is required for both basement membrane formation and endothelial cell adhesion, processes that are not facilitated by collagen types I and III. Ascorbate also prevents apoptosis of endothelial cells in culture induced by high glucose conditions, tumor necrosis factor-α, and lipopolysaccharide. In humans with congestive heart failure, an intravenous bolus of 2.5 g of ascorbate followed by 3 days of 2g vitamin C a day decreased plasma levels of circulating endothelial apoptotic microparticles to 32% of baseline levels, an effect also significant compared to a placebo-treated control group of the same clinical characteristics. Although the foregoing evidence favors a positive effect of ascorbate on endothelial growth and survival, ascorbate has also been noted as an angiostatic factor, inhibiting formation of

capillary-like structures in cultured endothelial cells and decreasing in vivo angiogenesis in chick chorioallantoic membranes. It is likely, however, that such effects relate to use of very high ascorbate concentrations, which have been shown to inhibit angiogenesis in excised aortic rings and in subcutaneous Matrigel plugs in vivo. Also, ascorbate deficiency in mice unable to synthesize vitamin C markedly impaired angiogenesis in a transplanted tumor model. Whereas ascorbate deficiency clearly impairs endothelial proliferation and angiogenesis, the effects of ascorbate may differ with the amount of ascorbate available. (36)

III.5 The effect on iron absorption:

The mechanism of action of AA is usually related either to its ability to form soluble iron complexes or its ability to reduce ferric to ferrous ions. Both mechanisms will reduce the probability that iron ions will be strongly bound to other ligands in the intestinal content, such as hydroxyl ions, and that iron will be prevented from being absorbed. Ascorbic acid will thus counteract the influence of ligands that bind iron ions and inhibit iron absorption. The most potent dietary inhibitors known are phytates and polyphenols. The amounts in different foods of such polyphenols that inhibit iron absorption are not precisely known. Phytates can be more easily quantified but the interplay between phytates and AA in determining the bioavailability of iron is not known.(37)

IV.Ascorbic acid deficiency

IV.1 Experimental scurvy

A study has been designed to highlight the manifestations of experimental scurvy in man and to obtain additional information regarding the size of the body pool of ascorbic acid, the rate of catabolism of this vitamin, the correlation between body pool size and blood plasma levels, the relationship of all three to clinical signs and symptoms of scurvy.

Table III: Signs and symptoms of scurvy in the five subjects by giving ^{14}C-labeled ascorbic acid

subjects	Symptoms
H	Hyperkeratosis Congested follicles Petechiae Ecchymoses Subconjunctival hemorrhage Joint effusions Dyspnea on exertion Edema Femoral neuropathy
MC	hyperkeratosis Congested follicles Petechiae Ecchymoses Swollen gums Arthralgia
P	Hyperkeratosis Congested follicles Coiled hairs Petechiae Bleeding swollen gums Joint effusions
R	Hyperkeratosis Congested follicles Coiled hairs Ecchymoses Joint effusions Dyspnea on exertion
S	Hyperkeratosis Redness of gum margins Petechiae

Finally, we wished to observe the rate of repletion of body pool size and the cure of scurvy in men receiving a greater range of daily doses of this vitamin, results (clinical) despite a somewhat shorter period of deprivation in the second scurvy study, (84 to 97 days) than in the

first (99 days), the subjects in the second study developed a more severe degree of scurvy. Joint effusions, petechial hemorrhages, small ecchymotic lesions, a large subconjunctival hemorrhage and edema and dyspnea were observed in addition to the hyperkeratosis, perifollicular congestion, gum changes, and occasional petechial hemorrhages observed in the first study. The signs and symptoms of scurvy noted in each subject are listed in Table III. (38)

IV.2 Some behavioral effects of ascorbic acid deficiency:

in a study of experimental human scurvy, detailed observations were made on the effects on behavior of deprivation of ascorbic acid. During deprivation, changes were found to occur in measures of personality and psychomotor performance, and in certain physical fitness tasks. Scores in the "neurotic triad" of the Minnesota Multiphasic Personality Inventory (a psychological test that assesses personality traits and psychopathology. It is primarily intended to test people who are suspected of having mental health or other clinical issues.)

the Hypochondriasis, Depression, and Hysteria scales became elevated as deficiency of ascorbic acid progressed, the personality changes preceded decrements in psychomotor performance that were associated with the reduced arousal or motivational level present during scurvy.

Impaired performance in measures of physical fitness occurred in those tasks that involved use of the legs and was due to scorbutic arthropathy or neuropathy, or both.(39)

IV.3 The manifestations of the lack of vitamin c:

The experimental scurvy model showed the delay of the apparition of clinical signs. If the restrictive diet begins at day0, ascorbemia becomes zero at day 41, depletion cell at day 121 and the first skin signs at day 132. The dental abnormalities do not appear until after six months, the clinical picture is constituted in one to three months of absolute deficiency when the total pool of the organism is less than 300 mg and that the ascorbemia below 2 to 2.5 mg/l, by creating an experimental scurvy in five patients, the first clinical signs of scurvy appear when ascorbemia is between 1.3 and 2.4 mg/l.(40)

Table IV: Clinical and biological manifestations of scurvy(40)

General signs
Asthenia anorexia
Osteoarticular manifestations
Arthralgia
Myalgias
Hemarthrosis
In the child:
Bone pain (sub-periosteal bleeding)
X-rays of the peri osteoplasty sleeve, enlargement of the extremity
Anterior to the ribs.
Hemorrhagic syndrome
Purpura
Ecchymoses
Hematomas

Hemarthrosis
Bleeding from nerve sheaths
Cerebral hemorrhages, gynecological
Stomatological manifestations
Hypertrophic and hemorrhagic gingivitis (absent in case of edentation)
Parodontolysis
Fall of teeth
Skin manifestations
Follicular hyperkeratosis
Pigmented ichthyosis
Swelling of the lower limbs
Damage to hair: «Corkscrew hair», alopecia.
Other
Dry syndrome
Parotid hypertrophy
Psychiatric disorders: depression
Cellular immunity deficiency and phagocytosis disorders
Convulsions
Heart damage
ST segment and T-wave changes
Sudden death
Biological signs
Anemia
Leukopenia
Hypocholesterolemia
Hypoalbuminemia
Ascorbemia less than 2 mg/l; decrease in leucocytic ascorbic acid.

V.Smoking and ascorbic acid:

V.1 Effect of smoking on plasma AA concentrations

Cigarette smoke contains a range of xenobiotics, including oxidants and free radicals that can increase lipid peroxidation. One estimate suggests cigarette smoke contains on the order of 10^4free radicals per inhalation. Free radicals are capable of directly and indirectly inducing oxidative stress in the body. Since ascorbic acid is a critical component of the body's antioxidant defense mechanisms, it has been investigated assessing a range of biomarkers of oxidative stress in smokers.(41)

An association between smoking and lower levels of serum ascorbic acid has been observed repeatedly, one explanation for lower serum levels of ascorbic acid observed in smokers would be an inadequate dietary intake. Among other plausible explanations, the lower serum ascorbic acid levels observed in smokers might reflect an altered metabolism of ascorbic acid. Decreased absorption, increased metabolic demand, increased elimination, or any

combination of these factors might influence the metabolic fate of ascorbic acid, resulting in the consistent observation of lower serum levels in the smoking population.(41)

Tobacco smoke contains a large number of toxic chemicals such as Nox and other oxidizing radicals that cause damage to cellular functions The high oxidant content of smoke explains the low antioxidant status and increased oxidative stress and damage that is consistently observed in smokers. On this basis, it has been suggested that smokers in particular would benefit from increasing their dietary intake of antioxidants.(42)

A combination of increased oxidative stress from tobacco smoke and low ascorbate plasma concentrations could lead to elevation of DHAA in plasma. Consequently, the ratio of DHAA to total ascorbate could be a marker of oxidative stress.(43)

A study shows relations between smoking status, sex, and ascorbic acid. The concentration of TAA (total ascorbic acid in plasma was lower in smokers than in nonsmokers and higher in women than in men, analysis of covariance showed that sex and smoking were significant independent predictors of plasma ascorbic acid concentrations whereas age was not a significant covariate. The concentration of DHAA was higher in smokers (0.78 ± 2.32 µmol/L) than in nonsmokers (0. 1 1 ± 2.36 µmoL/l) ($P < 0.05$). Further analysis of the factors predicting DHAA concentrations were done by analysis of covariance with sex and smoking as independent variables and age as a covariate. Regardless of whether the DHAA (dehydroascorbic acid) concentration or the fraction of DHAA (%DHAA) of total vitamin C was used as a dependent variable, here was a significant effect of smoking (P 0.0009 and P 0.048, respectively) but not of age or sex ($P = 0.59$ and $P = 0.58$, respectively). In these analyses the negative estimates of DHAA were used; however, if these negative values were substituted with zero the significance of the effect of smoking persisted as a significant difference (43)

V .2 Effect of smoking cessation on plasma ascorbic acid concentration

Numerous studies have shown that cigarette smokers have a lower plasma concentration of ascorbic acid than non-smokers, but only a few have considered the antioxidant status of ex-smokers.' This study reports the first controlled study monitoring the early effect of smoking cessation on the concentration of ascorbic acid in plasma. (44)

VI.Dosage of ascorbic acid:

VI.1 Titrimetric method:

Ascorbic acid is a very powerful reducing agent that oxidizes very quickly, especially at high temperatures and in alkaline solutions, the dosage of vitamin C is done by titrimetry using a

0.1 N iodine solution. starch with an iodine molecule reacting with a vitamin C molecule according to the following reaction: $C_6H_8O_6 + I_2 \leftrightarrow C_6H_6O_6 + 2HI$

Before dosing, a defecation is essential using the basic acetate of Pb (10%) to eliminate macromolecules. When there are no more molecules of vitamin C, the iodine molecules will accumulate in the solution. This accumulation indicates the end of titration and it is highlighted by the formation of a blue-violet compound of high intensity. This compound is formed by iodine and starch.(45)

VI.2 Spectrophotometric method:

Vitamin C has an absorption spectrum in aqueous solution with maximum absorption at 245nm. This technique requires the use of a high-performance spectrophotometer, relatively pure solutions of ascorbic acid and a high enough concentration to obtain readable results. The spectrophotometer is set to a wavelength $\lambda = 245$ nm, the blanked reagent is placed in the spectrophotometer, it is a cell of distilled water. The reading is done directly at the spectrophotometer. From the calibration curve obtained previously, the ascorbic acid concentration for each dilution is deduced.(46)

VI.3 Electrochemical methods

Many polarographic methods including that based on the reduction waves obtained from the condensation products of dehydroascorbic acid with *o*-phenylene diamine have been introduced for the determination of Vitamin C. The use of equimolar or lower concentration of *o*-phenylene diamine gives a dominant wave for dehydroascorbic acid where reductones,diketogluconic acid and reduced sulfhydryl compounds do not interfere.(47)

VI.4 *Chemiluminescent* methods

Different systems involving Cu(II)–luminol,Ce(IV)–rhodamine 6G, Fe(II)–luminol–O_2, $KMnO_4$–luminol, H_2O_2–hemin–luminol and H_2O_2–luminol–peroxidase CL form the basis of many chemiluminescent methods for ascorbic acid . The detection limit lies in the range $(1–6.2)10^{-7}$ mol l^{-1}
and the upper limit of the linear response range varies from 2.10^{-6} to 6.10^{-5} mol l^{-1} ascorbic acid.
Such methods have been used in the analysis of fruits, juices, vegetables etc.(47)

VI.5 Kinetic methods

Kinetics in analytical chemistry have been of limited importance except where a reaction is slow to reach equilibrium. Consequently, only a few kinetic methods have been developed for the determination of Vitamin C. Kinetic spectrophotometric methods with or without the application of stopped flow have been proposed using the reducing effect of ascorbic acid on DCIP (Dichlorophenolindophenol) (_maxD522 nm) or toluidine blue (_maxD600 nm). However, these methods are time-consuming as they take at least 20 min for one determination. (47)

VI.6 Infrared spectroscopy:

Fourier transform infrared spectroscopy is based on the absorption of infrared radiation through the sample. It makes it possible to identify the chemical functions present thanks to

the detection of the vibration's characteristic of the chemical bonds. This technique allows a qualitative characterization from its spectral and quantitative signature allowing the determination of a substance at very low levels.(48) (figure8)

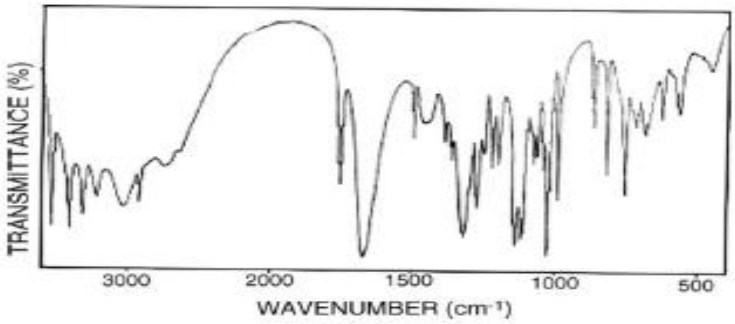


Figure 8: Specter FT-IR of ascorbic acid

VI.7 The nuclear magnetic resonance:

Allows the characterization of the structure of the molecular compound or compounds present in the analyzed sample. By means of tables, the chemical movements of the peaks can be identified in a grouping to identify the species analyzed. The NMR (Nuclear Magnetic Resonance) of proton 1h and 13c is the most used, 3d spectra can also be used to determine the molecular structure of more complex species. The NMR thus allows a qualitative but also quantitative analysis by assessing the proportion of the species according to the height of the peak in comparison with another species.(48)

VII.Applications of vitamin c:

VII.1 Antioxidant properties of ascorbic acid in foods

- Ascorbic acid was used to prevent the oxidation of olive oil, milk, arachis nut oil, lard, ethyl ester of lard, cottonseed oil, pork, and beef fat the role of ascorbic acid in enzyme systems has been know n for a long time. Th e ascorbic acid requirement was reviewed along with a-ketoglutaric acid, oxygen, and fe^{2+} in propyl and lysyl hydroxylases and the possibility that ascorbic acid's function is to keep to iron as fe^{2+} (30). More recently researchers claim that ascorbic acid does not participate in the hydroxylation reaction but is specifically required to keep the enzyme fe^{2+} in the reduced form***.(49)***
- There are several additives arisen from the ascorbic acid that nowadays we can find in trade: e300, ascorbic acid; e301, sodium ascorbate; e302, calcium ascorbate; e303, potassium ascorbate; e304, fatty acid esters of ascorbic acid (ascorbyl palmitate and ascorbyl stearate). These additives use the ascorbic acid itself (e300) or in the form of salts (e301, e302, e303) or lipophilic esters. Lipophilic esters (e304) are arisen with long fatty acid's chain to be able to use the effects of ascorbic acid even in lipidic foods, preventing the rancidification.(50)
- All additives based on ascorbic acid – except for the e303 potassium ascorbate – are approved in Europe, USA, Australia and New Zealand. In these nations the use of e303 is also approved. Additives, based on ascorbic acid, are used in production and transformation phases of several foods such as beer, gelatins, jam, sweets, bread and

baked products, fruit juices, wine, fishing products and meats. The use of additives based on ascorbic acid is approved by current regulations, even in products for infants and children's feeding.(50)

VII.2 Protecting the skin from free radicals:

- Vitamin C is a water-soluble AOx (antioxidant) and it is the predominant AOx in the skin based on molar concentrations.54 Vitamin C neutralizes free radicals in aqueous compartments of the skin, and also plays a role in regenerating vitamin E. Aside from serving as an AOx, it is also a cofactor for critical enzymes in collagen synthesis and can inhibit elastin biosynthesis to reduce elastin accumulation.56 It also reduces pigment darkening by inhibiting tyrosinase and maintains hydration by protecting the epidermal barrier of the skin.57 At the molecular level, addition of topical 1% vitamin C increases collagen synthesis and reduces MMP(matrix metalloproteinase) expression. (51) (figure 9)

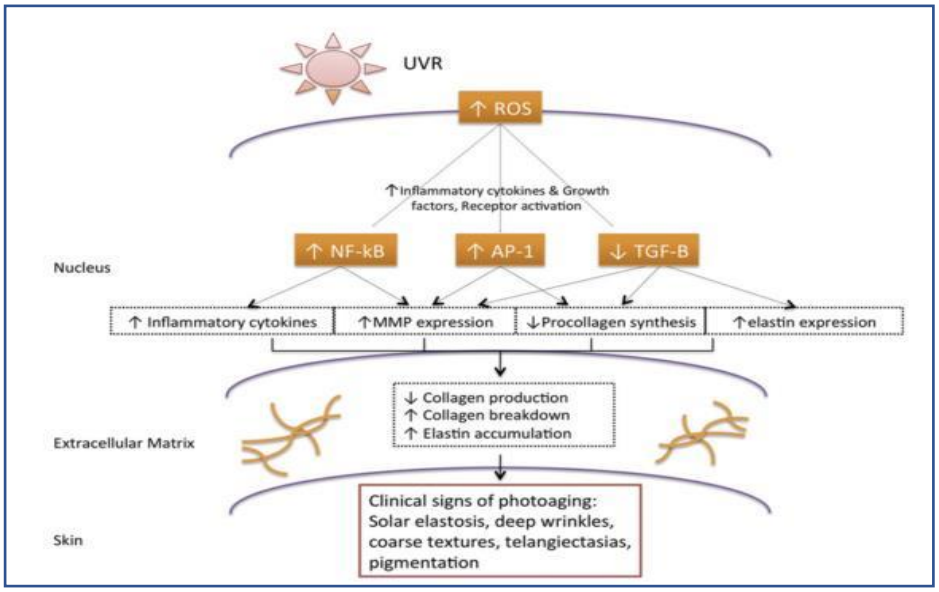


Figure 9: Role of reactive oxygen species in photoaging

VII.3 Drug Nanoparticle Formulation Using Ascorbic Acid Derivatives:

- Hydrophilic ascorbic acid derivatives have been used not only as antioxidants but also as food or pharmaceutical excipients. They are usually loaded into a nanoparticle formulation to prevent oxidation of the drugs and the components. Ascorbyl n-alkyl fatty acid derivatives have been well investigated as antioxidants for nanoparticle formulations, such as micelles, microemulsions, and liposomes.(52)

Research and methodology:

I. Objective:

Ascorbic acid is the most abundant and effective antioxidant in human plasma, it is considered to be essential for oxidative stress-related illnesses and degenerative processes.

Cigarette smoking has been linked to a decrease in the amount of ascorbic acid in the body for numerous years.

A study in this direction is necessary which sets as its objective:

- Determination of ascorbic acid levels in two groups of people smokers and non-smokers
- Observing a correlation link between smokers and lower plasma levels of ascorbic acid

II.Material and method:

II. 1. Type of study:

This is an observational, descriptive with an analytical focus ,comparative study, in which two existing groups differing in outcome are identified and compared on the basis of some supposed causal attribute

This study took place at the laboratory of toxicology in the department of pharmacy at Tlemcen Faculty of Medicine and in Toxi-Med Laboratory, over a nine-month period from October 2021 to June 2022.

II. 2. Study population:

- Group of smokers:

Healthy adults with no chronical or terminal illnesses, that are regularly smoking. with a history of consuming cigarettes or newly taking them.

- Group of non-smokers:

They are free from any pathology, each case was matched to a control, same sex, same age ±2 years and same body mass index (BMI).

II. 2. 1. Eligibility criteria:

Any person residing in Algeria may respond to the form and with a full agreement to participate in the study that we conducted.

II.2.2. Ineligibility criteria:

- Excluded from the study, for cases and witnesses
- Viral infection
- Current or historical chronic health problems
- Antioxidant supplements; or receiving prescription medication.

- Scarcity of clinical information.
- Consumption of alcoholic drinks.

II. 3. Ethical:

The study procedure was explained to the subjects, and they gave their free and informed consent to participate in the study.

II.4. Data collection:

Data collection (smokers and non-smokers) was done using a fact sheet with 03 components:

- Patient identification.
- Fagerstrom test for nicotine dependence (FTND) (test for nicotine dependence is a standard instrument for assessing the intensity of physical addiction to nicotine)
- Food survey. (Annex 1)

II.5. Collecting and storing samples.

Four (4) ml of venous blood was collected from each individual between 08:30 and 10:30h. into citrate tubes, the serum was separated to be conserved at 4°c for vitamin c determination (With-in 24 hours) and kept frozen at -80°C for delayed or rechecking analysis.

II.6. Parameter studied:

II.6.1. Main parameter:

Ascorbic acid.

Table V: Amount of intake for sufficient and deficiency states of ascorbic acid(53)

Normal value	Deficiency	Intake
0.5-2 mg/dL.	less than 0.3mg/dl	≥0.6 mg/dl

II. 7. Determination of ascorbic acid:

The AA had been dosed in plasma.

II. 7. 1. Reagents used:

The standard reagents used for analysis often contain trace impurities that are not important for most analytical uses, but may be too contaminant for trace and ultra-trace analysis. It is therefore necessary to use pure parent reagents of:

- Trichloroacetic acid (TCA)10%.
- Ascorbic acid analytical grade.
- Folin reactive 10%.

All solutions were prepared with ultra-pure water.

II.7.2. Machinery and equipment:

The analysis of the samples was realized in OPTIZEN POP (spectrophotometer) ,it can measure the transmittance or absorbance at each wavelength of a sample in ultraviolet and visible light bands to determine the quantitative characteristics such as concentration and purity. OPTIZEN POP, which can be used widely from general analytical experiments to professional research fields, guarantees accurate measurement and excellent reproducibility and provides reliable results in various fields such as the environment, biotechnology, and chemistry it also provides four measurement modes (Photometric Mode, Quantitation Mode, Spectrum Mode and Kinetics Mode), and users can select a mode according to the purpose of the measurement. The embedded S/W built into the equipment, touch screen interface and applications make it very easy for users to use the equipment. (table VI)

Table VI: Specifications of the device

Optical System	Single Beam Type
Spectral Band Pass Width	< 1.8nm
Wavelength	
Range	190 ~ 1100nm
Accuracy	<± 0.5nm (at D_2 peak 656.1nm, 486.0nm)
Reproducibility	<± 0.1nm
Setting	≤ 0.1nm
Slew Rate	About 7,800nm/min
Scanning Speed	Max. 4,000nm/min
Photometric	
Range	-3.0 ~ 3.0 ABS (Enable to Set Up)
Accuracy	± 0.005 ABS (at 1.0 ABS)
Reproducibility	± 0.003 ABS (at 1.0 ABS)
Stray Light	< 0.05%T (220nm, 340nm)
Baseline Stability	< ± 0.001 ABS/h (at 550nm)
Baseline Flatness	< ± 0.002 ABS (200- 1100nm)
Monochomater	Modified Czerny- Turner type with 1200 lines/mm blazed Grating
Light Source	Tungsten-halogen & Deuterium Lamp
Lamp Change Wavelength	340 - 410nm (Default 370nm), including Auto Position System
Standard Cell Holder	Rotary type 8 position Multi Cell Holder
Interface Ports	4 USB ports / 3 RS-323C ports
Power Requirement	Free Voltage

II. 7. 3. The method's concept:

The absorption maximum of the color developed by the interaction of ascorbic acid with Folin reagent is 769 nm. The technique obeys the Beer-Lambert law up to a concentration of 45 µg ascorbic acid as shown by the standard curve. The color developed (blue component) (annex 2) has been found to be stable up to 18 h. Recovery experiments showed that the technique is almost 100% efficient. The development of the color is not obstructed by glucose, glutathione, bovine serum albumin, urea, cysteine, adenine, guanine, cytosine, uracil, sulfosalicylic acid, thymol, or oxyhemoglobin, which are compounds suspected of interfering in routine analysis. The technique is simple, quick, and efficient and can be employed for the estimation of ascorbic acid in a wide variety of biological materials.(54)

II. 7. 4. Protocol of the assay:

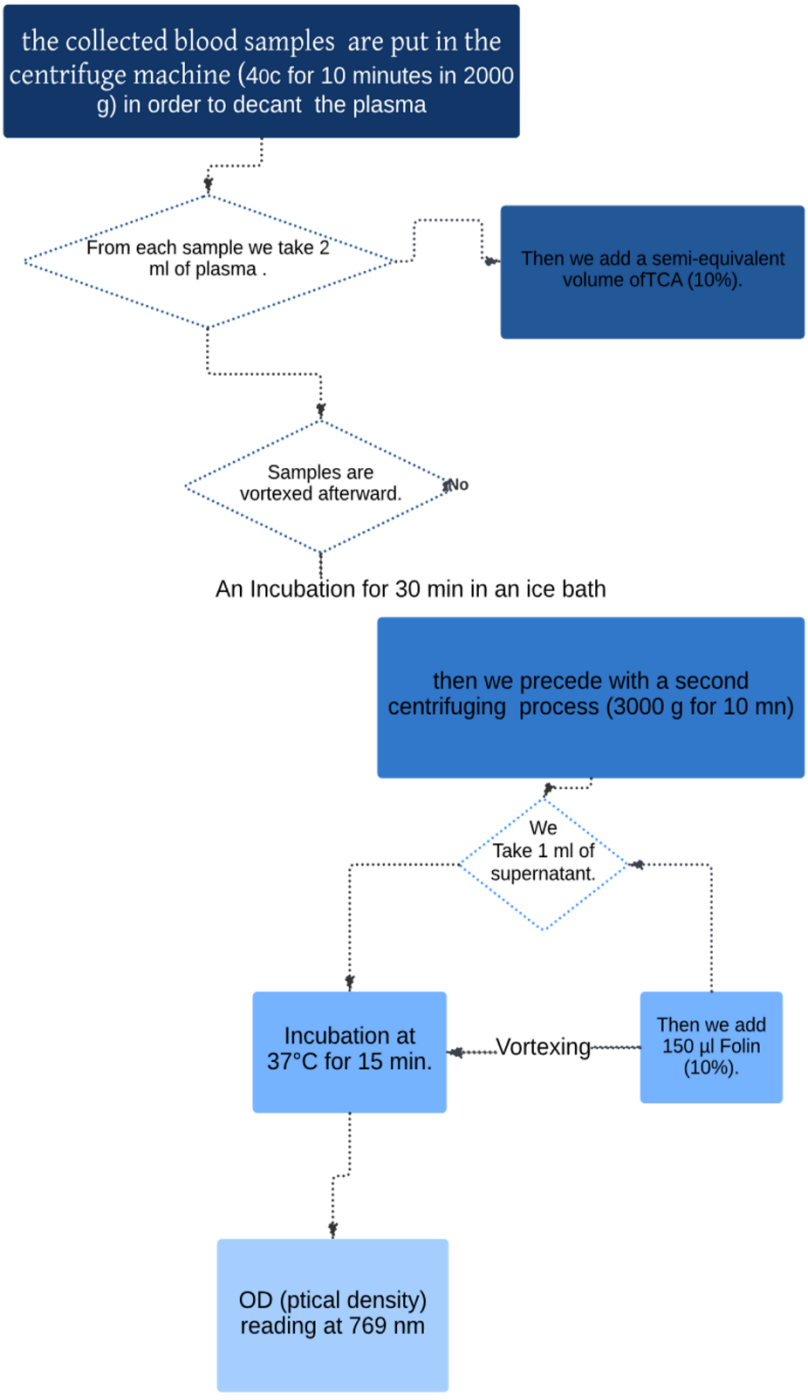


Figure 10: Different stages of the analysis

II. 7. 5. Method validation:

In order to certify our protocol a calibration range been prepared 3 times and the analysis process lasted for 3 days.

A curve of calibration was designed on the basis of the general means founded during the assay, demonstrated on figure 11.

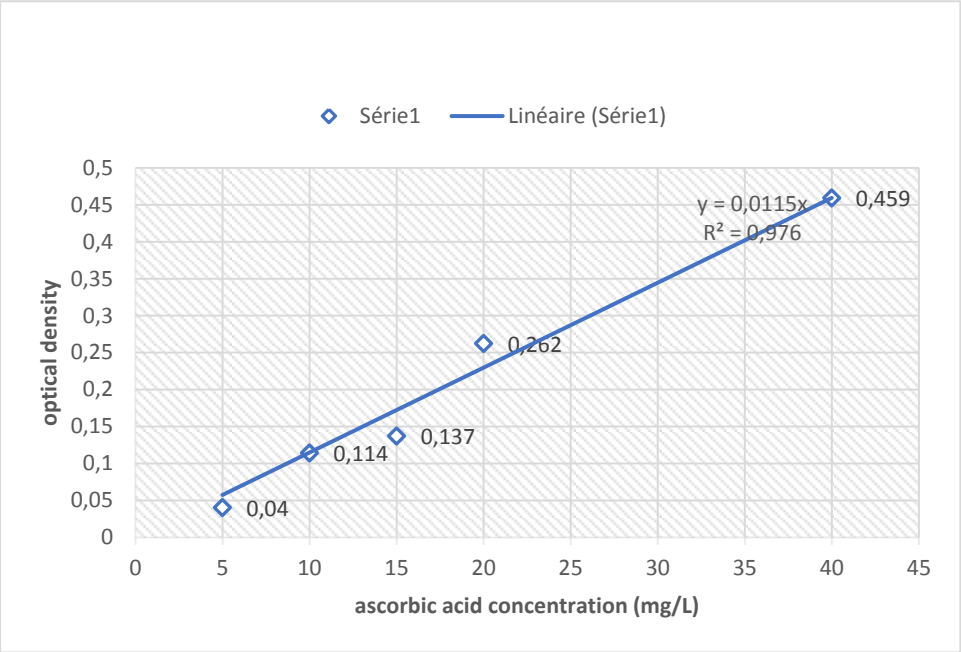


Figure 11: Curve of calibration of three days

II. 7. 6. Statistical analysis of data:

The results are expressed by the arithmetic mean plus or minus the standard deviation. Due to the normality of the distribution, the choice was on parametric tests the student t test to compare between two averages.

To assess the degree of relationship between ascorbic acid levels and smoking, we determined the Pearson correlation coefficient" r".

Excel 2007 and SPSS version 21 were used.

III.Results:

III.1 Descriptive characteristics of the population:

III.1.1 Socio-demographic data:

III.1.1.1 Geographical origin:

This study has been conducted on 62 participants.

The majority of participants are from the wilaya of TLEMCEN (77 %) and the wilaya of NAAMA (13%), demonstrated on figure 12.

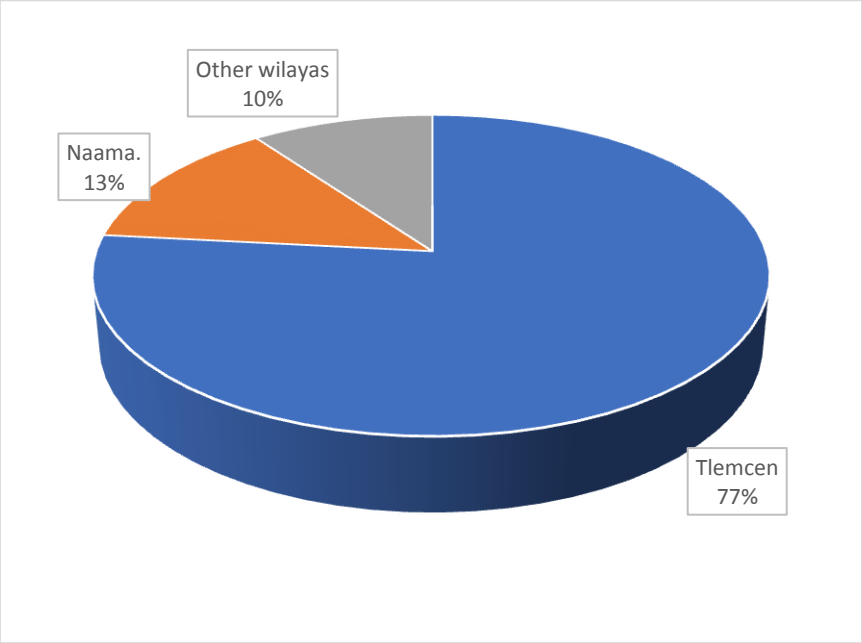


Figure 12: Distribution of the population according to geographical origin.

III.1.1.2 Age:

The age of study participants ranges from 19 years old to 65 years old, the average age is 33.62years old ± 13.44, demonstrated on figure 13

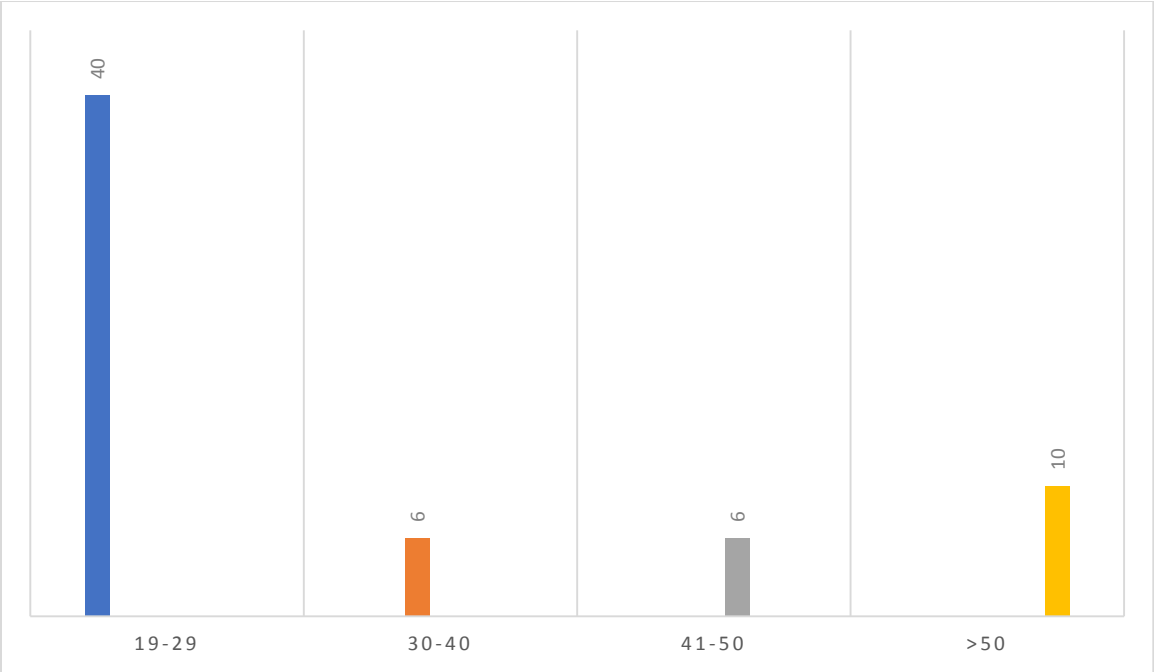


Figure 13: Distribution of the population by age.

III.1.1.2 Gender:

The study is conducted only on a population of males (62participants), demonstrated on figure14.

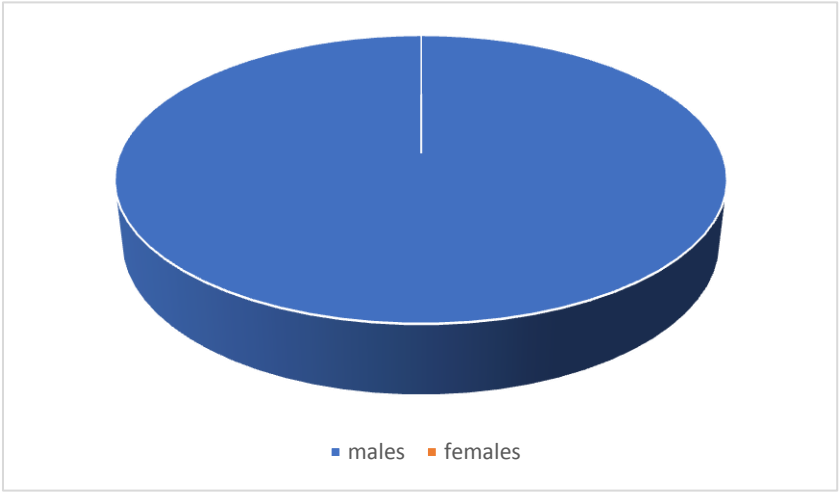


Figure 14: Distribution of the population by gender.

III.1.1.3 Education Level:

This study included 62 individuals which 66.12% of them have a diploma degree and 24.19% had an education in high school, demonstrated on figure15.

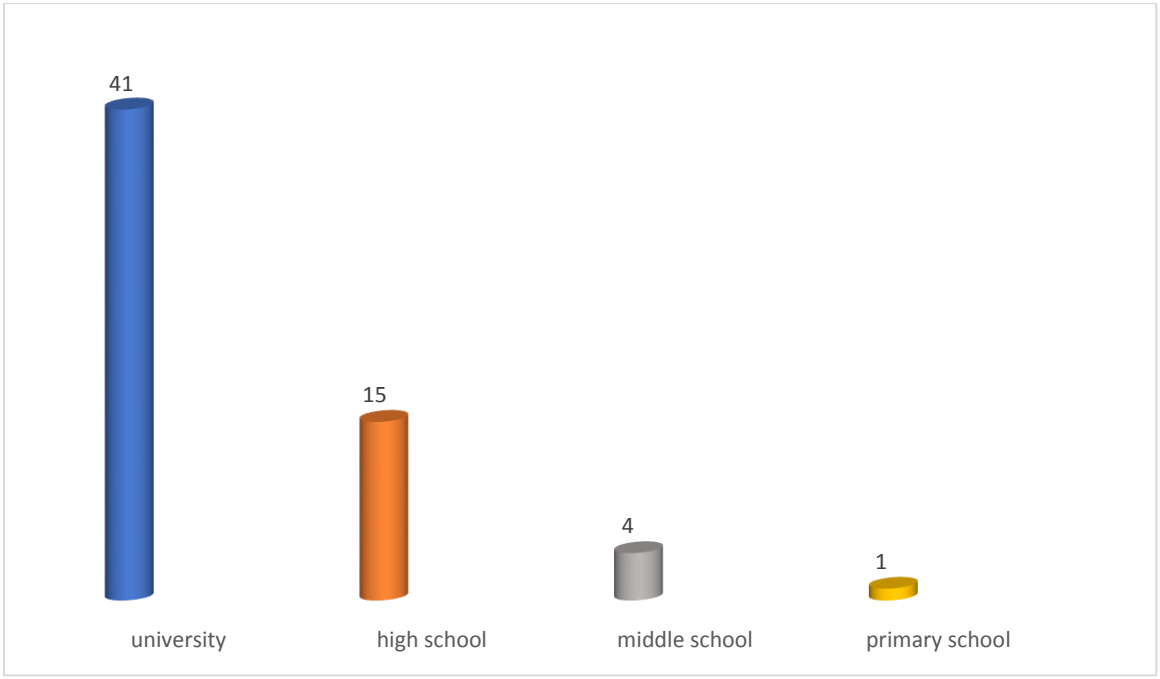


Figure 15: Distribution of persons by level of education.

III.2 Fagerstrom Test for Nicotine Dependence (FTND) Results:

The fagerström test for nicotine dependence is a standard instrument for assessing the intensity of physical addiction to nicotine. The test was designed to provide an ordinal measure of nicotine dependence related to cigarette smoking.

III.2.1 Number of cigarettes per day:

We note on this study groups that 47% belongs to class Ⅱ (16-25 cigarette), another 41% consume less than 16 cigarettes a day, while only 12% take more than 25, demonstrated on figure 16.

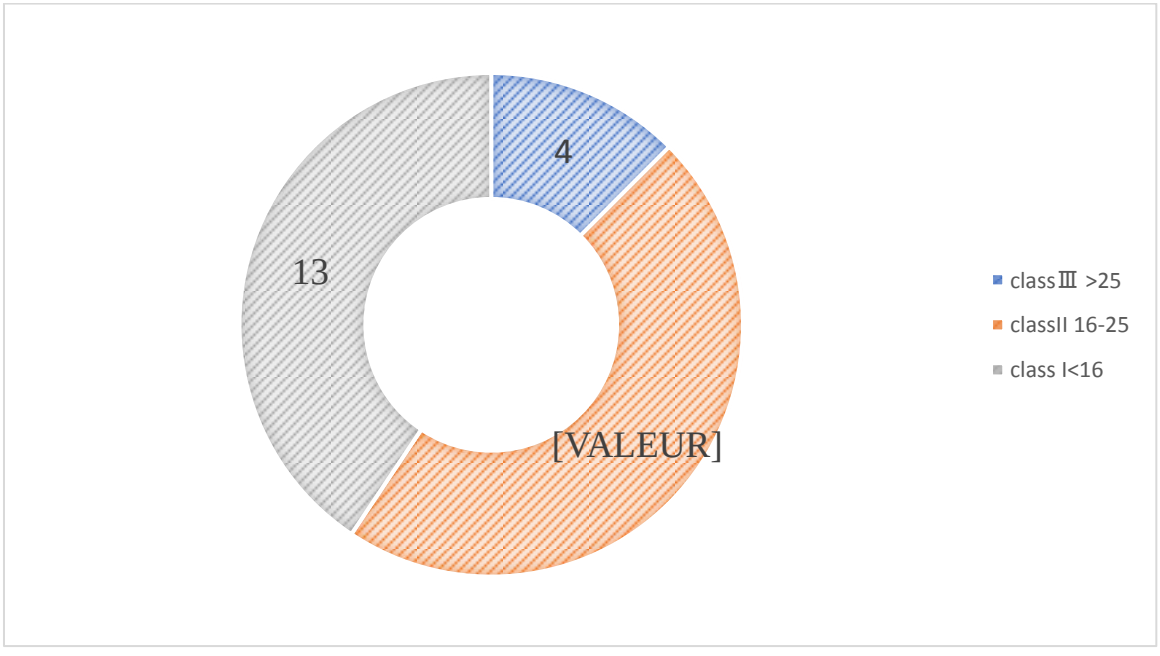


Figure 16: Distribution of the smoking population by number of cigarettes per day.

III.2.2 Nicotine levels contained in the cigarettes in milligram:

We remark that 46.87% of the smokers group consume cigarettes that contain a nicotine level between (0.8-1.5) mg, and 37.5% take cigarettes with nicotine amount less than 0.8mg, demonstrated on figure17.

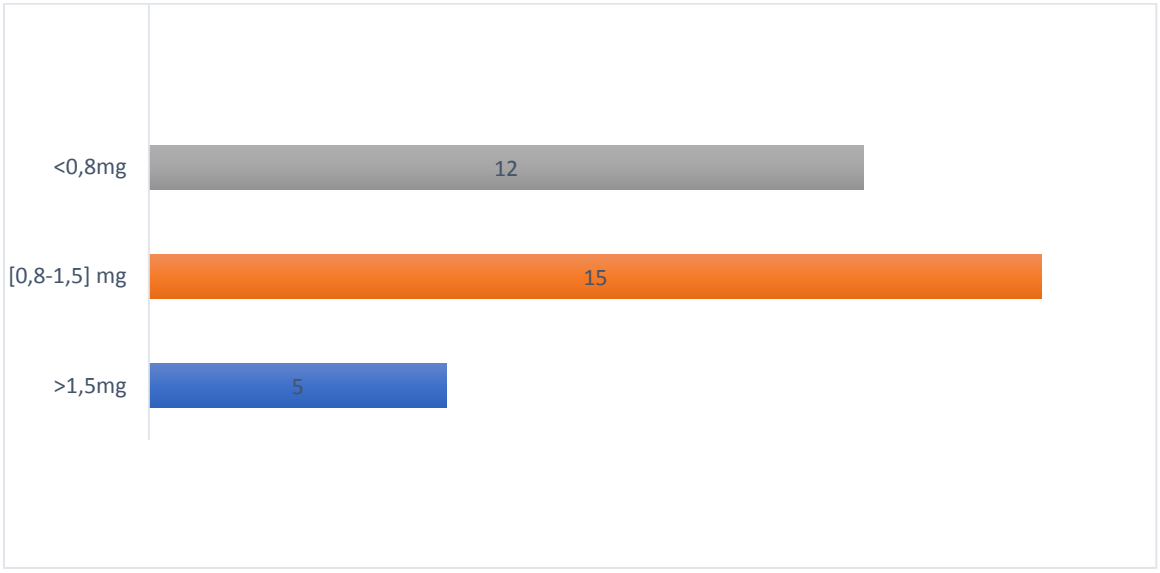


Figure 17: Nicotine Levels in Types of cigarettes consumed by participants

III.2.3 Frequency of inhaling cigarettes smokes:

The majority of the smoking participants (63%) constantly inhale their cigarettes smokes, demonstrated on figure18.

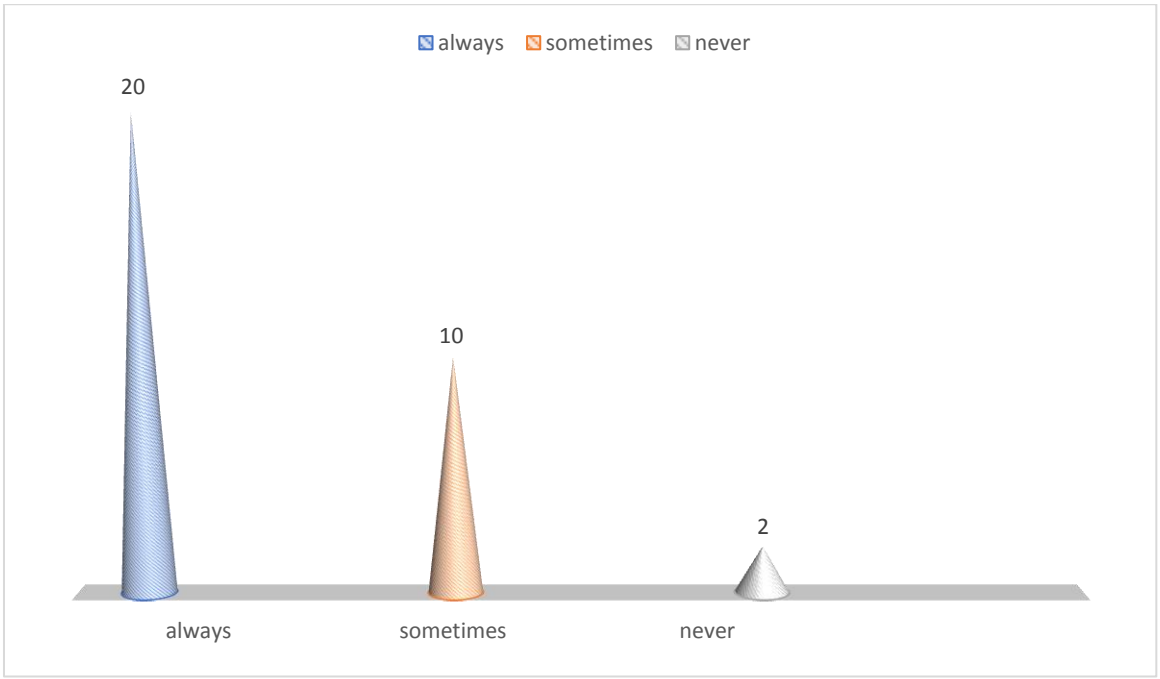


Figure 18: Frequency of inhaling cigarettes smokes according to participants.

III.2.4 Do you smoke more frequently during the first hours after waking than during the rest of the day?

We remark that 45.2% of participants smoke during the morning, while 29% prefer the evening, demonstrated on figure19.

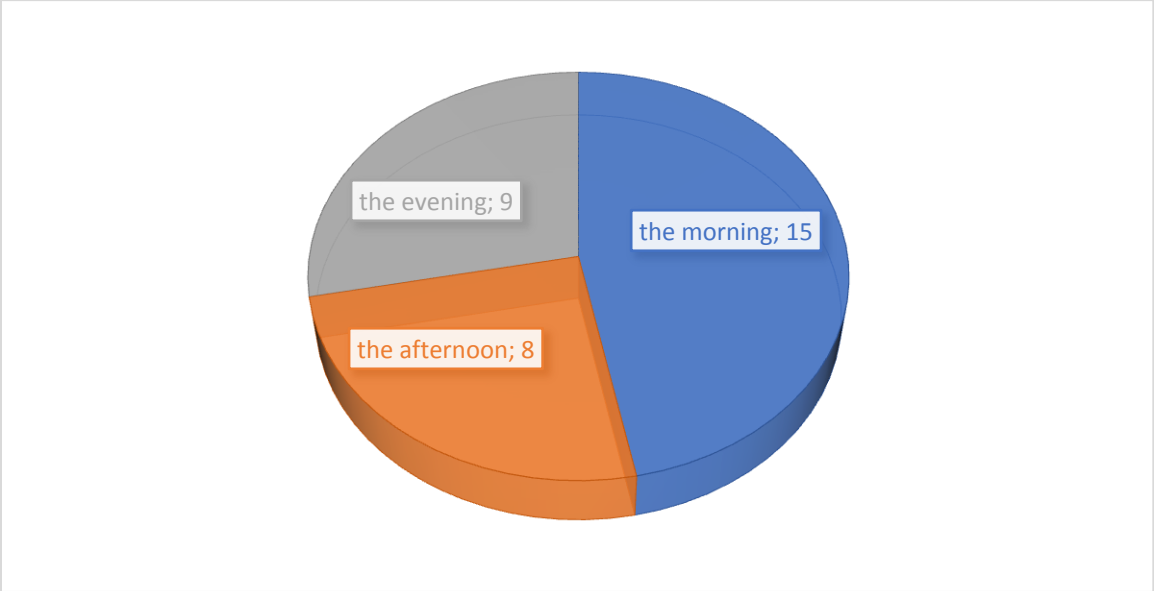


Figure 19: Distribution of the population by smoking periods.

III.2.5 How soon after you wake up do you smoke your first cigarette?

According to the obtained results there are 20participants of 30 take their first cigarette 30 minutes after awakening while the others (12) before 30 minutes, demonstrated on figure20.

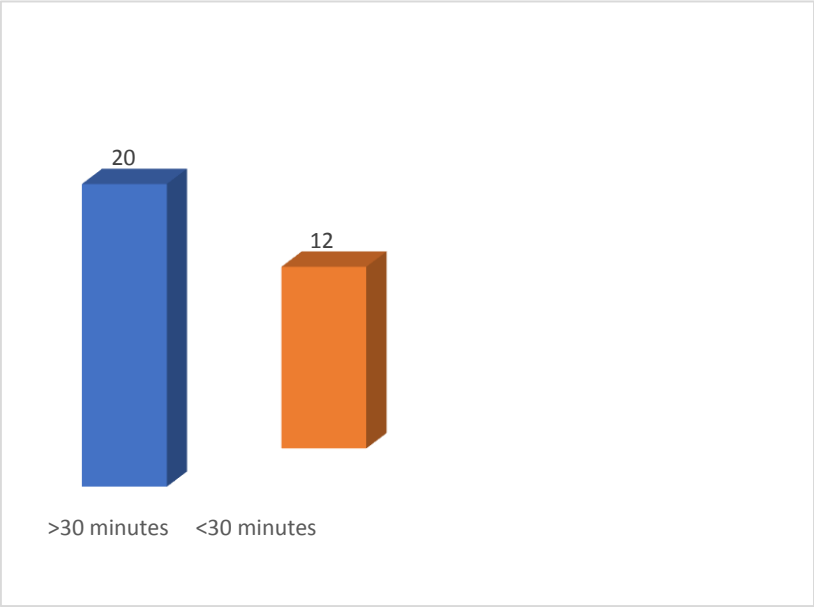


Figure 20: Distribution of the population by smoking gap after awakening.

III.2.6 Which cigarette would you hate most to give up?

We observe that 69% of participants find the first cigarette to be the most essential, demonstrated on figure21.

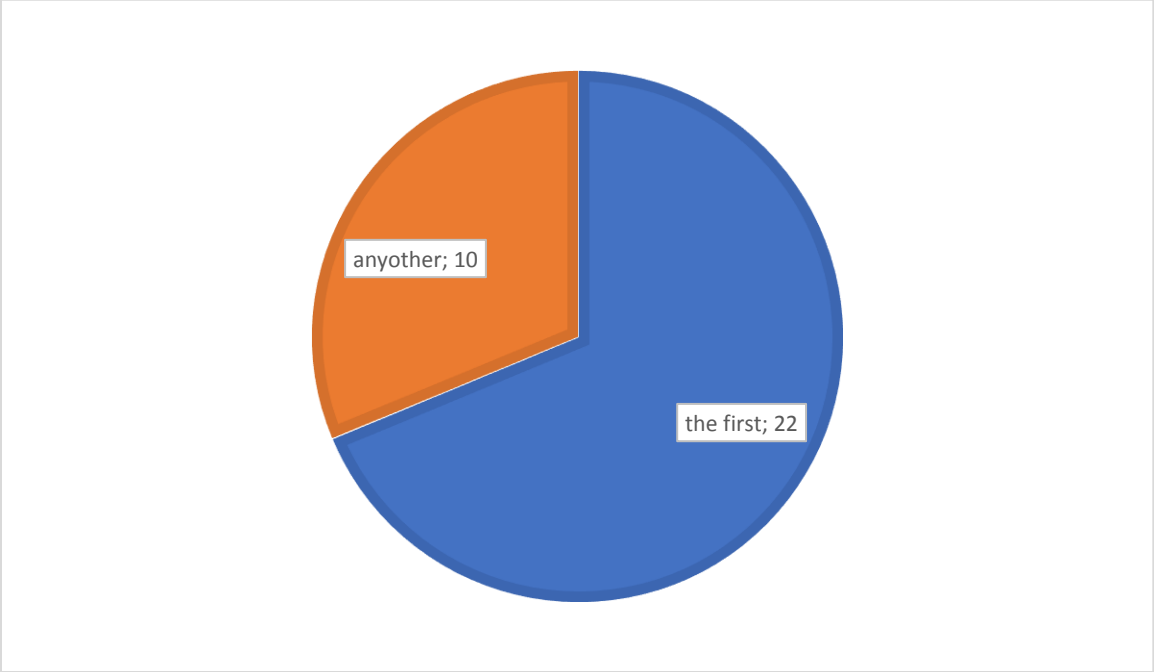


Figure 21: Repartition of the most necessary cigarette for the candidates.

III.2.7 Do you find it difficult to refrain from smoking in places where it is forbidden?

The majority of participants do not find any difficulties to abstain smoking in prohibited places, while 22% can't resist the urge of smoking in those places, demonstrated on figure22.

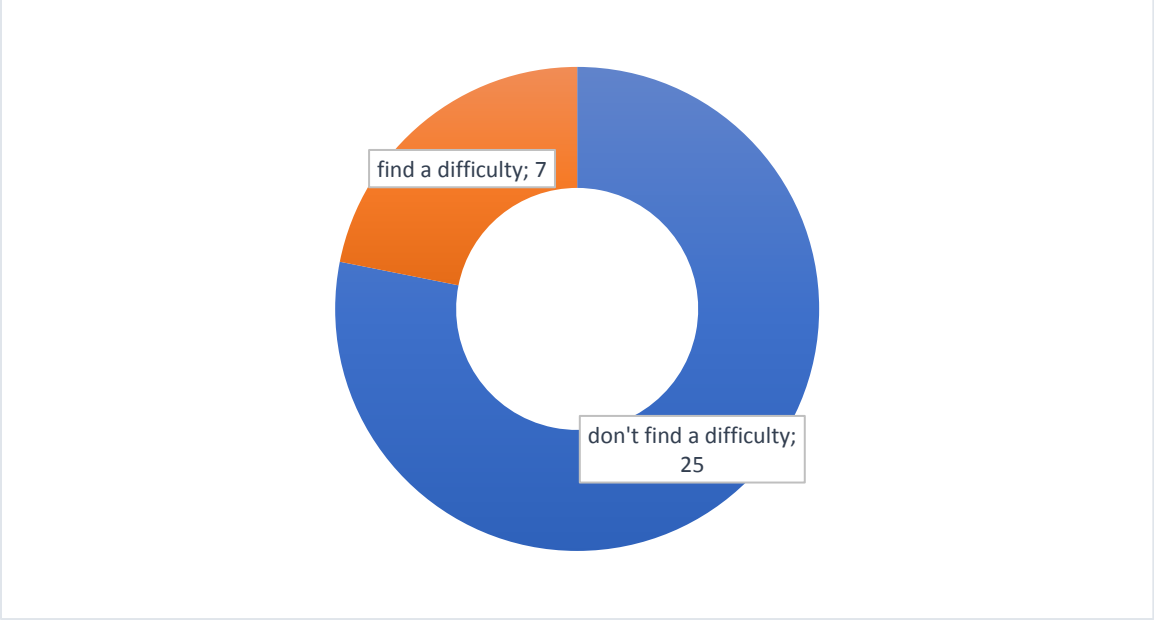


Figure 22: Distribution of participants according to the toughness to not smoke on prohibited places.

III.2.8 Do you smoke when you are so ill that you are in bed most of the day?

Responses shows that 25% (8) of the population smoke even if they are experiencing a sickness that demand staying in bed, demonstrated on figure23.

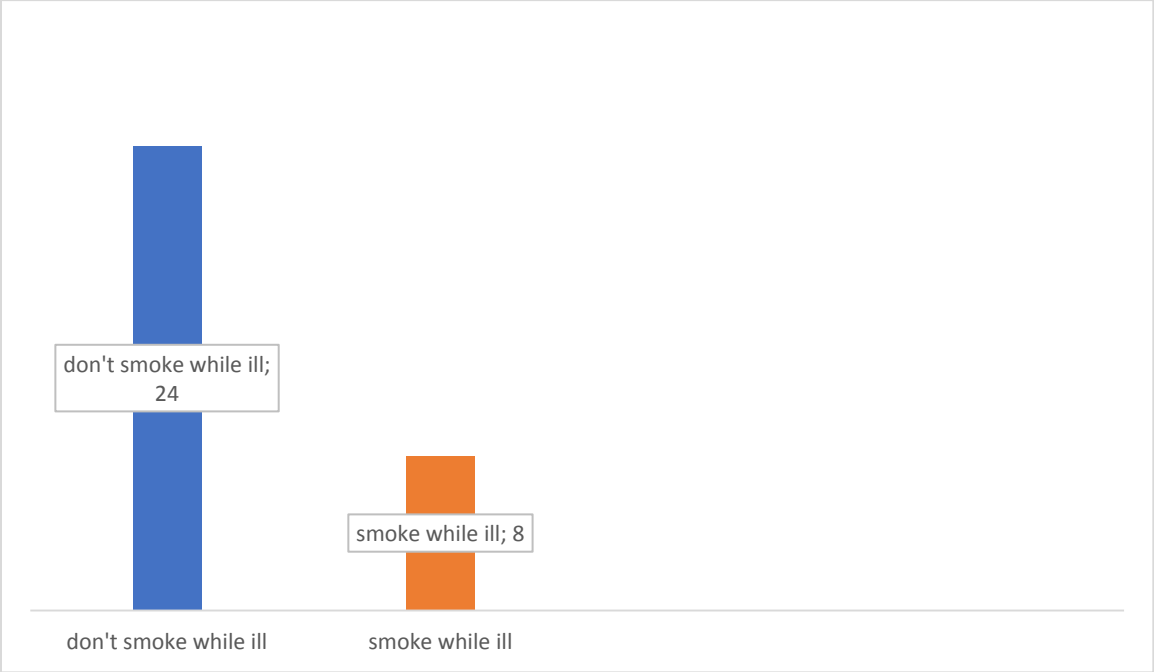


Figure 23: Apportioning of the population according to the decision of smoking while having an illness.

III.2.9 Supplementing on vitamin C:

Among the totality of the two groups 85% do not take any supplementation on ascorbic acid, the rest 15% did consume a sort of supplementation that contained vitamin c, demonstrated on figure24.

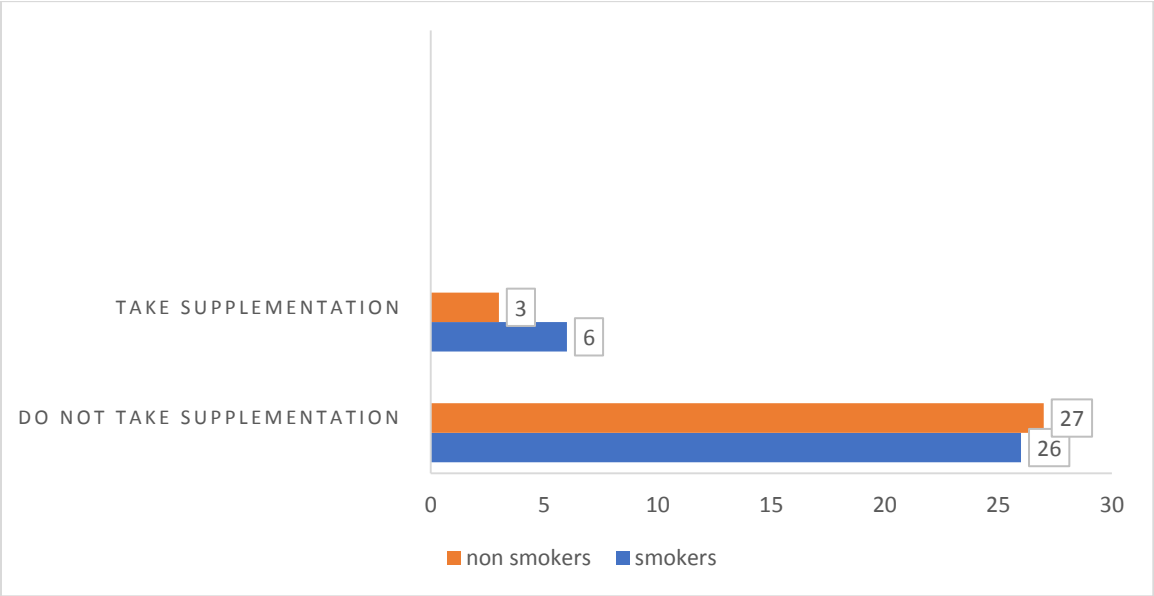


Figure 24: Partition of the two groups based on smokers, non-smokers and taking supplementation or not.

III.3 Survey on nutrition that include considerable amount of vitamin c:

In the following titles a graphic demonstration about the frequency of intake of some nutriments according to the participants of this study

III.3.1 Pepper:

We notice that 58% of participants eat pepper occasionally and 24.2% on a regular basis, , demonstrated on figure 26.

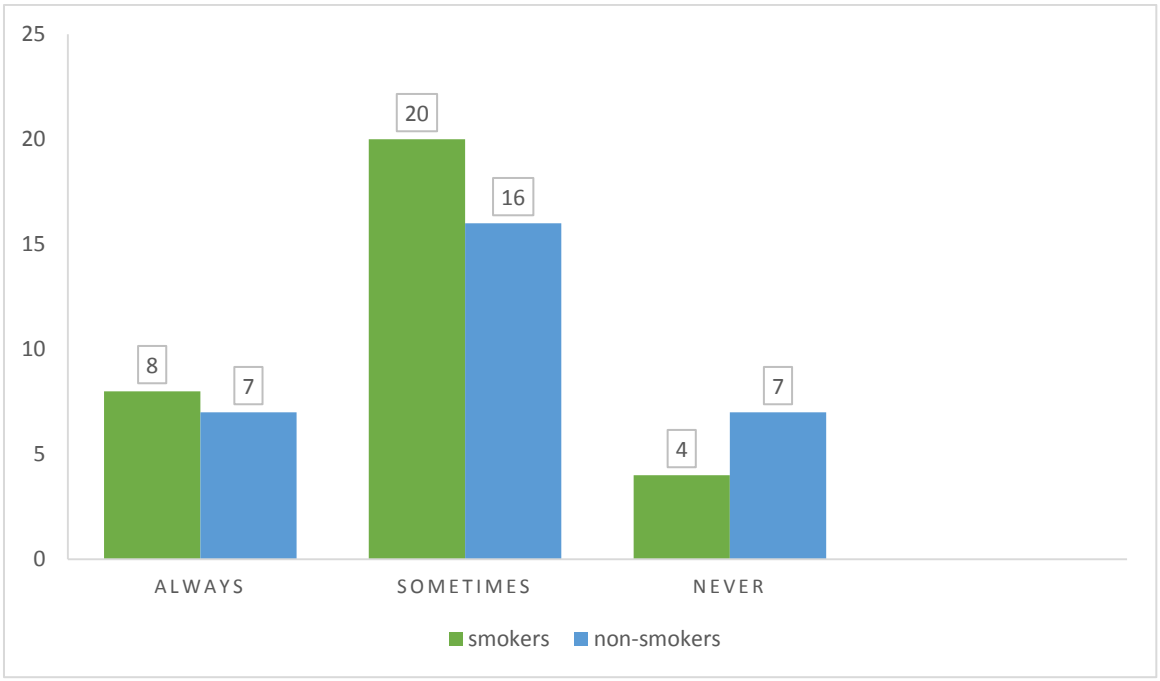


Figure 25: Frequency of eating pepper according to the participants.

III.3.2 OLIVES:

We note that 11.29% of candidates excludes olives from their die, demonstrated on figure 26.

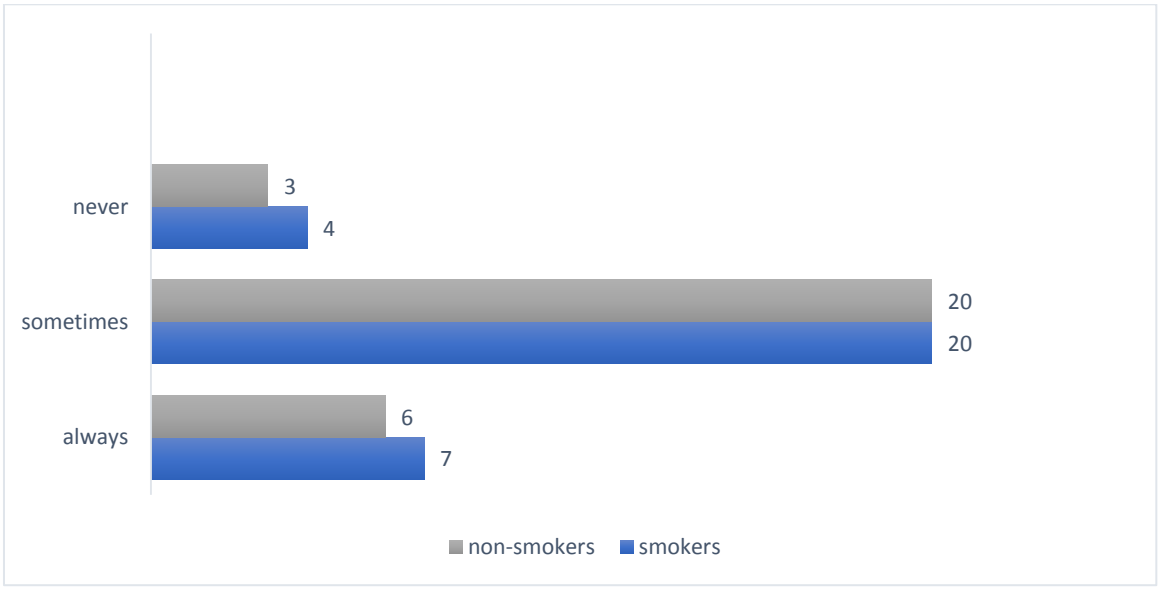


Figure 26: Frequency of eating olives according to the participants.

III.3.3 HORSERADISH:

In this study we observe that 5% of members eat HORSERADISH constantly, while 45.16% completely ruled out taking it, demonstrated on figure 27.

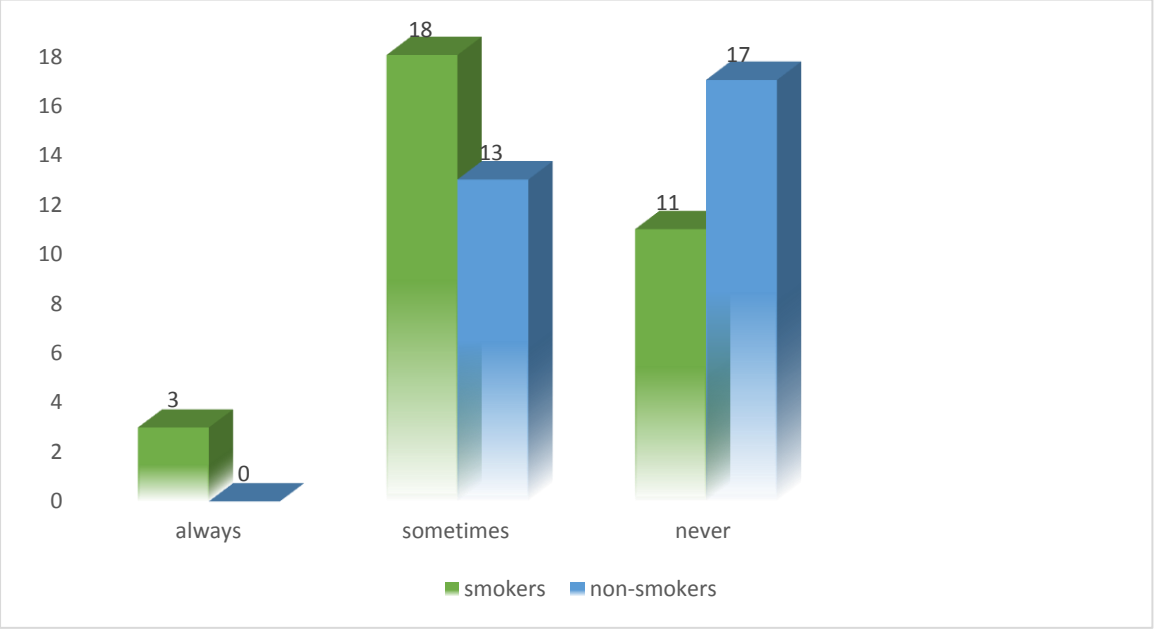


Figure 27: Frequency of HORSERADISH eating according to the participants.

III.3.4 SPINACH:

We note that 58% of candidates eat spinach once in a while, demonstrated on figure 28.

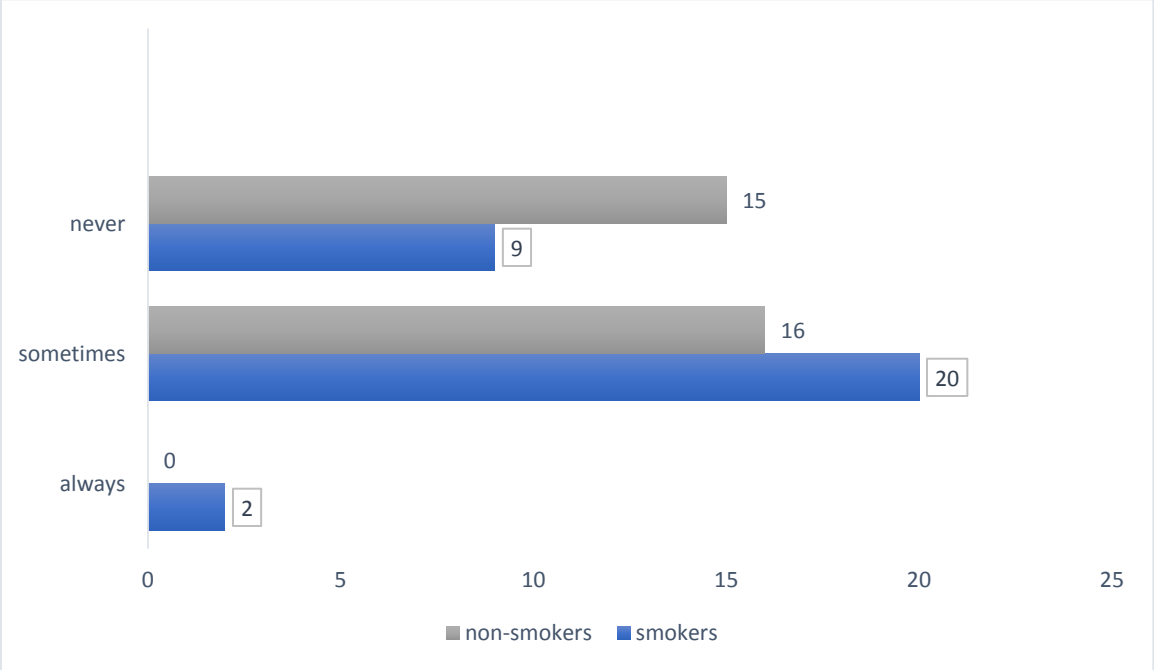


Figure 28: Frequency of eating spinach according to the participants.

III.3.5 PARSLEY:

We notice that 53% of the groups study eat parsley occasionally, demonstrated on figure 29.

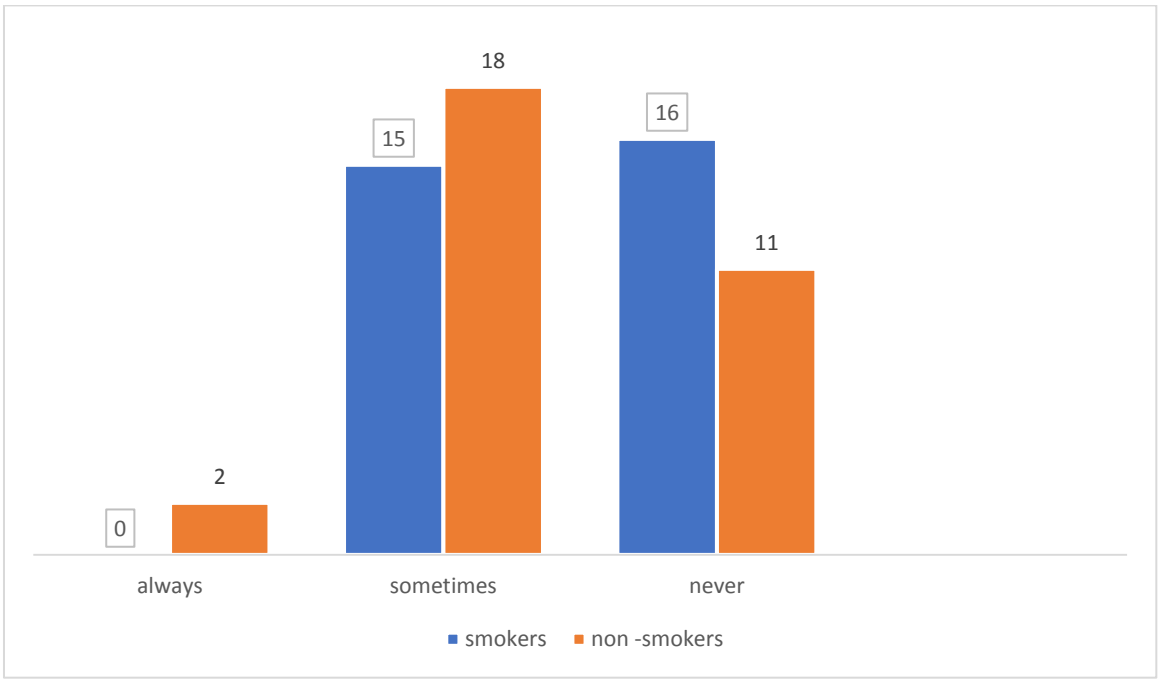


Figure 29: Frequency of eating parsley according to the participants.

III.3.6 CAULIFLOWER:

CAULIFLOWER is consumed by 19.53% of the research groups on a regular basis, demonstrated on figure 30.

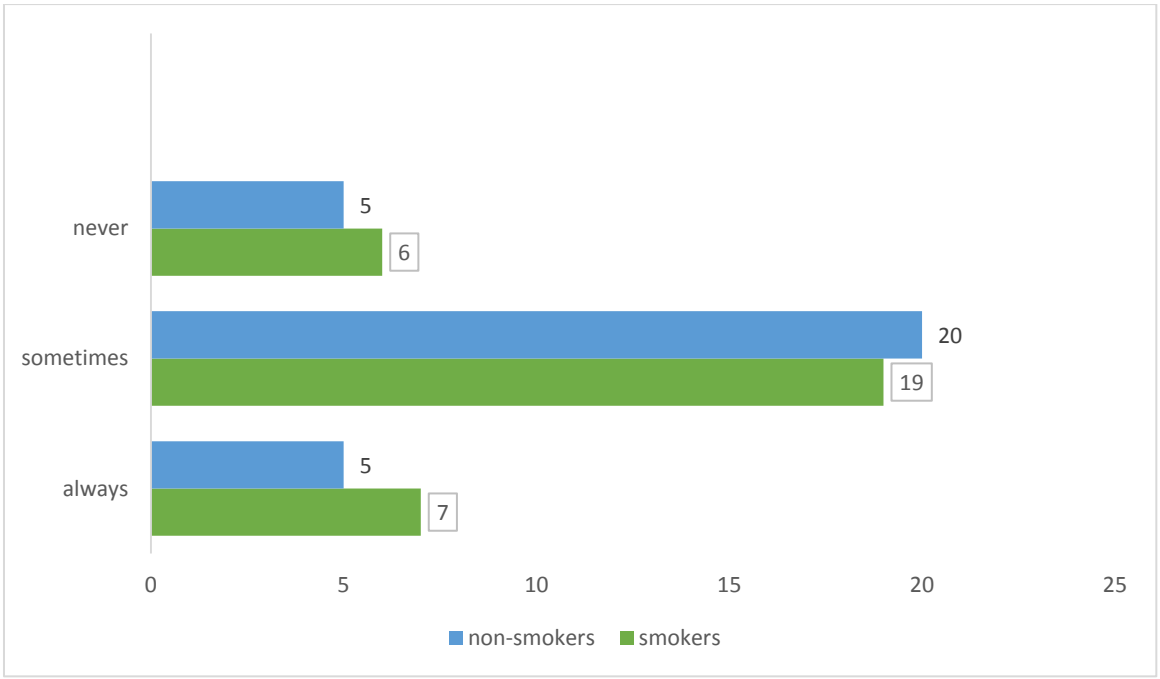


Figure 30: Frequency of eating cauliflower according to the participants.

III.3.6 CEREALS:

Cereals are avoided by 51.61 % of the total population in the study, demonstrated on figure 31.

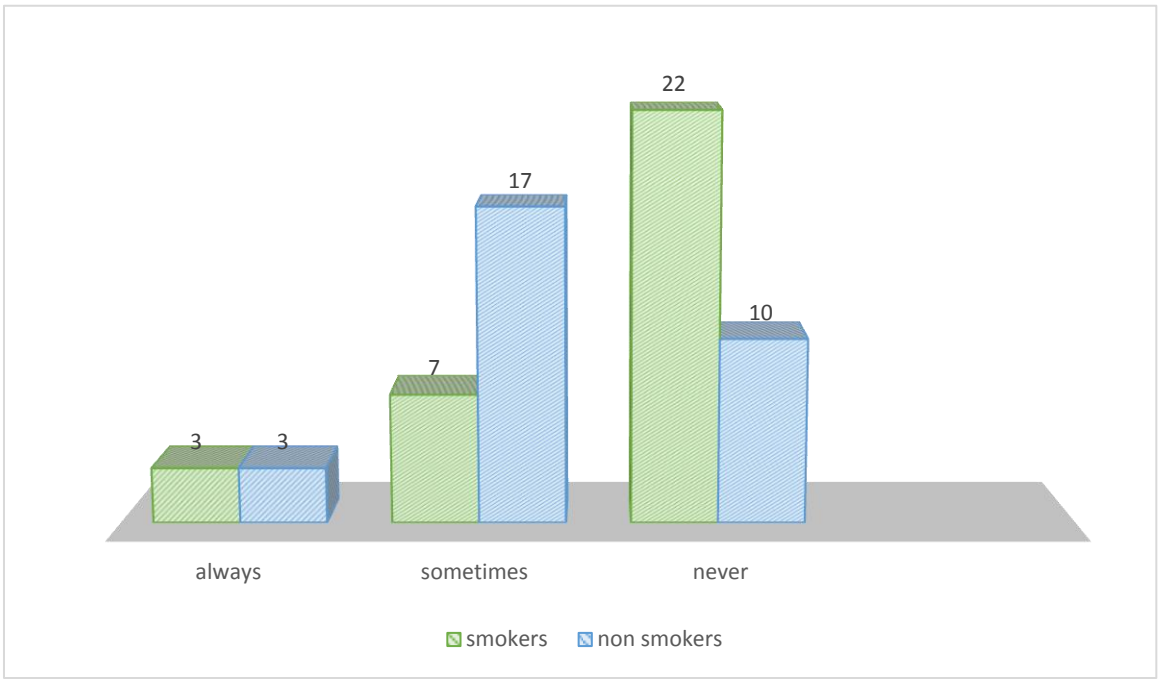


Figure 31: Frequency of eating cereals according to the participants.

III.3.7 LEMON:

We notice that 17.74% of the study population do not consume lemon as a fruit form or beverage, demonstrated on figure 32.

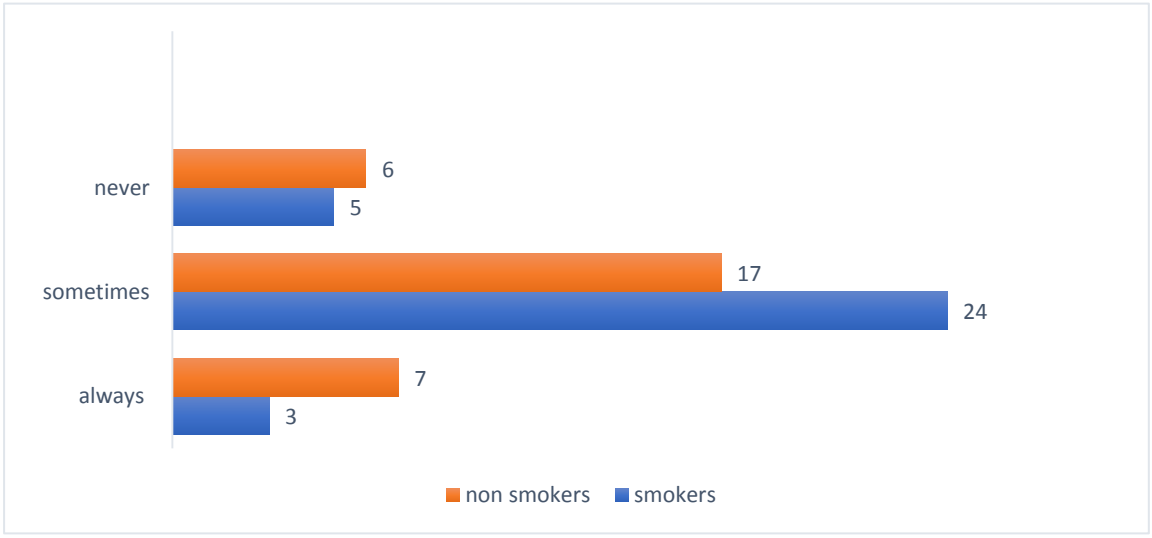


Figure 32: Frequency of eating lemon according to the participants.

III.3.8 Oranges:

Oranges are consumed by 25.8 % of the study participants on a regular basis, whether in the form of a fruit or a beverage, demonstrated on figure 33.

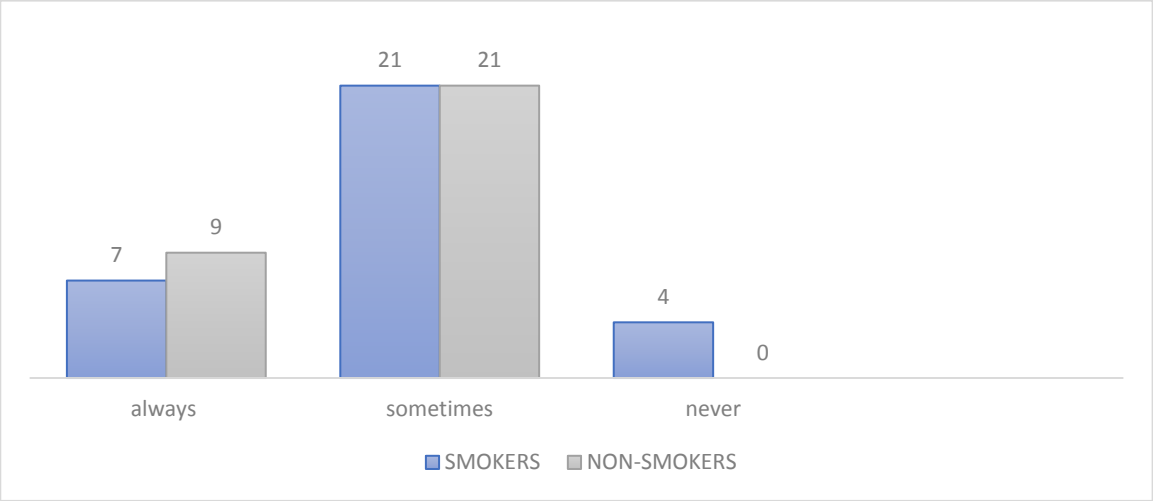


Figure 33: Frequency of eating oranges according to the participants.

III.3.9 Grapefruit:

Grapefruit is excluded by 74.19 % of candidates , demonstrated on figure 34.

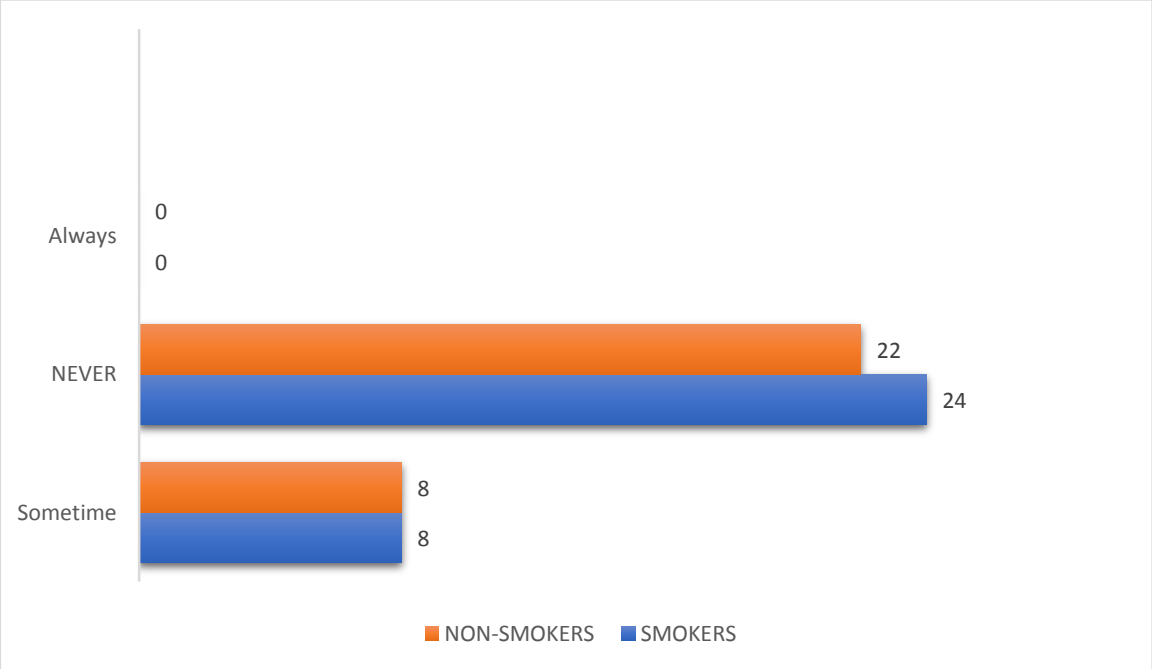


Figure 34: Frequency of eating grape fruit according to the participants

III.3.10 STRAWBERRY:

Strawberry is ingested on a frequent basis by 82.25% of the study participants, demonstrated on figure 35.

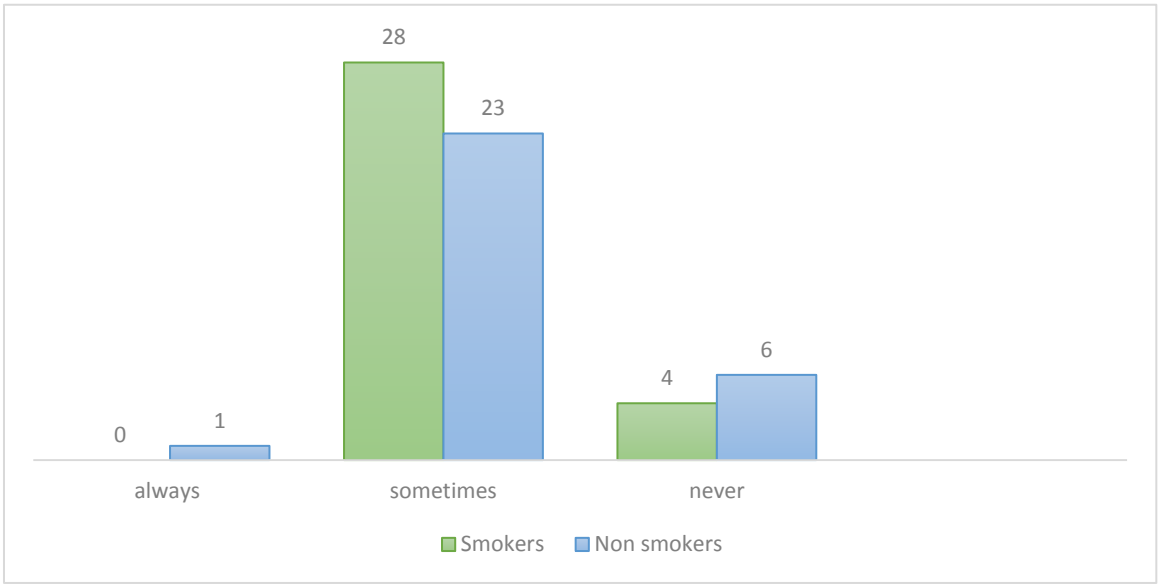


Figure 35: Frequency of eating STRAWBERRY according to the participants

III.3.11kiwi:

Kiwi fruits are ignored with71% of candidates, demonstrated on figure 36.

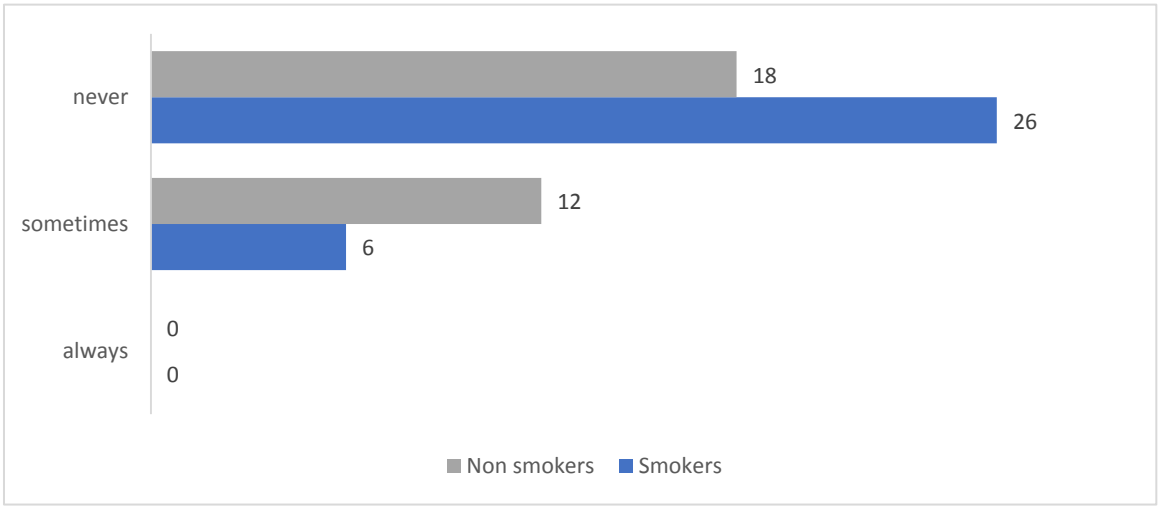


Figure 36: Frequency of eating kiwi according to the participants.

III.4 Biological results:

III.4.1 Ascorbic acid grade in both populations of study

The average ascorbemia level is 9,62 mg/l±3,47 for the non-smoking group, and 8.62mg/l±3,19 for the smoker's group, with a significant difference (p=0.000).

Table VII: The average of ascorbic acid levels for both populations of study.

	Average ± Standard deviation (mg/l)	Min	Max	*P*
Non- smokers' population (n=30)	9.62±3,47	5	18	0.000
Smokers' population (n=32)	8,62±3,19	3.6	16	

III.4.2 Ascorbic acid status:

The deficiency occurred only in the smoker's population with 3 participants below the normal range.

Table VIII: Population status in regard of AA rates.

	Deficiency	Normal
Non- smokers' population (n=30)	0	30
Smokers' population (n=32)	3	29

III.4.2 Ascorbemia of the smokers' participants by number of cigarettes per day:

A significant negative correlation was noted between the AA concentrations in the smokers' study group and the number of cigarettes consumed per day (p=0.01).

Table IX: Correlation between AA grade of smokers and the amount of cigarettes taken in a day

	Cigarettes-consumption	*P*
Smokers AA rate (n=32)	Pearson correlation (-,458**)	*0.01*

III.4.3 Ascorbemia of the smokers' participants by quantity of nicotine held in cigarettes:

An important significant negative correlation was noted between the AA concentrations in the smokers' study group and the rate of nicotine contained in cigarettes.

Table X: Correlation between the AA concentrations in the smokers' study group and the rate of nicotine contained in each cigarette.

	Nicotine concentration in cigarettes	*P*
Smokers AA rate (n=32)	Pearson correlation (-,550**)	*0.01*

III.4.4 Cigarettes consumption rate by age:

A negative significant correlation was noted between the age in the smokers' study group and the cigarettes consumption.

Table XI: Correlation between age and cigarettes consumption

	Age	*P*
Cigarettes consumption	Pearson correlation (-,577**)	*0.01*

III.4.5 Multiple correlation between Ascorbic acid smoker's status and different sorts of dietary intakes:

There is no significant correlation between AA status and the different nutriments.

Table XII:Correlation between Ascorbic acid smoker's status and different sorts of dietary intakes

	PEPPER	OLIVES	HORSERADISH	SPINACH	parsley	CAULIFLOWER	CEREALS
Ascorbic acid smoker's status	-,098	0.239	0,057	0,261	0,316	-0,017	0,20

	Lemon	Oranges	Grapefruit	Strawberry	Kiwi
Ascorbic acid smoker's status	0,207	0.076	0.193	-0.125	0.160

III.4.6 Correlation between Ascorbic acid smoker's grade and the results of Fagerström test:

There is no correlation between different smoking habits of the participants and ascorbic acid levels.

Table XIII: Correlation between Ascorbic acid smoker's grade and the results of Fagerström test

	1	2	3	4	5	6
Ascorbic acid smoker 'concentrations	-.291	-.103	-.061	.064	-.164	-.188

1: Frequency of inhaling cigarettes smokes.

2: Smoking more frequently during the first hours after waking or during the rest of the day.

3: Delay between awakening and the first cigarette consumed.

4: Most necessary cigarette.

5: Difficulty of smoking in prohibited places.

6: Smoking even throughout the sickness condition.

IV.Discussion:

The main objectives of our study was to assess and compare ascorbic acid level in smokers and non-smokers subjects.

For this we conducted an observational descriptive analytical comparative study.

The recruitment of participants proved to be difficult. since over During a period of 6 months we were able to recruit only 62 volunteers cause of reluctance among our population to participate where the number of people informed of the study was significantly higher than the number of people who responded positively to the survey.

Furthermore, COVID -19 pandemic was a limit factor of sample size due to the wide and consistent supplementation of ascorbic acid during these periods. Then we were obliged to recruit only after outside COVID-19 peak period. (55)

Analytical method was another problem. We opted first for HPLC method but results were not producible. After more than four months, we switched to a spectral method and certify its reproducibility and repeatability. Then we started recruiting participants over a period of 1 month.

Our study was carried out on a sample of 62 subjects of which 77% have an origin from Tlemcen The gender ratio of our population is 1.due to the conflict of socials conventions we did not have any female participants.

An average age of 33.62 years old and an average BMI of 23.62 kg/m^2

In order to pursuit our study blood samples have been collected into citrate vacutainers to avoid substantial losses of ascorbic acid that can occur during sample collection and handling. Specially for analysis procedures that took place long after the sampling.

And this was elaborated in a study about stability of whole blood and plasma ascorbic acid.

They highlighted the effect of different variables notably anticoagulants, acidification, storage and time were tested, illustrated on figure 37.

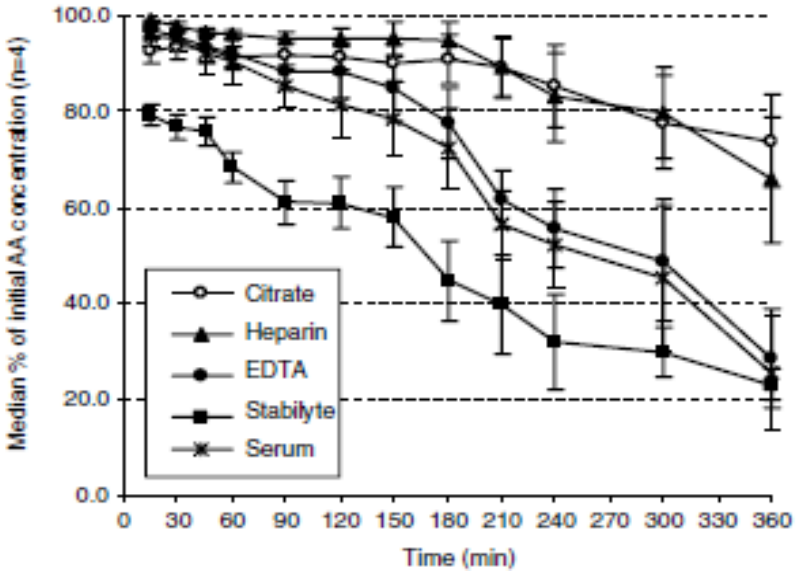


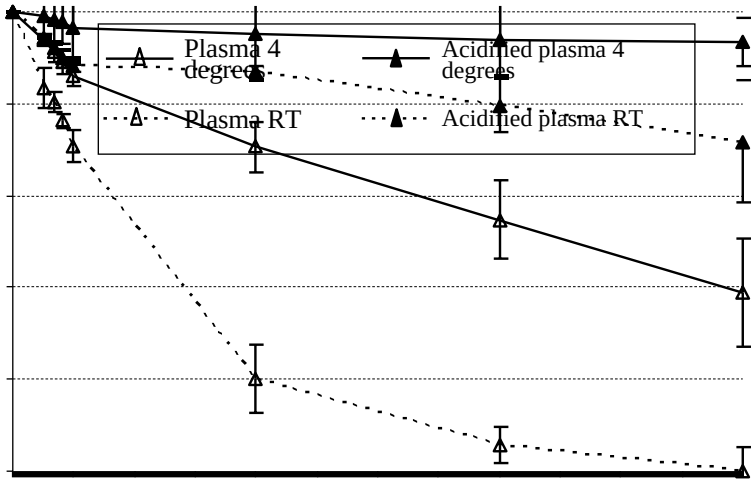
Figure 37: Initial levels and degradation of AA in whole blood at room temperature

Great care needs to be exercised to minimize this source of preanalytical error if meaningful results are to be obtained.(56)

Table XIV: Effect of delayed deproteinization and preservation on ascorbic acid concentration

Time (h)	EDTA				Heparin				Serum			
	Concentration (μmol/L)		Loss (%)*		Concentration (μmol/L)		Loss (%)*		Concentration (μmol/L)		Loss (%)*	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
2	37·5	4·5–132·9	36	13–71	55·1	14·8–146·5	12	4–28	50·5	13·6–138·5	15	7–33
5	16·5	1·1–93·1	67	39–93	50·0	13·6–143·1	16	13–33	49·4	11·4–135·1	17	12–33
8	6·8	1·1–52·2	83	57–95	51·1	12·5–138·5	21	10–33	51·1	11·4–132·9	21	13–34

Because AA degrades rapidly in whole blood and plasma, sample collection should be followed by centrifugation and plasma acidification as soon as possible. To avoid sample degradation during handling, samples should be stored at -70 °C as soon as possible and evaluated within 80 days, as shown in figure 38.(57)



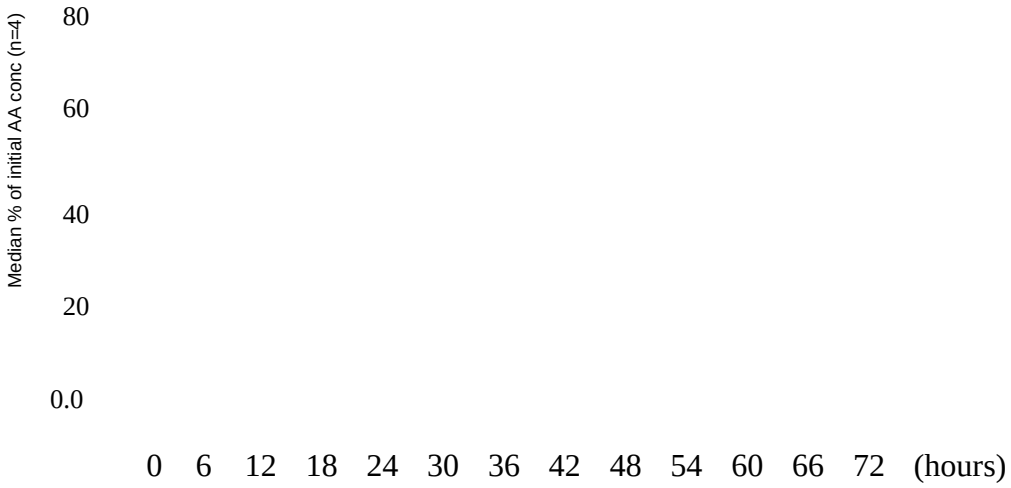


Figure 38: Degradation of AA in plasma and acidified plasma at room temperature (RT) and at 4°C.

A spectral technique was used to determine AA concentrations with a pretreatment approach to the collected samples.

Analysis of the AA rates in our samples revealed an average of 9,62 mg/l in the non-smokers group and an average of 8.62mg/l in the smokers' group with a statistically significant difference (*p=0.000)*, which is similar to the results found in a study carried out in Wisconsin USA on a general population including 11592 subjects (58)and another study carried out in California on a sample of 75 persons.(59)

Students from DHAKA Bangladesh also made a study on the same concepts with 88 participants.(60)

We also found another survey that was done on the pursuit of the same investigation with 2151 subjects in the USA "National Health and Nutrition Examination Survey" (NHANES) 2003–2004.(61)

Another matching assay was done in Scotland including 196 participants (62)

Table XV: Analogy of the average of total ascorbic acid in both groups in our study and other similar once

Study	Wisconsin USA	California USA	DHAKA Bangladesh	USA (NHANES)	Scotland	Our study
N	11592	75	88	2151	196	62
Average AA nonsmokers (mg/l)	11.5	7.2	5.3	8.2	6.5	9.12
Average AA smokers (mg/l)	8.3	4.7	3.9	6.19	3.2	8.62
P	0.001	< 0.01,	0.0004	0.001.	**0.001.**	0.000
Reference	Schectman G 1989	Lykkesfeldt J 2000	Faruque O 1995	Schleicher RL 2009	Bolton-Smith 1993	Algeria 2022

Our results concerning vitamin c status have demonstrated three cases with a deficiency in the smoker's group with a lower AA average of 5.48%. The same condition appeared in multiple studies such (Faruque O 1995) where total ascorbic acid was 26% lower in the smoking group with an average of 3.9 mg/l (60) , another study in Scotland found a low average of total ascorbic acid with value of 3.2 mg/l Which makes the result of deficiency clear. (62)

A Similar finding in a study about the influence of smoking on vitamin c status in adults have shown a deficiency odds ratio of 95% in the smoker's population. (58)

Moreover, speaking about correlation links in our survey, an important negative significant correlation (-,458**) was established between cigarette consumption and vitamin c grades, (p=0.01)

This result is confirmed by the study conducted in the USA on "The Influence of Smoking on Vitamin C Status in Adults" , they found a negative significant correlation, heavy smokers represented by a group of 1288 participants had a number of cigarettes surpassed one pack per day (ppd) have shown a level of ascorbic acid equal to 8.3 mg/l, while the moderate smokers represented by 1553 subjects who did consume less than one ppd had a level of 9.7 mg/l. (58)

This finding was supported by the results of «LYKKESFELDT ET AL 2000", which revealed a negative significant correlation. where only ascorbic acid among all the other antioxidants

was significantly depleted by smoking per se ($P < 0.01$). An ascorbic acid content of 4.7 mg/l±3.5 was recorded in smokers represented by a group of 37 participants who smoked an average of (23± 15) cigarettes each day. (63)

This result is in line with the study carried out in France 1998, they have demonstrated that plasma ascorbic acid concentration was reduced in smokers compared with nonsmokers and was inversely related to cigarette consumption. This study included 188 smokers which been segregated into two groups; "heavy smokers ≥ 20 cigarettes/day "with status of smoking duration over a period of 18.2 years, and the second group "moderate smokers <20 cigarettes/day" for a period of 15.9 years. The AA levels were respectively (5.5 mg/l - 6.7mg/l) for each group.(64)

Likewise NHANES III, 1988–1994 survey in the USA found out a significant ($P<.001$) inverse correlation link between serum vitamin C and cotinine level (which been defined on the foundation of the number of cigarettes) and classified into 4 groups (0 cigarettes < 14 ng/ml ; [14-100]ng/ml<10 cigarettes ; [100-200]ng/ml=[10-20] cigarettes; >200 ng/ml = more than 20 cigarettes)(65)

An additional study was performed to see if there was an association between lower levels of ascorbic acid and the number of cigarettes smoked by Dietrich M-2003 the results showed that Total ascorbic acid levels measured in plasma samples from 83 smokers (≥15 cigarettes per day), 40 passive smokers(passive smokers were eligible if they had not smoked cigarette, for 1 year and were exposed indoors to the smoke of 1cigarette/d on 5 d/week), and 36 nonsmokers, Smokers' plasma ascorbic acid concentrations were substantially lower than nonsmokers' and passive smokers' (ascorbic acid: 7.6, 9.6, and 9.5) mol/L, respectively.(3)

Another study exhibited, a significant dose-response relationship between smoking and vitamin C in Institute of Nutrition and Food Science, university of Dhaka, Dhaka-1000, Bangladesh -1994, including 88 participants separated between non smokers (n=44) and smokers (n=44), the last once have been divided on the ground of cigarette consumption heavy smokers >10 cigarettes /day and mild smokers ≤9 cigarettes/day, AA levels were (3.3-4.2)mg/l respectively for heavy and mild smokers.(60)

Table XVI: Correlation between number of cigarettes and AA grades in our study and other comparable researches

Study	Wisconsin USA		France		USA			THIS STUDY
Number of smokers	(>ppd) n=1288	(<ppd) n=1553	(>20 cigarette /day) n=84	(<20 cigarette /day) n=104	1484			32
					<10 c/d	[10-20] c/d	>20 c/d	
AA (mg/l)	8.3	9.7	5.5	6.7	6.5	5	4.6	8.62
P	0.001		<0.001		<0.001			0.000
References	GORDON SCHECTMAN-1989		Karine Marangon-1998		Wei W 2001			Algeria 2022

Free radicals in cigarette smoke may cause oxidative damage to macromolecules, contributing to decrease plasma antioxidant concentrations.(3) cotinine is the principal metabolite of nicotine, with a half-life of approximately 20 hours. Serum cotinine is directly proportional to the absorbed nicotine and has been used to measure tobacco exposure in epidemiologic studies, Serum cotinine is considered a better marker of smoking status than self-reported tobacco use.(65), our research illustrated a negative association between the amount of nicotine held in cigarettes and AA concentrations.

Speaking about correlations a further link was specified in our results concerning the amount of nicotine held by cigarettes and AA levels. Where we did find a negative association (-,550**/*p*=0.01). Schleicher, R. L designed a study in which smokers were characterized by maintaining a plasmatic cotinine level >10ng/ml, and this same group had much decreased AA rates, indicating the negative correlations (p=0.001).(61)

A second survey about the association of smoking with serum and dietary Levels of antioxidants in adults "NHANES III, 1988–1994" have pointed A significant (P<.001) inverse relation was found between serum vitamin C and cotinine levels independent of diet effect, where smokers have been defined by having plasmatic levels of cotinine higher than 14ng/ml.(65)

An earlier study done by Dietrich, M-2003 compared the effects on plasma antioxidant concentrations in cotinine-confirmed active and passive smokers independent of differences in dietary intakes and other covariates, plasma cotinine concentrations in the smokers (1469.3 nmol/l) were ≈400-fold, those in the passive smokers ($p < 0.0003$). Which did impact directly on AA concentrations, leading to a lesser level of 32.69% (3)

Dawson, E. B-1999 have carried out a study on 75 smokers in Texas USA. And his findings indicate that dietary levels of AA are inversely correlated to urinary excretion of nicotine(66)

Various studies searched for the impact of these factors age, gender, BMI on serum vitamin C the conclusions were controversial and the answers diverged on a wide spectrum

Our investigation led to a non-significant correlation between AA concentrations and variables such age and BMI.

A further analysis of the NHANES II data realized by Schectman-1989 confirms that cigarette smoking is associated with decreased serum vitamin C levels. This association persisted despite correction for factors which independently affected serum vitamin C levels such as age, gender, race, and BMI. (58)

A study about the same approach was performed in DHAKA BENGLADESH-1995, resulted that Mean age and BMI of smokers were 24.7 years and 19.7 kg/m^2 respectively and for the non-smokers were 24.8 years and 20.0 kg/m^2 respectively, differences between the groups were not statistically significant concluding that the effects of these various potentially confounding factors were minimal in their subjects as they were all young males, had similar age, body weight, BMI.(60)

Rosemary L-2009 fulfilled a survey about" Serum vitamin C and the prevalence of vitamin C deficiency in the United States" the outcomes revealed that the highest concentrations were found in children and older persons within each race-ethnic group, women had higher concentrations than did men (p= 0.05), as figure 39 revealed.

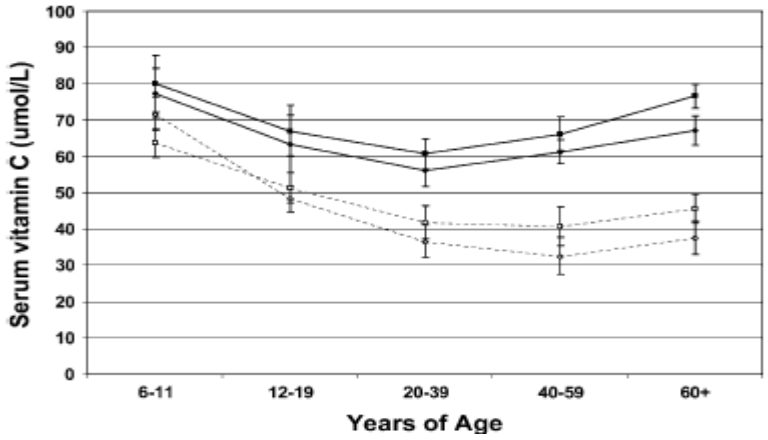


Figure 39: Mean serum concentrations of vitamin C in selected age groups were stratified by sex and any recent intake of vitamin C

Moreover, the significant association of smoking status and vitamin C deficiency persisted after controlling for the possible confounding effects of sex, age, race-ethnicity, BMI, income, use of vitamin C–containing supplements, dietary intake of vitamin C, and survey (odds ratio: 3.76; 95% CI: 3.08, 4.59; P= 0.001).(61)

A follow-up study of the same topic was undertaken in France-1998 providing the following results a decline in plasma ascorbic acid concentrations in smokers which persisted even after statistical adjustment for the confounders (vitamin intake, alcohol consumption, age, and plasma lipid concentrations)(64)

Some other study done by Wei, w-2001 discovered for certain measurements, a sex difference influence factor, males showed considerably lower mean serum vitamin c levels (p=0.01) than females, regardless of smoking status.(65)

Dietrich. M, 2003 ran a survey relating to smoking and exposure to environmental tobacco, plasma antioxidant concentrations in the nonsmokers, passive smokers, and active smokers even after the adjustments for each (race, age, sex, bmi, alcohol intake, fruit and vegetable intakes, and triacylglycerol) the differences in ascorbic acid levels between the groups remained significant.(3)

Another investigation accomplished in Denmark -1997 concluded these findings the mean plasma concentration of total ascorbic acid was higher in females than in males ($p < 0.005$); this difference persisted in multivariate analysis when smoking was adjusted, analysis of covariance showed that sex and smoking were significant independent predictors of plasma ascorbic acid concentrations whereas age was not a significant covariate ($p=0.59$).(63)

Our research reveals that there is no significant correlation between Ascorbic acid smoker's status and different sorts of dietary intakes.

This result is according to a study conducted in the United States -2009 including 1183 smoker, the significant association of smoking status and vitamin c deficiency persisted after controlling for the possible confounding effect of dietary intake of vitamin c, and ($p=0.001$).(61)

Another study was carried out in Bangladesh-1995 found that There was no significant difference in vitamin C intake between the various smoking groups, plasma vitamin C level was lowest in heavy smokers, highest in non-smokers and mild smokers had an intermediate level. The difference was highly significant ($P = 0.0005$).(60)

Nevertheless, this result is totally opposite with the findings of Bolton-smith. C ,1993 dietary intakes of the antioxidant vitamins differed significantly between the current, ex- and never smoking groups, for both men and women whether expressed as amounts per day or as nutrient densities. These differences were maintained after statistical adjustment for socio-economic status (occupation, education or housing tenure), age and BMI, the foods which contributed to vitamin c intake ($p=0.001$).(62)

In a previous report by smith and Hodges-1987 in which smoking status was assigned by response to the questionnaire, a larger portion of smokers had serum vit c less than 0.3 mg/dl than nonsmokers with similar dietary intake.(67)

Gridley, g-1990 carried out a study in the USA leading to an opposite result of our assay they found with increasing levels of cigarette smoking among the cohort's men, mean vitamin c intake shows a significant decreasing trend (p.001). All current smokers consumed significantly (p.001) less vitamin c than nonsmokers in the case-control study.(68)

Although our research did not include the influence of supplementation with vitamin c for the smokers, several studies have investigated this factor and whether it can restore the normal serum values.

A study in this approach conducted by Rosemary L-2009 have shown that recent vitamin c supplement use or adequate dietary intake decreased the risk of vitamin c deficiency (p=0.05), the significant association of vitamin c supplement use and low risk of vitamin c deficiency persisted after controlling for the possible confounding effects of sex, age, race-ethnicity, BMI, pir, smoking status, dietary intake of vitamin c, and survey (p=0.001).(61)

Smith, j. L-1987 ran a survey concerning “serum levels of vitamin c in relation to dietary and supplemental intake of vitamin c in smokers and nonsmokers” and came out with the next result the difference between smoker and nonsmoker mean serum c within any intake range was approximately 0.2 mg/dl serum c. The parallel bioassay method applied to mean serum vitamin C values estimated that smokers need to consume an additional 65.4 mg/day of vitamin c on the average to maintain serum values equivalent to nonsmokers.(67)

Lykkesfeldt, Jens-2000 in his research about repleting AA by moderate supplementation found that ascorbic acid was significantly depleted by smoking per se ($P < 0.01$). After the 3-months supplementation period, ascorbic acid was efficiently repleted in smokers ($P < 0.001$).(59)

Another investigation conducted in the USA-1989 revealed that smokers had a three-fold increase in risk of marginally or severely deficient vitamin C status. Approximately 35 per cent of smokers had low serum levels placing them within one of these two categories. Although smokers taking vitamin supplements were also at increased risk of hypovitaminosis C compared with non-smokers, the prevalence of low serum vitamin C levels was much reduced in both smoking and non-smoking individuals taking supplements. This finding suggests that the increased vitamin C intake associated with vitamin supplementation may frequently prevent the low serum levels from occurring in smokers. As estimated by the regression curves in Figure 43, approximately 130 mg of additional dietary vitamin C daily would be required to overcome the adverse effect of cigarette smoking on serum vitamin C levels.(58) (figure40)

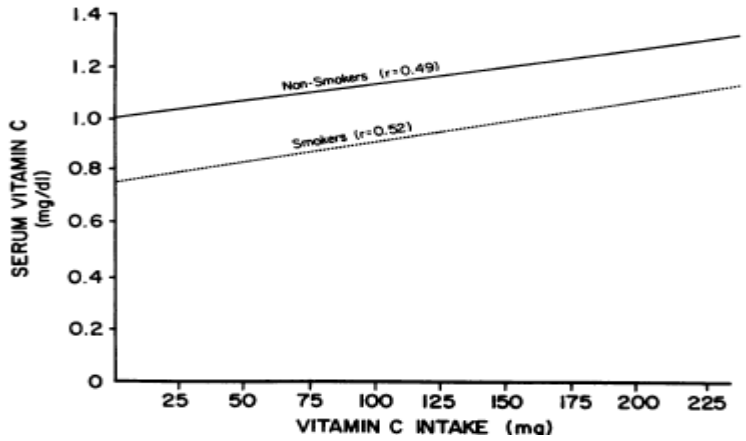


Figure 40: Relation between dietary vitamin C intake and serum levels. Adjusted for (age, gender, race, body mass and vitamin supplementation).

DAWSON ET AL-1999carried out a study resulting with this finding: the nicotine intake of the three study groups remained unchanged throughout the study. The differences in cotinine excretion between the placebo group and the supplemented groups demonstrated suppression of nicotine metabolism from increased AA intake, AA is a biological antioxidant and would depress the oxidative metabolism of nicotine(66)

V.Conclusion

This study reported for the first time, in our country, where our aim was to measure ascorbic acid levels in 62 participants divided into two populations (smokers and non-smokers)

An investigation was carried out in order to find any possible link between cigarette smoking and lower rates of vitamin c.

which was the case in the conducted study, a statistical significant difference was obtained between the average of both populations of the survey

Further we have observed in our research a widespread of outcomes regarding a variety of factors such higher frequency of cigarette consumption, nicotine concentrations, different sorts of dietary intakes.

A multitude of correlation tests were performed to expand the general knowledge about the theme, where we did draw the next results.

A negative significant association was determined between higher rate of cigarette consumption, elevated concentrations of nicotine and lower serum ascorbic acid

On the other side nutrition habits, and dietary intakes did not affect significantly ascorbic acid levels.

Lastly, we wanted to highlight the importance of the supplementation for the cases who showed a decreased concentrations under the normal range, multiple studies suggested a supplementation raise to correct serum vitamin c rates.

In this approach it would seem necessary to run a research among our population

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ANNEX

Copy of le tabac et vitamine c

le tabagisme favorise la génération des espèces radicalaire oxygènes et ces demiers diminuent la concentration plasmatique des antioxydants comme la vitamine c
Notre étude a pour but de vérifier s'il existe un lien entre le tabagisme et le déficit en vitamine c

---

* Required

1. Nom et prénom *

_____

2. niveau intellectuel *

Mark only one oval.

- ◯ primaire
- ◯ secondaire
- ◯ lycée
- ◯ université

3. Lieu et date de naissance *

_____

4. numéro de telephone

_____

les questions suivantes du (1-6) sont concernées pour les fumeurs

5. sexe *

Mark only one oval.

- homme
- Femme

6. V Combien de cigarettes fumez vous par jour

Mark only one oval.

- >25
- 16-25
- <16

7. 2/ TAUX DE NICOTINE DE VOS CIGARETTES (en mg)

Mark only one oval.

- >1.5
- 0.8-1.5
- <0.8

8. 3/ Inhalez vous la fumée?

Mark only one oval.

- toujours
- parfois
- jamais

9. 4/ Quand fumez vous le plus?

Mark only one oval.

- le matin
- après midi
- le soir

10. 5/ A quel moment apres le réveil fumez vous votre 1 "ere" cigarette ?

Mark only one oval.

- <30 min
- >30 mi,

11. 6/ Quelle cigarette vous parait la plus indispensable ?

Mark only one oval.

- la premiere
- une autre

12. 7/ Trouvez vous difficile de ne pas fumer dans les endroits interdits ? (bibliotheque ; magasin ;cabinet

Mark only one oval.

- oui
- non

13. 8/ Fumez vous meme si une maladie vous oblige a rester au lit ?

Mark only one oval.

- oui
- Non

III) enquête sur la supplémentation en vitamine c

14. 1/ Prenez vous une supplémentation en vit c (Cp ,sachet...) *

Mark only one oval.

- Oui
- Non

15. 2/ produits consommés par participent : (mentionner le nom de produit)

_____

cette partie en dessous concerne les aliments qui contient la vitamine c (veuillez répondre selon la fréquence de votre consommation/toujours-parfois-jamais)

3/ listes des aliments

16. • legumes (poivron rouge cru, poivron vert cru) *

Mark only one oval.

- toujours
- parfois
- jamais

17. olive vertes *

Mark only one oval.

- toujours
- parfois
- jamais

18. raifort cru *

Mark only one oval.

- toujours
- parfois
- jamais

19. épinard *

Mark only one oval.

- toujours
- parfois
- jamais

20. persil *

Mark only one oval.

- toujours
- parfois
- jamais

21. chou-fleur *

Mark only one oval.

- toujours
- parfois
- jamais

22. céréales pour petit déjeuner, céréales aux fruits, céréales au chocolat *

Mark only one oval.

- toujours
- parfois
- jamais

- **Fruits**

23. zeste de citron, citron, jus de citron pressé *

Mark only one oval.

- toujours
- parfois
- jamais

24. orange, jus d'orange pressé *

Mark only one oval.

- toujours
- parfois
- jamais

25. pamplemousse *

Mark only one oval.

- toujours
- parfois
- jamais

26. fraise *

Mark only one oval.

- toujours
- parfois
- jamais

27. kiwi *

Mark only one oval.

- toujours
- parfois
- jamais

The developed color after the incubation

Annex2

ABSTRACT:

Cigarette smoke is a significant source of oxidative stress, many studies have demonstrated that heavy smoking in males is associated with a decrease in serum ascorbic acid levels. The main of this study is the determination of ascorbic acid levels in two groups of people smokers and non- smokers

we designed an observational analytical comparative study, in which two existing groups differing in outcome are identified and compared on the basis of some supposed causal attribute, over a nine-month period from October 2021 to June 2022.

Serum ascorbic acid levels of 62 were analyzed via a spectrophotometric method, the average for smokers and non-smokers were respectively 8.62 mg/l and 9.62 mg/l. This deference was statistically significant ($p<0.000$). also, we noted an inverse association between both serum levels of vitamin c and smoking which was related to cigarette consumption, nicotine abundance. Moreover, multiple studies suggested a supplementation raise to correct serum vitamin c rates.

Key words: smoking, ascorbic acid, analysis.

ABSTRAIT :

La fumée de cigarette est une source importante de stress oxydatif, de nombreuses études ont démontré qu'une forte consommation de tabac chez les hommes est associée à une diminution des concentrations sériques d'acide ascorbique. Le principal élément de cette étude est la détermination des concentrations d'acide ascorbique dans deux groupes de personnes fumeurs et non-fumeurs.

Nous avons conçu une étude comparative analytique par observation, dans laquelle deux groupes existants dont les résultats diffèrent sont identifiés et comparés en fonction d'un attribut causal présumé, sur une période de neuf mois allant d'octobre 2021 à juin 2022.

Les concentrations sériques d'acide ascorbique de 62 candidats ont été analysées par spectrophotométrie, la moyenne pour les fumeurs et les non-fumeurs étant respectivement de 8,62 mg/l et 9,62 mg/l. cette déférence était statistiquement significative ($p < 0,000$). Nous avons aussi noté une association inverse entre les niveaux sériques de vitamine c et le tabagisme, qui était liée à la consommation de cigarettes, à l'abondance de nicotine. En outre, plusieurs études ont suggéré une augmentation de la supplémentation pour corriger les taux sériques de vitamine C.

Mots clés : fumer, acide ascorbique, analyse.

ملخص :

يعد التدخين عاملا رئيسيًا للإجهاد التأكسدي، وقد أظهرت العديد من الدراسات أن التدخين المفرط لدى الرجال مرتبط بانخفاض مستويات حمض الأسكوربيك في المصل. الهدف الأساسي من هذا البحث هو تحديد كميات حمض الأسكوربيك في مجموعتين من الناس: المدخنون وغير المدخنين.

على مدى تسعة أشهر من أكتوبر 2021 إلى جوان 2022، طورنا دراسة مقارنة تحليلية قائمة على الملاحظة يتم فيها تحديد مجموعتين موجودتين بنتائج مختلفة ومقارنتهما على أساس بعض المتغيرات المسببة ظاهريًا. واستخدمت طريقة قياس الطيف التصويري لفحص مستويات حمض الأسكوربيك في مصل 62 من مقدمي الطلبات؛ وبلغ المتوسط بالنسبة للمدخنين وغير المدخنين 8.62 ملغم/لتر و9.62 ملغم/لتر على التوالي. مع وجود اختلاف احصائي معبر(P=0.000).

اكتشفنا أيضًا علاقة عكسية بين مستويات فيتامين سي في المصل والتدخين، والتي كانت مرتبطة باستخدام السجائر ووفرة النيكوتين. علاوة على ذلك، أظهرت العديد من الدراسات أن المكملات بفيتامين سي يمكن أن تساعد في تطبيع مستويات فيتامين سي في المصل.

الكلمات الرئيسية : التدخين وحمض الأسكوربيك والتحليل