REPUBLIC OF TURKEY

ALTINBAŞ UNIVERSITY

Institute of Graduate Studies

Biomedical Sciences

EVALUATION OF THE RELATIONSHIP BETWEEN PESTICIDE EXPOSURE AND OXIDATIVE DAMAGE IN GREENGROCERS

Amenah Raad Muslim AL-KAZRAJI

Master's Thesis

Supervisor

Assoc. Prof. Dr. Şükriye KARADAYI

Istanbul, 2022

EVALUATION OF THE RELATIONSHIP BETWEEN PESTICIDE EXPOSURE AND OXIDATIVE DAMAGE IN GREENGROCERS

Amenah Raad Muslim AL-KAZRAJI

Biomedical Sciences

Master's Thesis

ALTINBAŞ UNIVERSITY

2022

The thesis titled “EVALUATION OF THE RELATIONSHIP BETWEEN PESTICIDE EXPOSURE AND OXIDATIVE DAMAGE IN GREENGROCERS” prepared by AMENAH RAAD MUSLIM AL-KAZRAJI and submitted on 11/08/2022 has been **accepted unanimously** for the degree of Master of Science in Science in Biomedical.

Asst. Prof. Dr. Şükriye KARADAYI
Supervisor

Thesis Defense Committee Members:

Asst. Prof. Dr. Şükriye KARADAYI	Faculty of Medical Laboratory Techniques, Altınbaş University	_____
Asst.Prof.Dr.Özlem Kurnaz GÖMLEKSIZ	Faculty of School of Medicine, Altınbaş University	_____
Assoc. Prof. Dr. Tülin ÖZBEK	Faculty of Arts & Science, Yıldız Technical University	_____

I hereby declare that this thesis meets all format and submission requirements of a Master’s thesis.

Submission date of the thesis to Institute of Graduate Studies: ___/___/___

I hereby declare that all information/data presented in this graduation project has been obtained in full accordance with academic rules and ethical conduct. I also declare all unoriginal materials and conclusions have been cited in the text and all references mentioned in the Reference List have been cited in the text, and vice versa as required by the abovementioned rules and conduct.

Amenah Raad Muslim AL-KAZRAJI

Signature

DEDICATION

Foremost, I would like to express my sincere gratitude to my advisor Assoc. Prof. Dr. Şükriye Karadayi for the continuous support of my M.Sc. Study and research, for her patience, motivation, enthusiasm, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my M.Sc study. A special thanks to all the academic staff in my university; it is your kindness and experiment that made me through my research. I want to give my deepest appreciation to my father, Asst. Prof. Dr. Raad Al-khazraji and Mother Rajaa Hassan for their continuous and endless support through this journey and for them to provide me this fantastic opportunity.A special thanks to my sisters Zainbe, Tuqa, and Aia for constantly reminding me that I took their parents away and that I have to finish my research fast so we can travel back to our sweet, lovely home. In addition, I would like to mention my deep thanks to Saif Nasser, Aydın Oğuzhan and Sabry Kuday, who helped me a lot with my sample collection. Finally, I want to give my deepest appreciation to Dr. ENG. Taha Salah, my best friend who has been with me from the first day of my journey to its end, the person that always been there for me.

ABSTRACT

EVALUATION OF THE RELATIONSHIP BETWEEN PESTICIDE EXPOSURE AND OXIDATIVE DAMAGE IN GREENGROCERS

Al-kahzraji,Amenah

M.Sc., Biomedical Sciences, Altınbaş University,

Supervisor: Assoc. Prof. Dr. Şükriye KARADAYİ

Date: August/2022

Pages: 79

The study aims to determine the concentrations of DNA oxidative stress among greengrocer store workers in Istanbul using urinary 8-Hydroxydeoxyguanosine (8-OHdG) concentrations and to determine whether there is a causal relationship between the oxidative stress of greengrocer workers and chemical pesticides as a result of their working with fruit and vegetables. To gather data that may be useful in conducting the research, we have prepared a form or a questionnaire for all sample members. Sixty urine samples were collected, 40 were from greengrocers, and the other 20 were workers in different jobs. Each of the 8-OHdG level and the creatinine levels was calculated using the ELISA commercial kits method in all sample. The 8-OHdG and creatinine concentrations of all participants (sample group and control group) were determined using the calibration curve. The resulting creatinine-corrected 8-OHdG concentrations (ng/mg) were used for biostatistical analyses. The research sample consisted of only men whose ages ranged between (16-73). The research results proved that there is a relationship close to the significance limits at $p \leq 0.05$, where it was 0.06. We did not find any significant relationship between the univariate and multivariate variables collected from the questionnaire with the concentrations of 8-OHdG/creatinine (ng/mg).

Keywords: 8-Hydroxydeoxyguanosine, Oxidative Stress, Greengrocer, Pesticides.

TABLE OF CONTENTS

Pages

LIST OF TABLES

Pages

LIST OF FIGURES

Pages

ABBREVIATIONS

8-OHdG	:	8-Hydroxydioxyguanousine
ELISA	:	Enzyme Linked Immunosorbent Assay
WHO	:	World Health Organization
GHS	:	Globally Harmonized System
ROS	:	Reactive Oxygen Species
PUFA	:	Polyunsaturated Fatty Acids
SOD	:	Superoxide Dismutase
CAT	:	Catalase
GPx	:	Glutathione Peroxidases
GR	:	Glutathione Reductase
G6PDH	:	Glucose-6-Phosphate Dehydrogenase
RNS	:	Reactive Nitrogen Species
AChE	:	Acetylcholinesterase
BLK	:	Blank
TA	:	Total Activity
NSB	:	Non-Specific Banding

1. INTRODUCTION

Many diseases afflict workers in the agricultural and marketing field in general, and diseases that affect workers in the field of trading, cultivating and servicing vegetable and fruit crops spread in most markets and centres for selling and buying these crops. These diseases are known as occupational diseases. Occupational diseases are diseases that cause chronic or temporary diseases or cause physical or mental disabilities to their workers. These diseases occur as a result of repeated or continuous exposure to certain conditions related to the nature of this profession or work performed by categories of workers in the workplace. These disorders affect many functional human systems, including the neurological, respiratory, muscular, skeletal, excretory, and circulatory systems [1]. Greengrocers who deal with pesticide and agricultural residues are among those who develop these diseases.

Due to the multiplicity of types of fruits and vegetables in agricultural production, which, when grown, are exposed to many types of pests, insects and pathogens, and to increase their production, pesticides must be used for the crisis of chemical substances that are used to eliminate and get rid of microorganisms, insect pests and pathogens, viral, and vectors of human, animal or joint diseases, as well as infections resulting from infection from the bush and harmful and unwanted plants during the period of cultivation and growth that harm the crops and fruits[2,3]. All of these undesired and pathogenic reasons create economic and agricultural harm, resulting in a decrease in the nutritional content of fruits and vegetables and the elimination of nutrients during cultivation, processing, consumption, storage, and other marketing processes. It can be said that insecticides are chemicals used to eliminate all pests that pose a threat to human health, animals and other living organisms. However, at the same time they pose damage to the agricultural environment as well as to human health, as these pesticides can cause oxidative stress, which leads to the formation of free radicals and Alterations in the antioxidants and the regularity of the body's enzymes, and the neurotoxicity of pesticides is usually in the form of occupational diseases. Oxidative stress and DNA damage are linked to health effects on categories of workers in this field, such as cancer and neurodegenerative diseases such as Alzheimer's and others due to continuous and indirect exposure to pesticides [4,5]. Therefore, studying and measuring oxidative stress is nowadays gaining increasing importance to maintain human health and the environment safe. Currently, there are many scientific studies that indicate the existence of a relationship

between oxidative stress and exposure to pesticides, as well as studies to determine oxidative damage, where some previous studies indicated the determination of DNA damage, especially in lymphocytes, in vivo and in vitro [5,6]. One of the primary biomarkers most consistently used to monitor oxidative stress is 8-hydroxydeoxyguanosine, an oxidized DNA nucleoside, commonly called 8-oxo-7,8-dihydro-2-deoxyguanosine using protein-associated immunosorbent assay methodology. choose an 8-OHdG marker with a fragile, easy-to-apply and negligible procedure.

The current study intends to identify the oxidative damage that may occur as a result of grocers' sellers' exposure to chemical pesticides, with the goal of demonstrating a causal association between the illnesses of the greengrocers' categories and their employment or profession.

2. GENERAL INFORMATION

2.1. OVERVIEW ON PESTICIDES

Pesticides are chemicals that humans employ to kill or repel creatures (pests) that pose a threat to our health and well-being, as well as the health and well-being of pets and animals, or cause agricultural damage. Showers, herbicides, fungicides, insecticides, fungicides, molluscicides and rodenticides, among others, are not allowed, but the harmful effects of insects are not allowed. Insecticides, herbicides, fungicides, herbicides, herbicides, molluscs and rodenticides are the most commonly used insecticides and herbicides with the best biological activity.

Pesticides have been utilized to dispense with bugs for quite a while. a long time back, Sumerians utilized sulfur mixtures to oversee bugs and vermin. For very nearly 2000 years, pyrethrum, a substance removed from the dried blossoms of Chrysanthemum cineraria folium, has been utilized as an insect spray. Weeds have been controlled with salt or seawater. Until the 1940s, inorganic pesticides, for example, sodium chlorate and sulfuric corrosive, as well as natural mixtures got from regular sources, were habitually utilized in bug the board. [8].

Pesticide development accelerated during World War II (1939-1945), as the need for expanded food production and the discovery of potential chemical warfare chemicals became more critical. Many examples exist throughout history of human activities that, when carried out in an ill-judged or uneducated manner, represent a threat to human existence within a few years. It's what Nobel Laureate Albert Schweitzer (1952) warned us about. His statements are referenced in Rachel Carson's well-known book, Silent Spring: "if man has lost his ability to predict and prevent events. He'll finish by destroying the planet" [9].

Farming pesticides have numerous essential and optional benefits as well as many drawbacks that make it hard to gauge the advantages and disadvantages to the utilization of pesticide. The essential advantages are improved and expanded both the quality and yields of harvest and animals [4]. Food security, higher commodity incomes, and diminished overall illness spread are instances of optional advantages that are less evident. Temporarily, pesticides save crops, land, water, time, and other significant assets. As per gauges, burning through $10 billion on pesticides every year saves $40 billion in crop misfortunes [10]. On the other hand, broad pesticide use has various

downsides or weaknesses. Homegrown creature tainting and mortality, the deficiency of regular bug bad guys, pesticide obstruction, Honeybee and fertilization declines, misfortunes to local yields, fisheries and bird misfortunes, and groundwater defilement are a couple of them [11]. Pesticides incite the passing or mischief of microorganisms, which influences soil richness. What's more, a few pesticides cause immunotoxicity in people, which can bring about immunosuppression, extreme touchiness (sensitivities), immune system problems, and irritation; youngsters are especially powerless against the pessimistic results of pesticide openness. Individuals who work with pesticides consistently, like ranchers, are bound to foster malignant growth. Pesticides are liable for large number of non-deadly poisonings and malignant growth cases every year [12]. It is vital to take note of that different researchers have underlined the adverse consequences of pesticides on human wellbeing when used for a lengthy timeframe. For instance, the sensational ascent in lymphoma patients and various ailments in organic entities and human wellbeing, an issue that has been bantered till now [13].

Pesticides are the subject of both pro and con arguments. It benefits crop and livestock production, but they can harm human and environmental health. Arias-Estévez et al. recommends that pesticides be used sparingly or not at all, except for the target species, to reduce the harmful effects of pesticides. Pesticide formulation and application improvements, such as microbially produced insecticides and precision band spraying, also could reduce pesticide side effects Furthermore, pesticide use has long-term negative consequences that must be considered [14].

Pesticides are widely used and help farmers and consumers save money by improving crop quality and productivity. Nonetheless, pesticide use has long-term negative consequences that must be considered.

Pesticides are classified by various elements, including poisonousness (destructive impacts), bother organic entities killed, pesticide capability, synthetic piece, instrument of passage, method of activity, how or when they work, plans, and provenance. Be that as it may, giving significance to general wellbeing, World Health Organization (WHO) and Globally Harmonized System (GHS) grouped pesticides as indicated by their harmfulness or unsafe impacts. Pesticide poisonousness still up in the air by two elements: part and receptiveness time Hence, how much the substance is involved (piece) and how habitually the receptiveness to the substance occurs (time) achieve two unmistakable kinds of hurtfulness extreme and steady destructiveness [15].

Intense harmfulness alludes to how unsafe a pesticide is to a human, creature, or plant following a solitary momentary openness. Regardless of whether just a limited quantity is retained, a pesticide with a high intense poisonousness is deadly. Intense oral poisonousness, intense cutaneous harmfulness, and intense inward breath harmfulness are three kinds of intense poisonousness that can be measure.

Constant harmfulness is the noxious impact of a pesticide after it has been presented to it for quite a while. Due to likely openness to pesticides on/in food items, water, and the air, constant poisonousness of pesticides undermines the overall population as well as people who manage pesticides [16].

It's sorted by WHO into two classes: intense oral and intense cutaneous poisonousness, in light of the assessed lethal portion LD50 (the pesticide portion that is expected to kill half of the tried creatures while entering the body by oral or dermal course) [17]. As appearing in table 2.1 the order of the pesticide by WHO and in table 2.2 by the GHS.

In my investigation, we'll study one of the health issues that could be linked to pesticides causing oxidative damage in greengrocers. Pesticides produce free radicals in our bodies, which induce oxidative stress and eventually result to oxidative damage.

Table 2.1: Pesticides Classification as indicated by WHO Hazard Class 2009[18].

aWHO Classe		sLD 50 for rats (Mg/kg body wt.)		Examples
		Orals	Dermals	
I_a	Incredibly Hazardous	<.5	<.500	Parathion, Dieldrin, Phorate
I_b	Exceptionally hazardous	5 to 50	500 – 2000	eAldrin, Dichlorvos
II	Moderately hazardous	50 to.2000	2000-20000	aDDT, Chlordane
III	Slightly hazardous	Over, 2000	Over 20000	Malathions
U	Improbable to introduce intense hazard	50000 or more		Carbetamides, Cyclosporins

Table 2.2: GHS Classification of pesticides [19].

aGHS aClassification	xClassification Criteria			
	Oral		fDermal	
	LD_{50} (mg/kg bw)	sHazard aStatement	LD_{50} (mg/kg bw)	Hazard Statement
Classificationf1	< 5	sFatal if fswallowed	< 50	Deadly in touch with skin
Classificationf2	05 to 500	gFatal if swallowed	050 to 200	Deadly in touch with skin
Classificationf3	50 to 300	xToxic if xswallowed	200 to 1000	Poisonous in touch with skin
Classificationf4	3000 to 20000	Harmful if swallowed	01000 to 2000	Unsafe in touch with skin
Classificationf5	2000 to 5000	xMay be charmful	2000 to 5000	Might be unsafe

2.2 OXIDATIVE STRESS

The saying oxidative stress implies an unsettling influence yet to be determined between the improvement of reactive oxygen species (free radicals) and cell support monitors, which can achieve tissue hurt [20]. Any substance with unpaired electrons is alluded to as a free Radical. Unpaired electrons increment an iota's or alternately atom's compound reactivity. It is odd that oxygen, which is essential forever, can be destructive to the human body in specific cases. The creation and initiation of a gathering of compound particles known as Reactive oxygen species (ROS), which have areas of strength for a to move oxygen to different substances, are liable for most of oxygen's possibly unsafe impacts. The hydroxyl fanatic (OH), superoxide anion (Oz-), progress metals like iron and copper, nitric oxide (NO), and peroxynitrite (ONOO -) are instances of this. Many xenobiotics' harmfulness is connected to the development of free Radicals, which are noxious, yet additionally engaged with the pathophysiology of different problems. For

instance, there is a ton of proof that oxidative pressure plays a part in Alzheimer's sickness neurodegeneration, Parkinson's illness, waterfalls, atherosclerosis, neoplastic problems, diabetes, and ongoing provocative diseases of the gastrointestinal framework, skin maturing, asthma, and different infirmities are among the others [21]. Outside wellsprings of Free radicals and other receptive oxygen species, (ROS) are gotten either from typical fundamental digestion in the human body or from outer sources, for example, openness to beams, ozone, cigarette smoking, certain medications, pesticides, air poisons, and modern synthetic substances. Pesticides are one of the sorts of synthetics that are intentionally released into the climate in light of their known capacity to hurt natural frameworks; subsequently, their risk potential has been very much explored [22]. For as long as decade, pesticide-incited oxidative pressure has been a focal point of toxicological exploration as an expected component of damage. A few examinations have been done to check whether oxidative pressure in individuals or creatures is brought about by the different substances in this gathering and is connected to their hurtful impacts. As they continued looking for security, revolutionaries' assault nearby atoms to get another electron, actually hurting the particle's construction and capability. Free revolutionaries' substance reactivity can harm every single cell macromolecule, including proteins, sugars, lipids, and nucleic acids, in the event that they are not inactivated [23]. Their harming impact on LDL cholesterol, for instance, is probably going to be the reason for arteriosclerosis [24]. Besides, revolutionaries can modify the construction of DNA and cause genotoxicity, which can act as a forerunner to disease [25].

2.2.1. Free Radicals

A free fanatic can be portrayed as an engineered creature bunch having an unpaired electron. It can in like manner be considered as a piece of a molecule. In this way, free fanatics can be conveyed in three ways:

a) By hemolytic cleavage of a typical particles covalent bond, with each piece holding one of the matched electrons.

b) By the departure of a solitary electron from an ordinary particle.

c) By the expansion of a solitary electron to a typical particle [26].

Superfree progressive substances in normal structures are progressive helpers of oxygen. Reducing oxygen by exchanging a single electron for it will make the superoxide anion is awful.

$$O_2 + e^- \longrightarrow O_2^{-\bullet}$$

In organic frameworks, hydrogen peroxide is habitually created by the response of two superoxide particles, which produces hydrogen peroxide and oxygen. In spite of the fact that hydrogen peroxide is certainly not a free extremist, it is named a 'responsive oxygen animal varieties' (ROS), which contains oxygen free Radicals, yet in addition non-revolutionary oxygen subordinates engaged with the formation of oxygen revolutionaries.

$$2O_2^{-\bullet} + 2H^+ \longrightarrow H_2O_2 + O_2$$

Hydrogen peroxide is a fundamental compound in free extreme organic chemistry since it might rapidly break down, particularly within the sight of change metal particles creating hydroxyl revolutionary ($^{\bullet}$0H) which is the most receptive and unsafe of the oxygen free revolutionaries:

$$H_2O_2 + Fe^{2+} \longrightarrow {}^{\bullet}OH + OH^- + Fe^{3+}$$

Without any metal impetuses, superoxide and hydrogen peroxide are promptly taken out and are essentially innocuous [27].

2.2.2 Production of Free Radicals in Cells

As demonstrated by KH Cheeseman and TF Slater free radicals are normally generated in cells by electron migration reactions. They can be mediated by the movement of synthetic or non-enzymatic compounds, usually through redox studies of metal particles. Under normal circumstances, an important source of free radicals in the cell is the 'overflow' of electrons from electron transport chains, such as those in the mitochondria and in the endoplasmic reticulum. matter. substance that, for nuclear oxygen, produces superoxide. Different mixtures can resemble superoxide or hydrogen peroxide distributions, for example, levels of Flavin oxidants arranged in peroxisomes. Another source of superoxide in animal cells is the apparent mixed auto-oxidation,

These autoxidation responses can be extraordinarily upgraded by the association of progress metal particles [28]. Certain perilous unfamiliar synthetic compounds can decisively help the age of free extremists in cells. Carbon tetrachloride, for instance, was the main such atom to be demonstrated

to cause harmfulness by a free extreme system, as it is used to the trichloromethyl free revolutionary in the liver by cytochrome P-450. The liver's cancer prevention agent guards are overpowered by responsive free extremists, bringing about oxidative annihilation of cell films and huge tissue harm [29].

2.3 DAMAGE MECHANISM BY OXIDATIVE STRESS

a) Damages In lipids

Free radicals can focus on any of the significant kinds of biomolecules; however, lipids are presumably the most defenseless. Polyunsaturated unsaturated fats (PUFAs) are bountiful in cell films and are effortlessly harmed by oxidative extremists. Lipid peroxidation, the oxidative breakdown of PUFAs, is especially unsafe in light of the fact that it is a self-sustaining chain response [30]. The general course of lipid peroxidation, as described by KH Cheeseman and TF Slater, can be viewed as in the following embodiment, where LH targets PUFAs and oxidation-fanatic initial R1. The oxidation of PUFAs produces a spectrum of unsaturated fats, which rapidly add oxygen to approach progressive peroxyl unsaturated fats. Peroxyl progressives carry the chain reaction, can oxidize more PUFA particles and initiate new chains, breaking down lipid hydroperoxides into even more extreme species and a significant number of mixtures, aldehydes dramatically.

$$LH + R^{\bullet} \longrightarrow L^{\bullet} + RH$$

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$$

$$LOO^{\bullet} + L'H \longrightarrow LOOH + L`^{\bullet}$$

$$LOOH \longrightarrow LO^{\bullet}, LOO^{\bullet}, \text{aldehydes}$$

Progress metal particle catalysis is oftentimes used to separate lipid hydroperoxides, delivering lipid peroxyl and lipid alkoxyl revolutionaries in processes like those depicted above for hydrogen peroxide. Aldehydes are constantly shaped when lipid hydroperoxides separate and large numbers of them are organically dynamic, these mixtures can diffuse from the first site of the assault and spread the harm to different pieces of the phone.

All in all, lipid peroxidation is of especially significant as a harming response coming about because of free extreme creation in cells since: (I) it is a logical event, given the accessibility and powerlessness of PUFA in films; and (ii) an exceptionally horrendous chain-response can straightforwardly harm the layer structure and in a roundabout way harm other cell parts by delivering receptive aldehydes [31].

b) Damages in protein

Free extreme harm to proteins is simply prone to be destructive to cell suitability on the off chance that it is permitted to aggregate, which is far-fetched in many cells, or on the other hand assuming the harm is centered on unambiguous areas of specific proteins. Assuming a protein ties a change metal particle at a particular site, for example, copper restricting by a histidine buildup, the harm may be centered around unambiguous destinations of that protein [28]. In this situation, the progress metal's response with hydrogen peroxide will deliver hydroxyl revolutionaries that respond at or close to the metal-restricting site; this is alluded to as ' site-explicit harm [32].

c) Damages in DNA

Radiation researcher have demonstrated that DNA is handily obliterated by oxidizing revolutionaries assuming they are framed in its area. Subsequently, it should be considered as a touchy and fundamental objective. There have all the earmarks of being little likelihood of quick chain responses, and we should believe that for harm to be huge, it should either be 'site-explicit' for the situation that it is engaged and of focused energy, bringing about strand breaks, or it should escape the maintenance frameworks before replication, coming about a transformation. The presence of “oxidized nucleobases in human” pee has been thought of as confirmation of a consistent oxidative assault on DNA [19]. Regardless of whether fix proficiency is incredibly high, critical harm can gather over a long period to cause changes and, at last, disease.

Commonly, oxidative harm happens in atomic and mitochondrial DNA from tissue and blood lymphocytes [27]. Guanine is the most inclined to oxidation of all purine and pyridine bases. A hydroxyl bunch is added to the eighth place of the guanine particle when it is oxidized, and the oxidative changed produce 8-OHdG (Fig. 2.1) which is one of the most well-known free revolutionary incited DNA injuries. How much oxidative harmed DNA, known as 8-OHdG, can be estimated to decide the degree of DNA harm. To measure 8-OHdG in tissue or lymphocytes,

compounds such endonuclease and glycosylase should initially sever the oxidized item and delivery it into arrangement. Human tissues, particularly tumors, have been found to contain expanding levels of oxidative harmed DNA [34].

“8-hydroxy-2'deoxyguanosine (8-OHdG, or 8-oxodG)” has as of late arisen as a marker of oxidative pressure. Since it is painless and in fact less confounded, urinary 8-OHdG has been utilized most ordinarily to decide how much oxidative harm. The investigation of oxidative DNA harm is by and large settled to be clinically significant. Various investigations have connected oxidative DNA harm to various maturing related degenerative issues, including malignant growth, coronary illness, and diabetes. A straightforward ELISA can be utilized to evaluate urinary 8-OHdG, specific DNA fix item in the pee, on the grounds that oxidized atomic DNA is somewhat water-dissolvable, the maintenance results of oxidative DNA harms, for example, oxidized nucleosides and bases, will be discharged in the pee without being additionally corrupted. [35].

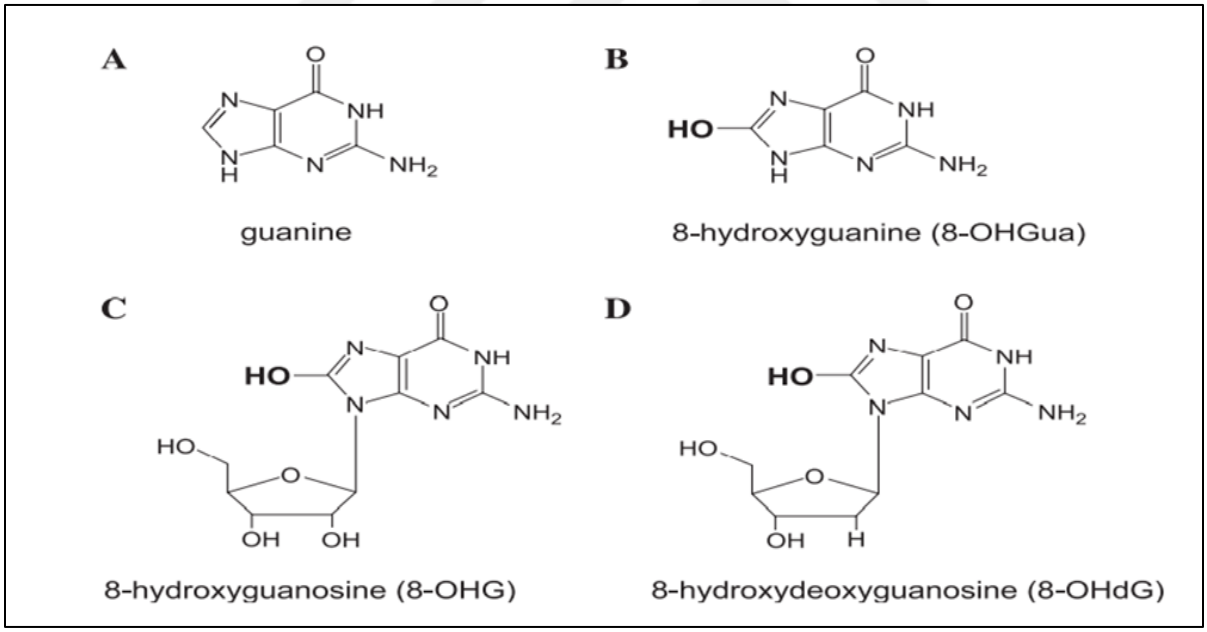

Figure2.1: Chemical design of 8-OHdG and its analogs: (A) unmodified guanine base; (B) oxidized guanine base; (C) homologue of 8-OHdG produced from RNA; (D) construction of 8-OHdG got from DNA [36].

2.4 TYPES OF DNA DAMAGE CAUSED BY OXIDATIVE MECHANISMS

The hydroxyl extremist responds with constituents' DNA close or at dispersion-controlled rates, making harm the “heterocyclic DNA bases and the sugar moiety by different systems”. Among oxygenating-inferred species, the hydroxyl revolutionary (OH), superoxide extremist (O2), and non-extremist H2O2 are of specific significance. Nonetheless, O2 and H2O2 have almost no substance reactivity and don't respond with DNA, proteins, or lipids. Moreover, the response between these two species is incredibly sluggish, with a rate consistent close to nothing. Just change metal particles, like iron and copper particles, catalyze the cycle, which produces OH (Haber-Weiss response) [37]. Just the limiting locales in the nitrogenous bases of DNA and the sugar particle will be momentarily portrayed in the accompanying segment. These connections will check the beginning of the creation of a few of the items that would ultimately cause DNA harm and, thus, a few of illnesses and changes, including growths and diseases.

2.4.1 The DNA Base Damage

Guanine

Guanine has the most negligible reducing ability among all DNA bases. As such it is the best electron contributor and exceptionally oxidized. Gua interfaces with hydroxyl, which proceeds at a consistent rate and a controlled extent of scattering. “The hydroxyl fanatic contributes to the C4-, C5- and C8-dots of Gua, as well as to a lesser extent the C2-position. There is increasing evidence for the easy oxidation of 8-OH-Guanine by multiple oxidants to give different things, the decreasing capacity of 8-OH-Guanine equals 0.74 V when it stands out from 1.29 V for Guanine”. The oxidation can be carried out by experts in different oxidizing subjects, for example ionizing radiations, singlet oxygen, metal particles, per oxynitrate and IrCl 6 2, among others.

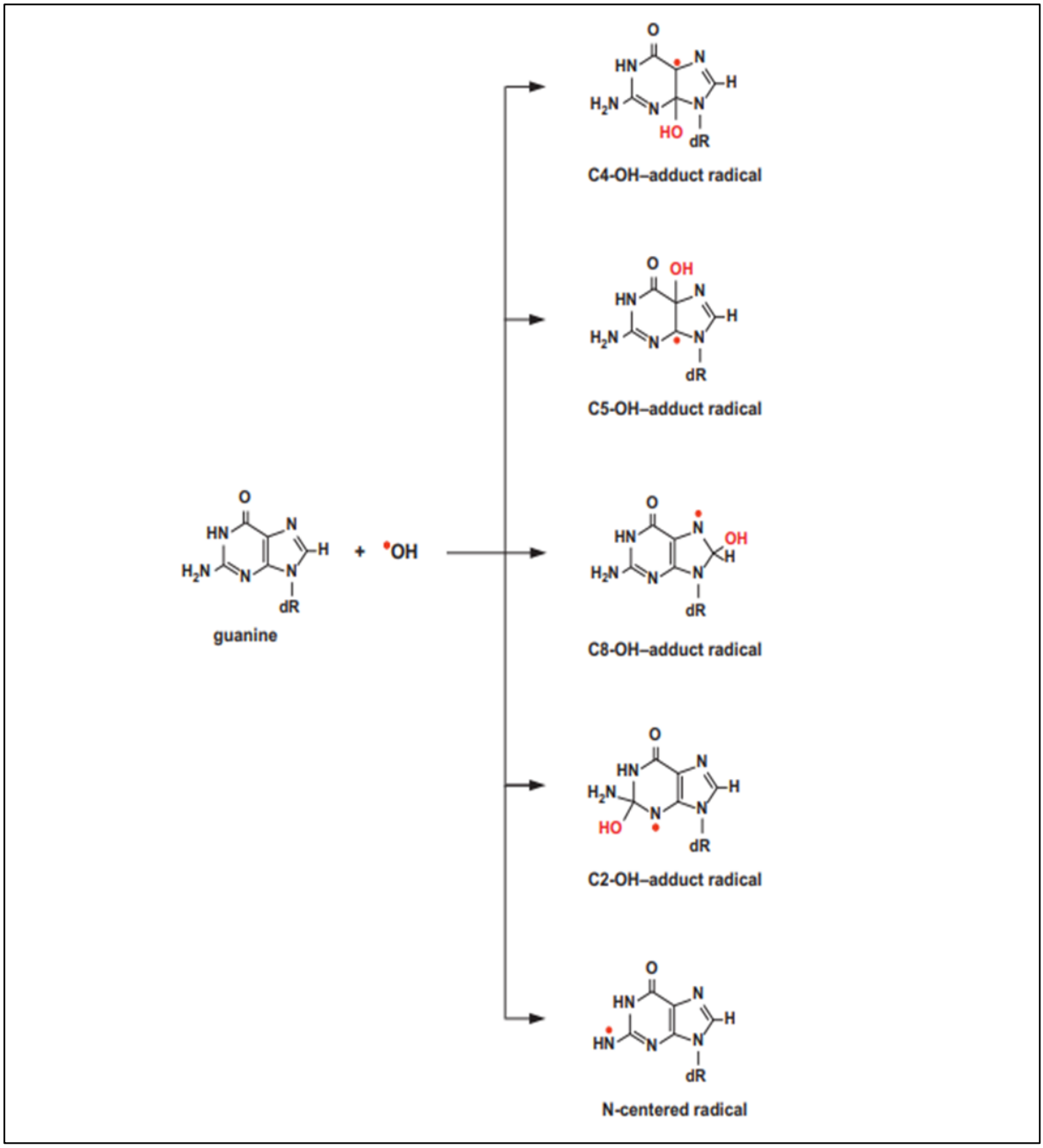

Figure 2.2: **“**Reactions of ˙ OH with Gua. Dr means 2 ′-deoxyribose here and in any remaining applicable figure legends”. (Adjusted from [40, 41]).

Adenine : Adenine's decrease potential (1.56 V) is essentially higher than Guanine's (1.29 V), making it less quickly oxidized. • OH, responds with Adenine by adding to its twofold bonds, as it does with Guanine, as displayed in Figure (2.3). The dispersion of increases, then again, is somewhat unique. “Thus, the expansion at C4 and C8 is half and 37%, separately, bringing about the C4-OH and C8-OH adduct revolutionaries [42,43]”. • OH, expansion to C5 will in general give the lessening C5-OH - adduct revolutionary at a pace of 5%, though expansion to C2 is anticipated to be something like 2% because of lower electron thickness at this site [42].

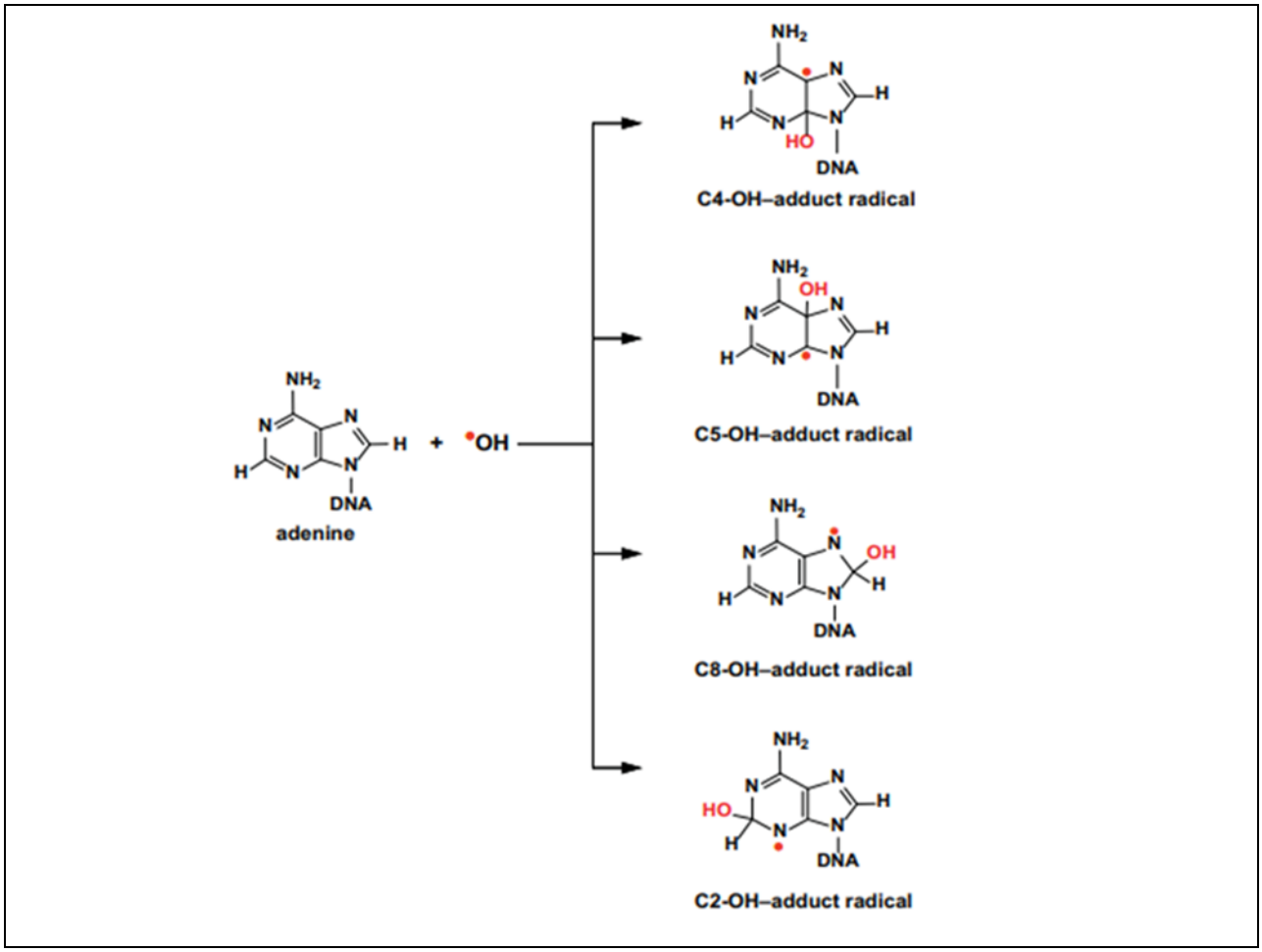

Figure 2.3: Reactions of • OH with Adenine [42].

Thymine : The hydroxyl radical doubles Thy's C5 - C6 - obligation to the extent of 60% at C5 and 30% at C6, and, moreover, removes H from methyl aggregation to much less extent

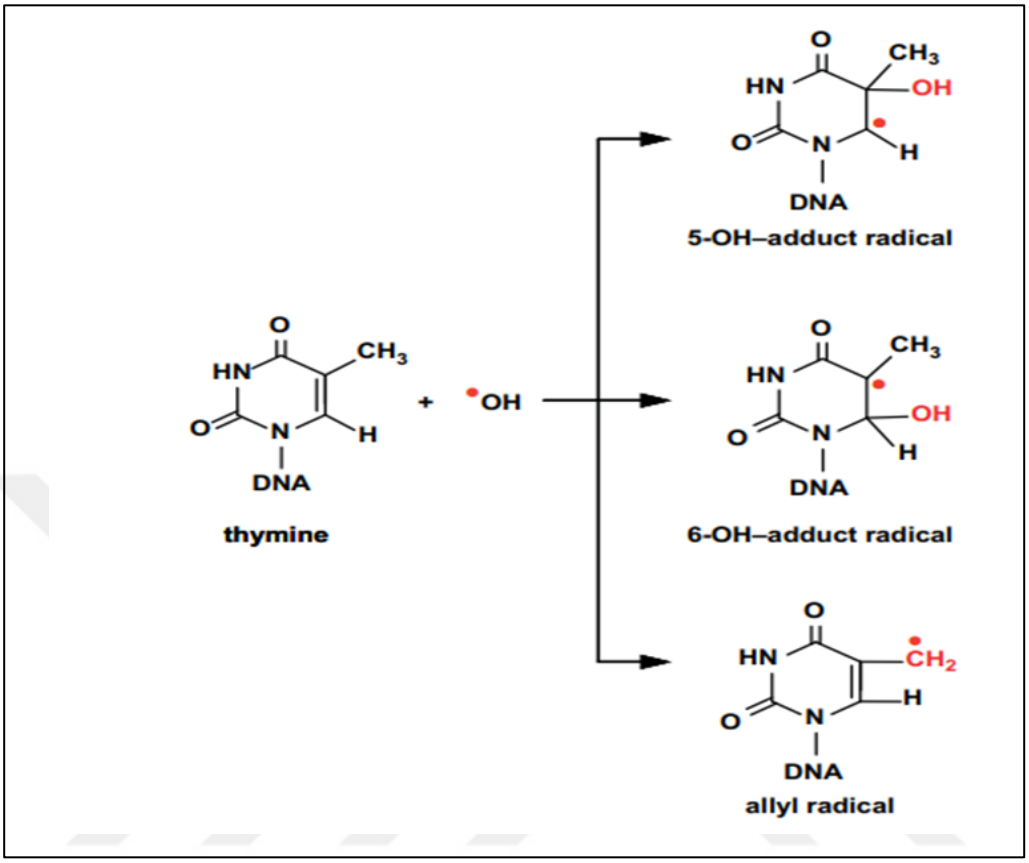

Figure 2.4: Reactions of • OH with Thymine [42].

Cytosine : By adding to the C5 - C6 twofold security, the Hydroxyl revolutionary joins with Cytosine at a dissemination-controlled rate, framing the C5-OH and C6-OH adduct extremists (Figure 2.5). Due to the very high electron thickness at C5 contrasted with C6, the circulation of • OH expansion at Cytosine varies fundamentally from that at Thymine, with expansion happening at C5 and C6 in how much 87% and 10%, separately [44, 45]. Albeit the expansion at N3 has been proposed, it is even doubtful to happen than different augmentations. Over 80% of hydroxyl extremists respond with the base in cytosine nucleosides, with the excess hydroxyl revolutionaries responding with the sugar moiety.

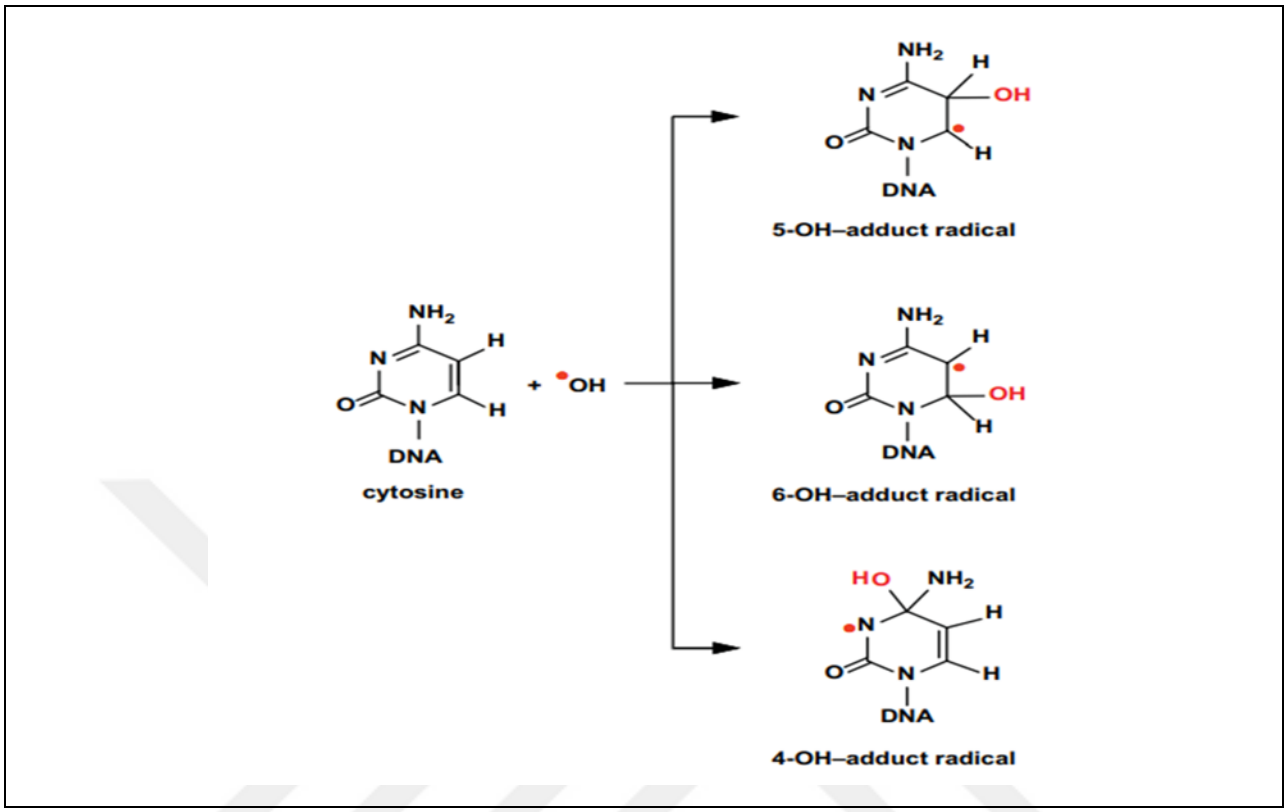

Figure 2.5: Reactions of • OH with Cytosine [42].

2.4.2 Damage to the Sugar Moiety of DNA

Progressive hydroxyl responds with 2′-deoxyribose in DNA by H • thought from all its carbons causing five C-centered fanatics as shown in figure. The overall reliable rate of this reaction is 2.5 x 10^{9} cm^{3} Mol^{-1} s^{-1}. Either way, the rate could depend on the C particles. center of the cell. In poly, for example, how much attack is figuratively 7%. In any case, the DNA strand break is surprisingly perfect considering the amount of •OH assault on 2'-deoxyribose, demonstrating a potential revolutionary exchange from an extremist base to 2'-deoxyribose.

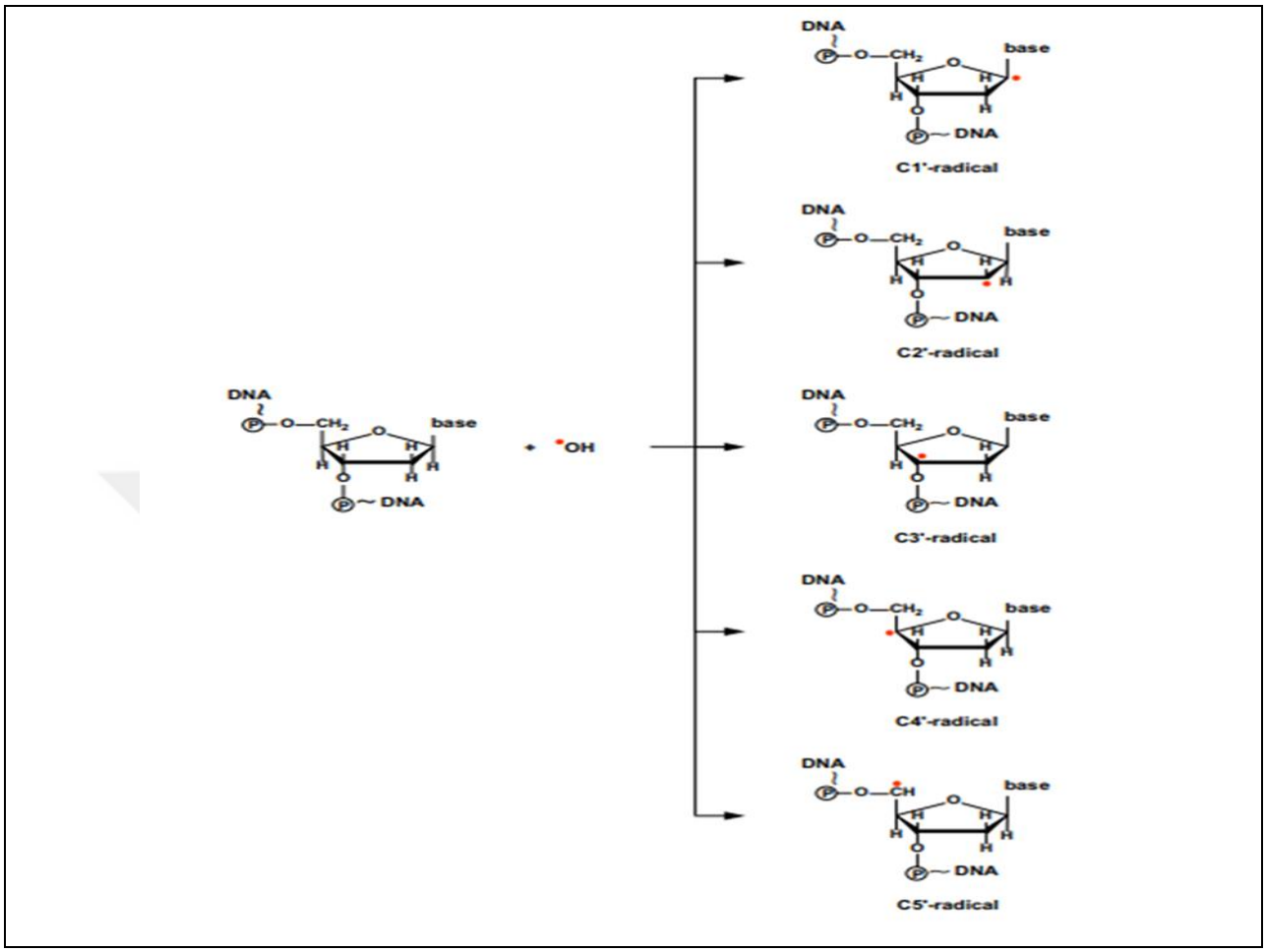

Figure 2.6: H• - Reflection by • OH from 2′-deoxyribose in DNA [41].

2.5 DNA REPAIR AND DNA REPAIR MECHANISMS:

DNA harm brought about by oxidation can bring about hereditary flimsiness, which is a sign of malignant growth. “The proteins engaged with DNA replication, DNA fix, apoptosis”, cell cycle guideline, and chromosomal steadiness might be impacted by hereditary unsteadiness, which can prompt disease [47]. Living creatures have advanced cell strategies to fix DNA harm to keep up with hereditary trustworthiness for endurance. Inability to fix DNA sores can bring about transformation, cytotoxicity, cell demise, and, therefore, obsessive circumstances like malignant growth. Different fix components can kill the DNA harm and reestablish the DNA construction to its unique design [48]. “Two significant instruments exist to fix oxidatively actuated DNA injuries. These are base-extraction fix (BER) and nucleotide-extraction fix (NER)”. Both BER and NER incorporate various cycles and compounds. Jumble fix (MMR) is a strategy for fixing DNA sores

that are mispaired with a DNA base. Furthermore, nucleotide pool fix happens to keep DNA polymerases from integrating changed 20-deoxynucleotides into DNA.

The DNA Mechanisms repair are:

A. Base excision repair BER

“In BER, a DNA glycosylase hydrolyzes the N-glycosidic associate and creates an abasic site to remove a DNA wound. Some DNA glycosylases additionally have AP-lyase activity which hydrolyzes the 3'phosphodiester security of the AP site through a β or β elimination framework, obtaining 3', β-unsaturated aldehyde and 5' phosphate things, and thus strand breaks. To fully restore DNA structure, AP objections are processed by AP endonucleases, DNA polymerases and DNA ligases. Fpg/Nei family and Nth superfamily are two sets of DNA glycosylases”.

In *E.coli*, three huge DNA glycosylases exist to dispense with oxidatively incited DNA base wounds:

a) “Formamidopyrimidine DNA glycosylase (Fpg, generally called MutM)” is a person from the Fpg/Nei gathering of synthetics that separates “FapyAde, and 8-OH-Gua from DNA” with practically identical extraction energy [50].

b) Endonuclease VIII (Nei), which has a place with a similar family, fundamentally targets pyrimidine sores and FapyAde [51, 52].

c) Endonuclease III (Nth) has a place with the Nth superfamily and eliminates pyrimidine injuries and FapyAde. It has covering substrate explicitness with Nei [53].

B. Nucleotide excision repair

The NER chemical is accountable for eliminating massive DNA-mutilating harms [53,54]. Worldwide genomes fix and record coupled fix are two unique NER processes answerable for the maintenance of the whole genome and particular fix of deciphering DNA strands, separately [55]. Excinuclease, a multi-subunit enzyme system, removes an oligodeoxynucleotide carrying the lesion by making twin incisions in the DNA strand. Polymerases then fill and ligate the remaining gap to finish the repair. Prokaryotes and eukaryotes have different lengths of deleted oligodeoxynucleotide [53, 55]. NER is also said to repair the oxidatively generated damages Thy

gly and 8-OH-Gua. Because of the 8,5` -covalent bond, BER is unable to repair 8,5` -cyclopurine-2` -deoxynucleotides. NER is the primary cellular repair mechanism for these injuries [56,57].

C. Mismatch repair

Bungle fix (MMR) is utilized to fix confounds like the 8-OH-GuaAde jumble. MutY, a DNA glycosylase in *E. coli*, eliminates Adenine from this befuddle, permitting 8-OH-Gua to couple with the homologous nucleotide Cytosine, which is then fixed by BER [58]. The core and the mitochondrion are designated by the human homolog MUTYH, which has a place with the Nth superfamily [58, 59]. What's more, MUTYH kills 2-OH-Adenine from each of the four unblemished DNA bases [60]. There is proof that MUTYH changes are connected to colorectal disease and physical G→T transformations, suggesting that MUTYH assumes a significant part in malignant growth avoidance [58, 61]. MUTYH has not been recognized to be a substrate for some other oxidatively caused DNA base sores in confounds. FapyGua, which moreover miss pairs with Ade and causes G→T changes, is a potential substrate of MUTYH and subsequently of MUTYH. "Is a potential competitor to be a substrate of MUTYH" and consequently to expect a section in colorectal threatening development along with 8-OH-Gua.

D. Repair in the nucleotide pool

Modified 20-deoxynucleoside triphosphates are immobilized by a special immobilization procedure in the cell's nucleotide pool before they can be facilitated into DNA-by-DNA polymerases and are capable of inducing changes change. MutT hydrolyzes 8-OH-dGTP to 8-OH-dGMP in *E. coli* bacteria, preventing it from being incorporated into DNA. MutT homologs have been found in warm-blooded individuals and animals. 8-OH-dATP and 2-OH-dATP are relatively hydrolyzed by human MTH1. Mth1 undoing in mice comes to fruition during unrestricted carcinogenesis in the lung, liver, and stomach, representing this qualitative seizure in a harmful developmental balance.

E. Repair of DNA strand breaks

Single and twofold strand breaks in DNA are likewise brought about by oxidatively actuated harm, bringing about hereditary flimsiness and hurtful organic impacts [49, 50]. Comparable techniques to those referenced on account of BER are utilized to "fix single-strand breaks. Homologous

recombination (HR) or non-homologous end-joining (NHEJ)" techniques are normally used to fix DSBs.

HR proteins, for example, BRCA1 are initiated when NHEJ is blemished, and DSBs are handled by HR, inferring a connection between the BER and HR/NHEJ pathways [66]. BRCA1 or DSB fix lack influences the handling of oxidatively produced DNA harms Repair of DNA strand breaks have been all around covered already [48,67,68], and it won't be talked about additional here.

2.6 ANTIOXIDANT

Cell reinforcements are a gathering of substance parts found normally in food that can assist with forestalling or limit oxidative stress in the body. Because of the consistent utilization of oxygen, the body is continually delivering free extremists. These free revolutionaries cause cell harm in the body and play a part in an assortment of medical problems, including coronary illness, diabetes, macular degeneration, and cancer [69]. Cancer prevention agents, which are fantastic free extreme scroungers, help in the counteraction and fix of the cell harm brought about by these free revolutionaries. Cancer prevention agents are plentiful in plants and creatures that combine them normally. Cell reinforcements can likewise be made utilizing a synthetic technique or a natural interaction from a few sorts of agro-related squanders [70]. Cell reinforcements are separated into two classes in light of their solvency: water-solvent and lipid dissolvable. Water-solvent cancer prevention agents, for example, ascorbic corrosive, glutathione, and uric corrosive, for instance, play parts in both the cell cytosol and the blood plasma. Ascorbic corrosive is a redox impetus that decreases and kills receptive oxygen species (ROS), glutathione is a cell reinforcement that goes about as a diminishing specialist and can be oxidized and diminished in a reversible way, and ubiquinol, tocopherol, and carotenoid are lipid-dissolvable cancer prevention agents that shield cell layers from lipid peroxidation. One more method for arranging cell reinforcements is by their method of activity, for example, essential or chain-breaking cancer prevention agents versus optional or protection cell reinforcements [71]. At the point when cell reinforcements are absent in the right region at the ideal focus brilliantly, they can go about as prooxidants. Flow research is taking a gander at the general significance of a cell reinforcement's cell reinforcement and prooxidant activities.

2.6.1 Levels of Action of Antioxidants

In living frameworks, the cancer prevention agent particles that make up the cell reinforcement safeguard matrix work at different levels. These levels could be an extreme decrease, revolutionary rummaging, or extremist-initiated harm fix. Cell reinforcements can be grouped into four classes in light of their line of safeguard: first-line guard cancer prevention agents, second-line protection cell reinforcements, third-line protection cancer prevention agents, and fourth-line safeguard cancer prevention agents [72].

a) Front line disease defense specialists: These are cellular fortifications that act in cells to reduce or thwart the period of free progressive or open species. They kill any molecule that can form into a free progressive or any free radical, with the ability to set off the game plan of various fanatics in a very short time. The top three pulses are superoxide dismutase, catalase, and glutathione peroxidase. These mixtures independently break down superoxide progressives, hydrogen peroxides and hydroperoxides into harmless particles. A molecule of metal limiting proteins such as transferrin and ceruloplasmin chelate or sequestering iron and copper independently suppresses free inflammatory formation.

b) Second-line guard cancer prevention agents: Scavenging cell reinforcements are the name given to this class of cell reinforcements. They search dynamic revolutionaries to stop chain inception and spread processes. They rummage or kill free revolutionaries by moving one electron to them, and simultaneously, they become free extremists with less destructive impacts. Different cell reinforcements in this class can without much of a stretch kill these "new extremists" and render them totally innocuous. This gathering incorporates hydrophilic cancer prevention agents, for example, ascorbic corrosive, uric corrosive, and glutathione, as well as lipophilic cell reinforcements like alpha-tocopherol (vitamin E) and ubiquinol [72].

c) Third-line assurance malignant growth avoidance specialists: After free outrageous mischief has occurred, this class of cell fortifications enters the picture. These are again proteins that protect the damage to biomolecules made by free progressives and patch the damaged cell layer. It is a collection of mixtures that protect damaged DNA, proteins and lipids. Similarly, they deliver a 'clean' capacity to see, isolate and dispose of oxidized or damaged proteins, DNA

and lipids to prevent their social situations that may be risky for human tissues. They are typical patterns “in both the cytosol and mitochondria of mammalian cells”.

d) Fourth line safeguard cell reinforcements: The activity of these 'cell reinforcements' depends on a variation component in which they utilize the signs expected with the expectation of complimentary revolutionary creation and reaction to forestall free extreme development and response. The sign given by the free extreme makes the creation and conveyance of a cancer prevention agent the appropriate area [73].

2.6.2 Classification of Antioxidants

Normal and manufactured cell reinforcements are the two significant types of cell reinforcements that can be sorted in view of their source (a schematic representation of the characterization of cell reinforcements is displayed in Figure 2.7).

A. Natural Antioxidants:

Regular cancer prevention agents are either made in the human body through metabolic cycles or acquired from other normal sources, and their action is to a not entirely settled by their physical and compound elements as well as their component of activity. Enzymatic cell reinforcements and nonenzymatic cell reinforcements are the two sorts of cancer prevention agents that can be found.

a) Enzymatic Antioxidants

Enzymatic cancer prevention agents are made solely in the human body and are delegated either essential or auxiliary cell reinforcements.

Primary antioxidants:

As recorded underneath, essential cancer prevention agents incorporate “superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)”.

Superoxide dismutase (SOD): The expert avoids malignant growth best and is the first detoxifying compound in the cell. The primary endogenous cell support dynamics fills as a component of the body's primary defense against open oxygen species. It catalyzes the decomposition of two iotas of the superoxide anion into hydrogen peroxide and subnuclear oxygen. Thus, the damaging

superoxide anion is conceivably reduced to less dangerous levels. Since SOD is a metalloenzyme, it needs a metal cofactor to function. Turf appears in different designs depending on the type of metal molecule it expects as a cofactor. Iron, zinc, copper and manganese are the metal particles to which the SOD binds overall. In that way, SOD is divided into three categories: I Fe-SOD, found in prokaryotes and chloroplasts of specific plant species; Mn-SOD, found in prokaryotes and eukaryotic mitochondria; and Cu/Zn-SOD, mainly found in the cytoplasm, furthermore in chloroplasts and peroxisomes. [76].

$$2O_2^{*-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Catalase (CAT): Is a far-reaching cell reinforcement catalyst found in basically all organic tissues that utilization oxygen as a wellspring of energy. “The compound catalyzes the degradation or reduction of hydrogen peroxide to water and subnuclear oxygen”, including iron or manganese as a cofactor, completing the detoxification connection started by SOD.[77]. Cat is exceptionally compelling, prepared to corrupt a large number of hydrogen peroxide particles in a singular second. The impetus is basically found in peroxisomes; notwithstanding, it isn't tracked down there of brain of mammalian cells. The main special case in the mitochondria of the rodent's heart is [78].

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

Glutathione peroxidases (GPx): Is a fundamental intracellular catalyst that breaks down hydrogen peroxides (H2O2) to water and lipid peroxides to their comparing alcohols, Reduced glutathione (GSH) catalyzes the collaboration of H2O2 with diminished glutathione (GSH); accordingly, oxidized glutathione It (GSSG) is framed, which is then reused back to its decreased structure “by glutathione reductase (GR) and diminished nicotinamide adenine dinucleotide phosphate (NADPH)”. Basically, in the mitochondria yet additionally in the cytosol [79]. Selenium, a micronutrient cofactor, is typically expected for its action. GPX is known as a selenocysteine peroxidase along these lines. The catalyst shields cells from oxidative pressure by smothering the lipid peroxidation process [80].

$$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$$

Secondary Antioxidant

“Glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH)” are instances of optional cancer prevention agents. NADPH is created by the chemical G6PDH. The optional chemical GR and NADPH are expected to reuse decreased glutathione (GSH).

$$\mathbf{GSSG+NADPH \xrightarrow{GR} NADP+2GSH}$$

“Glutathione is a cysteine-containing peptide-type cell reinforcement that is delivered in the cells of the body”. Its cysteine moiety's thiol bunch is a lessening specialist that can be oxidized and diminished in a reversible way. The cells contain a lot of glutathione. “Hissin and Hilf (1976)” kept in the diminished structure (GSH) is diminished by the chemical GR, which diminishes different metabolites and protein frameworks, for example, ascorbate. Due to its high focus and significance in upkeep, it is one of the main cell cancer prevention agents because of its redox state [81].

b) Nonenzymatic Antioxidants

Non-enzymatic cancer prevention agents, which are compounds with the capacity to quickly inactivate revolutionaries and oxidants, are the second line of protection against ROS. These proteins and little atomic mass mixtures give compelling intracellular and extracellular guard instruments against ROS and receptive nitrogen species (RNS) because of their area (Table 3.). The non-enzymatic cancer prevention agents are isolated into four significant subcategories which are nutrients, minerals, carotenoid, polyphenols, and different kinds of cell reinforcements.

Table 2.3: Intra- and Extracellular Non-Enzymatic Antioxidants [82].

INTRACELLULAR	**EXTRACELLULAR**
Melatonin	Uric acid
Glutathione	Bilirubin
Polyamines	Albumin
Myoglobin	Transferrin
Ferritin	Lactoferrin
Metallothionein’s	Ceruloplasmin
Coenzyme Q10	

a) Vitamin: Nutrients are complicated synthetic substances present in food varieties that are essential for a solid digestion. Absence of these supplements could create some issues, despite the fact that resupply of these supplements can assist with lessening the side effects of lack [83]. Nutrients are recognized from other food supplements by their natural structure, and their still up in the air by their compound nature and capability [84]. Nutrients are partitioned into two classes overall. Nutrients that are water-dissolvable and nutrients that are fat-solvent. Nutrients, for example, vitamin A, L-ascorbic acid, vitamin E, and vitamin B, are fundamental for the typical working of the body's cell reinforcement protein framework. They can't be created by human bodies and must hence be acquired through dietary supplementation.

Vitamin A: Vitamin A guides in the support of epithelial cells in mucous layers and skin, as well as it's significant for night vision. It comes in three essential structures: “retinol, 3,4-didehydroretinol, and 3-hydroxyretinol”, all of which have cell reinforcement characteristics that help the safe framework. Yams, carrots, milk, egg yolks, and mozzarella cheddar are the absolute best wellsprings of this nutrient. A potential cell reinforcement job was portrayed in a couple of concentrates in a circuitous technique. It was found to limit thioredoxin-collaborating protein, which represses the actuation of hepatic stellate cells (an effector of hepatocellular disease) and brings down oxidative feelings of anxiety [85]. Retinoic corrosive, a vitamin A metabolite, has likewise been displayed to upregulate cell reinforcement quality articulation in mature bison oocytes in vitro [86]. Besides, in both solid and varicocele sperm, all-trans retinoic corrosive expanded superoxide dismutase and glutathione transferase movement while diminishing malondialdehyde and responsive oxygen species, suggesting that retinol increments cell reinforcement compound action [87].

L-ascorbic acid: Vitamin C, frequently known as ascorbic corrosive, is a water-solvent nutrient. It very well may be found in different food varieties, including citrus organic products, vegetables, cereals, meat, poultry, and fish. It helps with the counteraction of some DNA harm created by free revolutionaries, which can add to the maturing system and the improvement of infections like malignant growth, coronary illness, and joint inflammation [88].

Vitamin E: Vitamin E is a strong cell reinforcement. Vitamin E is a lipid-solvent nutrient, and that implies it could be consumed through the skin. There are eight unique kinds of tocopherol, including α-, β-, γ-, and δ-tocopherol. δ-Tocopherol has the most elevated bioavailability and is

the main lipid-solvent cell reinforcement that responds with the lipid extremist and safeguards the layers from lipid peroxidation; accordingly, oxidized α-tocopheroxyl revolutionaries are created, which can be reused to the decreased structure through decrease by different cell reinforcements, for example, ascorbate and resveratrol [89].

B) Minerals: Minerals are co-variables of cell reinforcement proteins. Their nonappearance influences the digestion of numerous macromolecules like sugars, proteins, and lipids. Models incorporate copper, iron, manganese, selenium, and zinc.

C) Carotenoid : Plants and organisms produce carotenoids, which are isoprenoids. The majority of them have a skeleton comprised of extended carbon chains with a progression of formed carbon twofold bonds. Lycopene, lutein, - carotene, and - carotene are only a couple of models.

D) Polyphenols : Polyphenols are normally happening substances that can be tracked down in different food sources, including natural products, vegetables, oats, and beverages. For example, grapes, apples, pears, cherries and strawberries may contain 200-300 mg of polyphenols per 100 grams of new weight. Polyphenols are a gathering of phytochemicals with solid cell reinforcement properties. Their cancer prevention agent not set in stone by their synthetic and actual properties, which impact digestion through their sub-atomic designs [90]. Phenolic acids, flavonoids, gingerol, curcumin, and different mixtures are kinds of them [91].

Flavonoids are a kind of polyphenolic part that can be found in different food sources like vegetables, normal items, grains, seeds, leaves, blooms, and bark, and anything is possible from that point. While ginger is extracted from the ginger's rhizomes, curcumin is the main bioactive part of turmeric and is seen as serious solid space for action. Curcumin is an amazing ROS scavenger that causes oxidative tension just like O2. Progressives, lipid peroxyl radicals, OH fanatics and nitrogen dioxide progressives. “Curcumin has been shown” to reduce “lipid peroxidation and increase GSH levels in epithelial cells”, resulting in a reduction in ROS formation. [92].

E. Other Antioxidants : Progress Metal-Binding Proteins will be proteins that tight spot to change metals. The progress metal-restricting proteins egg whites, ceruloplasmin, haptoglobin, and transferrin are tracked down in human plasma and predicament to change metals to restrict the arrangement of metal catalyzed free extremists.

Non-Protein Antioxidants Bilirubin, uric acids, and ubiquinol are non-protein cell fortifiers that control oxidative processes by removing free progressives. [93].

Bilirubin: The mononuclear phagocyte framework produces bilirubin (BIL) as a breakdown result of hemoglobin and other heme proteins. uBIL has been displayed to have huge cancer prevention agent impacts in natural trials [94,95]. In polar media, for example, fluid lipid bilayers, BIL has been displayed to have serious areas of strength for a potential against peroxyl revolutionaries.

Uric Acid: Uric corrosive (UA) is a low sub-atomic weight synthetic particle that is delivered during purine digestion. UA is a scrounger of various ROS, including peroxynitrite, hydroxyl extremist, singlet oxygen, and lipid peroxides, and makes up 66% of the cell reinforcement. It can presumably search nitrogen dioxide and carbonate particles also [96], and structure stable edifices with iron and copper particles, hindering free extreme cycles like the Fenton response and the Haber-Weiss response [97].

Coenzyme Q: is an oil-soluble antioxidant that goes by the name ubiquinol (Co Q). The heart, liver, kidneys, pancreas, and other organs create this by a monovalent pathway. There are two possible mechanisms for action: In the essential part, the decreased sort of ubiquinol (CoQH) capacities as a chain-breaking cell support, diminishing peroxyl (ROO.) and alcoxyl (LO.) progressives [87].

CoQH + → ROO Q + ROOH

In the subsequent component, it responds with vitamin E revolutionary (TO.) and recovers vitamin E.

CoQH + → TO Q R+ OOH

b) Synthetic Antioxidants

Engineered cell reinforcements are made by different techniques. They are polyphenol radicals that end chain responses overall. More than one hydroxyl or methoxy bunch is normally seen in polyphenolic compounds. The main heterocyclic, N-containing atom used as a cancer prevention agent in food, especially creature feed, is ethoxy quinine. Engineered phenolic cell reinforcements are quite often p-subbed, though regular phenolic compounds are quite often o-subbed. Due to

their diminished harmfulness, the p-subbed compounds are leaned toward. To further develop dissolvability in fats and oils and limit poisonousness, engineered phenolic cancer prevention agents are constantly supplanted with alkyl gatherings [98]. These cancer prevention agent dynamic manufactured synthetic compounds are broadly utilized in drugs, as surface level additives, and to protect fat, oil, and lipid in food [92]. These new disclosures concerning engineered cell reinforcements have prompted the advancement of novel manufactured cancer prevention agents with further developed water solvency, steadiness, and non-poisonousness.“Butylated hydroxyanisole, butylated hydroxytoluene, ethylenediaminetetraacetic disruptor, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, propyl gallate and tertiary butylhydroquinone are part of known cell supplements”. [99].

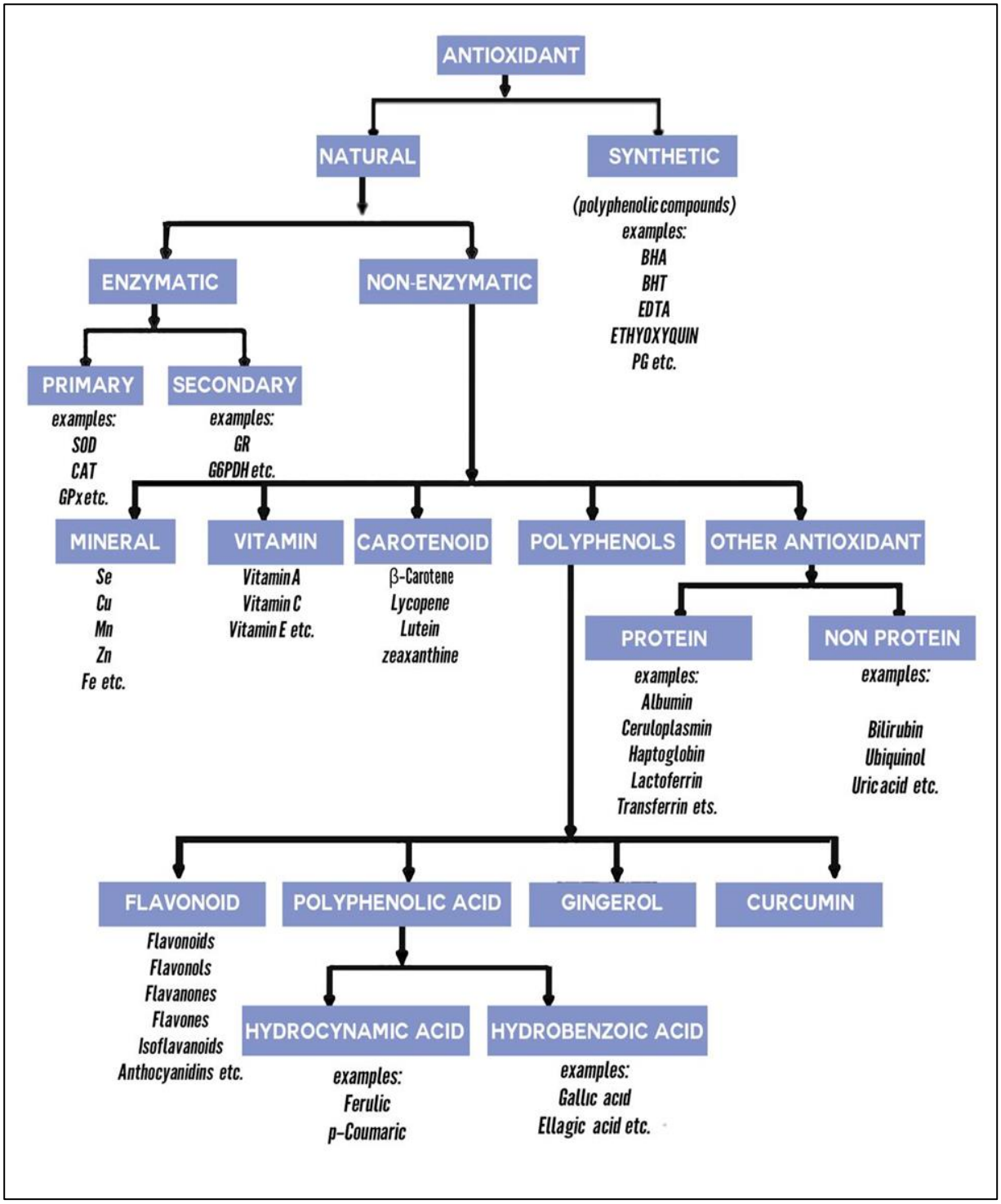

Figure 2.7: Classification of Antioxidants [71].

3. METHODOLOGY

3.1 RESEARCH COMMUNITY AND SAMPLE

The research community consists of the owners of shops and grocery markets in different regions of Istanbul (as shown in Table 3.1, the sample group and Table 3.2 the control group), where a random sample was selected from them, where the number of the sample was (40) greengrocers, as well as 20 individuals working in different jobs as a control so that the total sample community was 60 person, whose ages ranged between (16-73) years.

Table 3.1: Greengrocers' Markets and Region Information for the sample group.

SAMPLE NUMBER	GENDER	SAMPLE MARKET INFORMATION	CITY/REGION
SAMPLE 1	Male	small street greengrocer	İstanbul/Avcılar
SAMPLE 2	Male	Small market greengrocer	İstanbul/Avcılar
SAMPLE 3	Male	small street greengrocer	İstanbul/Avcılar
SAMPLE 4	Male	Small market greengrocer	İstanbul/Avcılar
SAMPLE 5	Male	small street greengrocer	Istanbul /Avcılar
SAMPLE 6	Male	small street greengrocer	İstanbul/Aksaray
SAMPLE 7	Male	small street greengrocer	İstanbul/ Yusufpaşa
SAMPLE 8	Male	small market greengrocer	İstanbul/Fatih
SAMPLE 9	Male	big market greengrocer	Istanbule / Beylikdüzü
SAMPLE 10	Male	small street greengrocer	Istanbul/Beylikduzu
SAMPLE 11	Male	small street greengrocer	İstanbul /Esenyurt
SAMPLE 12	Male	small street greengrocer	İstanbul/Esenyurt
SAMPLE 13	Male	small street greengrocer	İstanbul /Bahçelievler
SAMPLE 14	Male	small street greengrocer	İstanbul / Bahçelievler

Continue to Table 3.1

SAMPLE 15	**Male**	**small street greengrocer**	**İstanbul / Bahçelievler**
SAMPLE 16	Male	small street greengrocer	İstanbul / Bahçelievler
SAMPLE 17	Male	small street greengrocer	İstanbul / Bahçelievler
SAMPLE 18	Male	small street greengrocer	İstanbul / Bahçelievler
SAMPLE 19	Male	small street greengrocer	İstanbul / Bahçelievler
SAMPLE 20	Male	small street greengrocer	İstanbul /Sefaköy
SAMPLE 21	Male	small street greengrocer	İstanbul / Sefaköy
SAMPLE 22	Male	small street greengrocer	İstanbul / Sefaköy
SAMPLE 23	Male	small street greengrocer	İstanbul /Küçükçekmece
SAMPLE 24	Male	small street greengrocer	İstanbul / Küçükçekmece
SAMPLE 25	Male	small street greengrocer	İstanbul / Küçükçekmece
SAMPLE 26	Male	small street greengrocer	İstanbul / Küçükçekmece
SAMPLE 27	Male	small street greengrocer	Istanbul / Şirinevler
SAMPLE 28	Male	small street greengrocer	Istanbul /Florya
SAMPLE 29	Male	small street greengrocer	İstanbul/Florya
SAMPLE 30	Male	small street greengrocer	İstanbul/Yenibosna
SAMPLE 31	Male	small street greengrocer	İstanbul/Mustafa Kemal Paşa
SAMPLE 32	Male	small street greengrocer	İstanbul/Avcılar
SAMPLE 33	Male	small street greengrocer	İstanbul /Beşiktaş
SAMPLE 34	Male	Sunday Pazar	İstanbul / Beşiktaş
SAMPLE 35	Male	Sunday Pazar	İstanbul / Beşiktaş

Continue to Table 3.1

SAMPLE 36	Male	Sunday Pazar	İstanbul / Beşiktaş
SAMPLE 37	Male	Sunday Pazar	İstanbul / Beşiktaş
SAMPLE 38	Male	Sunday Pazar	İstanbul / Kadıköy
SAMPLE 39	Male	Friday Pazar	İstanbul / Kadıköy
SAMPLE 40	Male	Friday Pazar	İstanbul /Kadıköy

Table 3.2: The Control Sample group region information.

CONTROL NUMBER	GENDER	CITY /REGION
CONTROL 1	Male	İstanbul/Zeytinburnu
CONTROL 2	Male	İstanbul/Avcılar
CONTROL 3	Male	Istanbul / Avcılar
CONTROL 4	Male	İstanbul/ Esenyurt
CONTROL 5	Male	Istanbul / Esenyurt
CONTROL 6	Male	Istanbul/Esenyurt
CONTROL 7	Male	İstanbul/Fatih
CONTROL 8	Male	Istanbul/ Fatih
CONTROL 9	Male	Istanbul/ Fatih
CONTROL 10	Male	Istanbul/ Fatih
CONTROL 11	Male	İstanbul/Aksaray
CONTROL 12	Male	Istanbul/ Aksaray
CONTROL 13	Male	Istanbul/Faith
CONTROL 14	Male	İstanbul/Sefaköy

Continue to Table 3.2

CONTROL 15	Male	Istanbul/ Sefaköy
CONTROL 16	Male	İstanbul/Kadıköy
CONTROL 17	Male	İstanbul/Nişantaşı
CONTROL 18	Male	İstanbul/Ümraniye
CONTROL 19	Male	Istanbul/ Ümraniye
CONTROL 20	Male	İstanbul/Göztepe

3.2 DATA COLLECTION

To reach the desired results from conducting the research, a questionnaire was prepared to measure some individual and demographic characteristics such as (gender, age, weight and height), lifestyle (type of food consumed, diet and sports), some work-related features, such as (the number of daily working hours and Since when they are practicing this profession), and some information about the medical record of the sample members, including (infection with diseases and viruses in the last year or the present or absents of cancer in the family but not in the individual, even if they take certain medications on an ongoing basis). The data collection process was carried out in three months by direct interview. All smokers, alcohol consumers, and those with chronic diseases such as pressure, diabetes, heart disease, as well as neurological disorders and cancer were excluded from all members of the research sample for not overlapping the characteristics and effects caused by these diseases and habits with measuring biochemical information and obtaining precise results. The research sample size was 73 respondents, but ten males were excluded from the sample community because they did not meet the required criteria. Three females were also excluded due to the difficulty of taking samples from them and finding women working in this field, and others also refused to participate in the research. Thus, the final sample number was 60 males only. The research work plan was “reviewed and approved by the Ethics Committee of Altınbaş University (Ethics Committee number: 2021/107).

3.3 SAMPLE COLLECTION:

Urine samples were collected from all greengrocers and other individuals included in this study using special sterile 10 ml tubes. The samples were preserved in the laboratories of Altınbaş University at a temperature of -20 °C.

Urine test aliquots were used in an ELISA competitive assay kit (Cayman Chemical, Ann Arbor, MI, USA) to quantify 8-OHdG (ng/mL). The urinary creatinine (mg/dL) was estimated using another ELISA analysis Kit (Cayman Chemical, Ann Arbor, MI, USA). The 8-OHdG concentrations of all participants (sample group and control group) were determined using the calibration curve. The resulting creatinine-cured 8-OHdG (ng/mg) were used for analyses. ELISA assay was carried out in laboratory of Farmasina Tıbbi ve Kimyevi Ürünler firm.

3.4 TOOLS AND PROCEDURE

A. Creatinine (Urinary) colorimetric assay kit part:

a) Equipment's and devices used:

   a. Urine container

   b. Test tubes Beakers

   c. Graduated cylinder

   d. Test tube racks

   e. Microplate Shaker

   f. Vortex

   g. Pipette

   h. Plate reader (DAR800)

   i. Incubator

   j. Refrigerator

k. 96-well solid plate (colorimetric assay)

l. 96-well cover sheet

b) Liquids used (Solutions and Reagent):

a. Creatinine standard

b. Creatinine color reagent

c. Creatinine sodium hydroxide

d. Creatinine acid solution

e. Creatinine sodium borate

f. Creatinine surfactant

g. Alkaline picrate solution

h. UltraPure water

c) Reagent and sample preparation

a. All the Reagent (creatinine standard, creatinine color reagent, creatinine sodium hydroxide, creatinine acid solution, creatinine sodium borate, creatinine surfactant) are ready to use except Alkaline picrate solution for the preparation of volume enough to 96-well plate of it, we mix (3.6 ml of creatinine NaOH, 10 ml of creatinine color reagent, 6 ml of creatinine surfactant and 2 ml of creatinine sodium borate) together.

b. For the sample preparation, the first thing after samples were collected in a clean container was stored on ice until their transfer to the refrigerator at -20 ◦C. when we ran the experiment, the urine samples were dissolved into their liquid state at room temperature. After that, a 1:10 diluted with water was made right before assaying.

c. Standard planning: for the creatinine standard readiness, step through 8 examination cylinders and name those from A-H, then, at that point, add how much creatinine standard to refined water as indicated by table 3.3.

Table 3.3: Concentration of Standard.

Tube	Creatinine standard (µl)	Pure water(µl)	Final concentration (mg/dl creatinine)
A	O	5OO	O
B	5O	45O	2
C	1OO	4OO	4
D	15O	35O	6
E	2OO	3OO	8
F	25O	25O	1O
G	300	2OO	12
H	375	125	15

Performing the Procedure of the assay:

a) As saw as in Table 3.3, we add 15µl of creatinine standard (from tubes A-H) to each well in the exhibited well on the plate.

b) We add 15 µl of the Sample to one well, as displayed in (fig.3.1).

c) Proceed with the response by adding 150 µl of Alkaline picrate solution or reagent for each well.

d) We covered the plate with a plate cover and incubated for 10 minutes at room temperature on a shaker.

e) "We removed the plate cover and read the absorbance at 490-500 nm using the Plate reader; this review considers baseline absorbance".

f) We add 15 ml of the creatinine corrosive answer for every one of the wells.

g) Seal the "plate with the plate cover and gantry on the shaker for 10 minutes at room temperature".

h) Remove the plate cover and read the absorbance at 490-500 nm using a plate peruser; this examination will refer to the last absorbance examination.

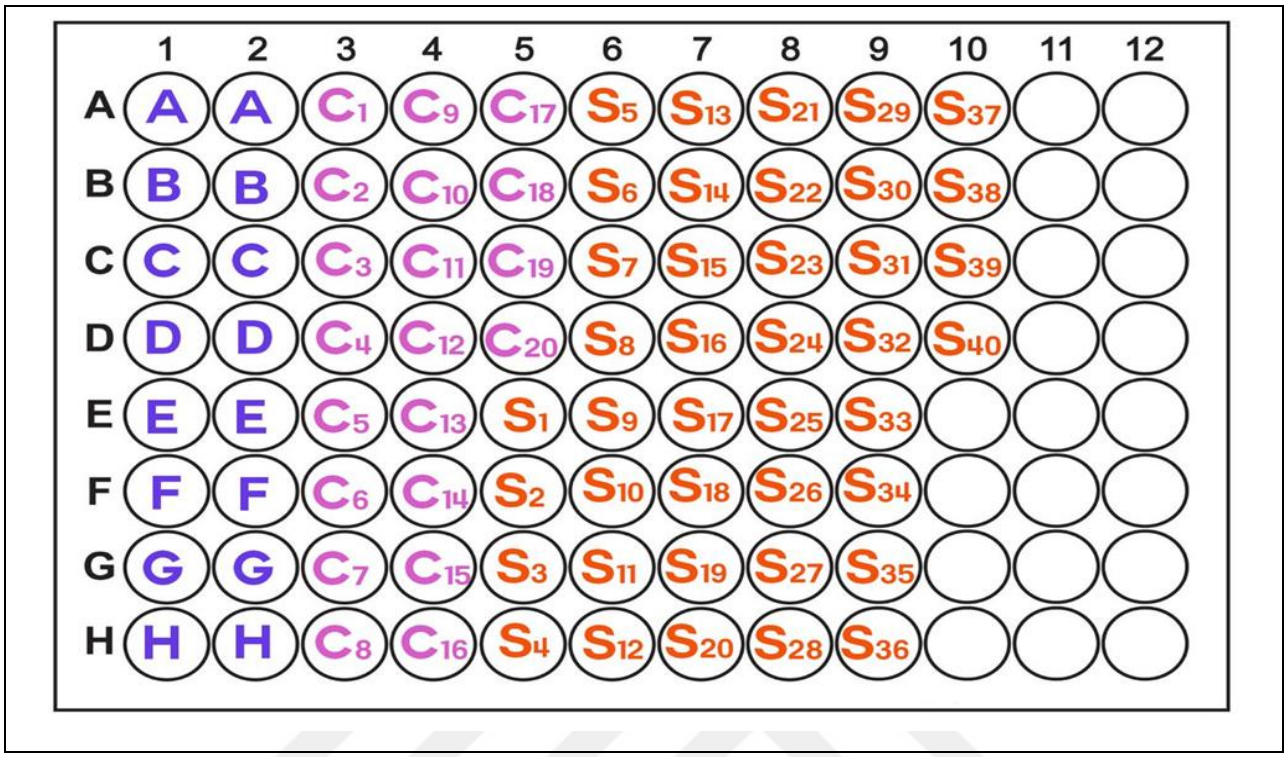

Figure 3.1: Plate Form Disruption of Creatinine (urinary) colorimetric assay kit.

B. "DNA/RNA Oxidative Damage (high responsiveness) ELISA Kit" part:

a) Equipment and devices used:

   a. Urine container.

   b. Test tubes.

   c. Beakers.

   d. Graduated cylinder

   e. Test tube racks

   f. Microplate Shaker

   g. Vortex

   h. Pipette

   i. Plate reader (DAR800)

j. Incubator

k. Refrigerator

l. 96-well solid plate (oxidative damage assay)

m. 96-well cover sheet

b) Liquids used (Solutions and Reagent):

a. “DNA/RNA Oxidative Damage ELISA Monoclonal Antibody”

b. “DNA/RNA Oxidative Damage AChE Tracer”

c. “DNA/RNA Oxidative Damage ELISA Standard”

d. “ELISA Buffer Concentrate (10X)”

e. “Wash Buffer Concentrate (400X)”

f. “Ellman's Reagent”

g. “ELISA Tracer Dye”

h. 'Ultrapure' water

Reagent and sample preparation:

A. Buffer preparation:

a. LIASA Buffer Preparation: we add 1 vial of ELISA buffer concentrated 10 X to 90 ml of ultra-pure water to for dilution.

B. Sample preparation: Pee tests ought to be put away at - 20°C following assortment. Tests ought to be weakened to 1:450 and fall inside the standard bend range. Urinary groupings of oxidized Guanine differ extensively, and “similarly as with any urinary marker”, we suggest normalizing the qualities got by ELISA to creatinine levels.

C. Readiness of Assay-Specific Reagents:

DNA/RNA Oxidative Damage ELISA Standard:

To set up the standard for use in ELISA, follow these methods: Obtain eight clean test chambers and print them with numbers #1 through #8. Aliquot 900 µl of ELISA buffer into tube #1 and 500 µl of ELISA buffer into tubes #2-8. "Move 100 µl of the mass standard into tube #1 and mix thoroughly". Debilitate the standard consecutively by taking 400 µl from tube #1 and implanting it in tube #2; totally mix. "Then, at this time, move 400 µL from tube #2 to tube #3 and mix thoroughly. Repeat steps 4-8 for tubes #4-8. Weakened standards can be stored at 4°C for up to 24 hour

Plate set up:

The 96-well plate included in this package is ready to use. Each plate or set of strips should contain somewhere around two blanks, two B_0 wells, the two TA wells, and an eight-well standard curve run in duplicate. As shown in the (Figure 3.2).

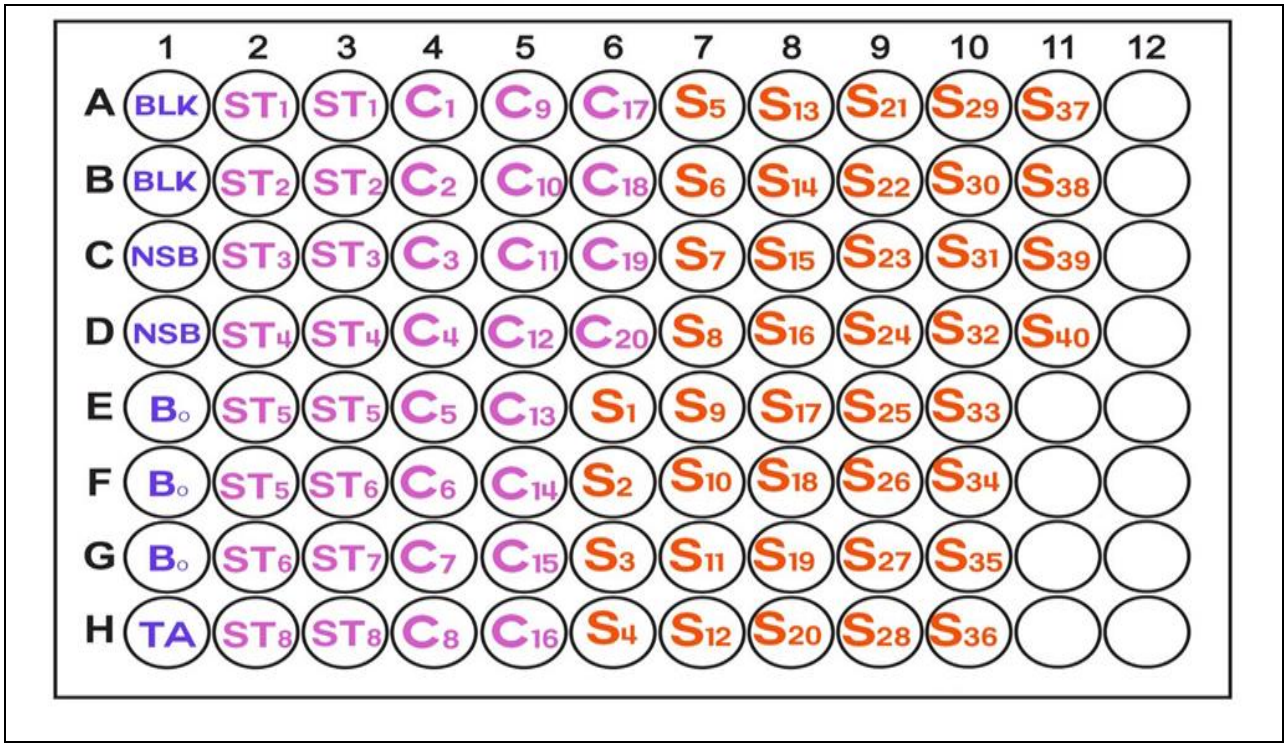

Figure 3.2: Sample Plate Format of DNA/RNA oxidative damage ELISA Kit, where BLK is the blank, TA is the total activity, NSB is non-specific banding, B0 is the maximum banding,

ST1-ST8 is the standards 1-8, C1-C20 is the controls and S1-S40 is the samples.

Performing the Procedure of the assay:

a) We added 100 µl, ELISA Buffer, to NSB wells. Add 50 µl, of ELISA Buffer, to B0 wells.

b) 50µl of "DNA/RNA Oxidative Damage ELISA Standard from Tube #8" were added to the most non-standard wells. "50 µl of DNA/RNA Oxidative Damage ELISA Standard" from tube #7 was added to the two standard wells. Review this strategy until all standards are split.

c) 50 µl of test per well was added to specific test wells and 50 µl of control test per well was added to each control test well.Add 50 µl DNA/RNA Oxidative Damage AChE Tracer to each well next to TA well and Blk well.

d) We added 50 µL of ELISA Monoclonal Antibody for Oxidative DNA/RNA Damage to each well next to the TA, NSB and Blk wells.

e) We covered the plate with plastic film and trapdoor for 18 hours at 4°C.

f) Ellman's Reagent was reconstituted speedily before use (100 dtn vial Ellman's Reagent reconstituted with 20 ml Ultrapure water).

g) We empty the wells and flush on different occasions "with Wash Buffer".

h) "Add 200 µl of Ellman's Reagent to each well".

i) "Add 5 µl of tracer to the TA wells".

j) "We covered the plate with plastic film" and trapdoor it on the shaker in lack of definition for 90-120 minutes.

k) Wipe the lower part of the plate with an ideal tissue to dispose of fingerprints, soil, etc.

l) Read the plate at a repeat somewhere in the range of 405 and 420 nm. Absorbance may be checked erratically until the B0 wells have shown something like 0.3 AU. The plaque should be seen when the absorbance of the B0 wells is in the range of 0.3-1.0 AU. Accepting that "the absorbance of the wells exceeds 2.0, wash the plate, add new Ellman's reagent and allow it to grow again".

3.5 THE DATA COLLECTED FROM THE BGOF FOR INDEPENDENT VARIABLES

The results obtained from conducting the experiment are consider the dependent variables as for the independent variables xi obtained from the information form of the sample members or the questionnaire that was prepared, the results were as shown in Tables 3.4 and 3.5.

Table 3.4: The Distribution of The Independent Variables of Control group sample.

Control sample number	**Age (years)**	**BMI Kg/m^2**	**Present or absence of cancer in the family**	**Present or absence of regular used medication**	**Present or absence of virial infection in the last 1 year**	**Sport type activity**	**Eating habits**
C1	25	24.7	Non	Non	Non	Little	Vegetable and fruit-based diet
C2	35	24.6	Non	Non	Non	Little	Mixed
C3	26	28.5	Non	Non	Percent (Covid-19)	Little	Mixed
C4	25	21.6	Non	Non	Non	Little	Mixed
C5	26	21	Non	Non	Non	Middle	Mixed
C6	27	26.2	Non	Non	Non	Many	Vegetable and fruit-based diet
C7	57	23.2	Non	Non	Non	Little	Meat-based diet
C8	41	34.6	Non	Non	Non	Little	Vegetable and fruit-based diet
C9	40	39.8	Non	Non	Percent (Covid-19)	Little	Meat-based diet
C10	31	25.1	Non	Non	Non	Many	Mixed
C11	3	32.7	Non	Non	Percent (Covid-19)	Little	Mixed
C12	73	31.2	Non	Non	Percent (Covid-19)	Little	Vegetable and fruit-based diet

Continue to Table 3.4

C13	32	20.8	Non	Non	Percent (Covid-19)	Middle	Meat-based diet
C14	33	27.7	Non	Non	Percent (Covid-19)	Little	Vegetable and fruit-based diet
C15	24	31.1	Non	Non	Non	Middle	Mixed
C16	36	26.8	Non	Non	Percent (Covid-19)	Little	Mixed
C17	69	31.2	Non	Non	Percent (Covid-19)	Little	Mixed
C18	24	23.4	Non	Non	Non	Little	Mixed
C19	65	25.4	Percent	Non	Percent (Covid-19)	Little	Mixed
C20	22	27.5	Non	Non	Non	Middle	Mixed

Table 3.5: The Distribution of The Independent Variables of the research Sample group

sample number	Age (years)	BMI Kg/m^2	Duration of working in the profession (years)	Present or absence of cancer in the family	Present or absence of regular used medication	Present or absence of virial infection in the last 1 year	Sport type activity	Eating habits
S 1	39	20	15	Non	Non	Non	Little	Mixed
S 2	52	31.9	1	Non	Non	Non	Little	Mixed
S 3	25	24.7	2	Non	Non	Non	Little	Vegetable and fruit-based diet
S 4	53	27.5	20	Non	Non	Non	Little	Mixed
S 5	35	29.2	5	Non	Non	Percent (Covid-19)	Middle	Mixed
S 6	28	30.8	13	Non	Non	Percent (Covid-19)	Many	Vegetable and fruit-based diet
S 7	57	34.7	35	Non	Non	Non	Little	Mixed
S 8	32	27.3	16	Non	Non	Percent (Covid-19)	Middle	Mixed
S 9	60	30.9	40	present	Non	Percent (Covid-19)	Little	Vegetable and fruit-based diet
S 10	52	23.1	25	Non	Non	Percent (Covid-19)	Middle	Mixed
S 11	45	26.9	20	present	Non	Percent (Covid-19)	Little	Mixed
S 12	48	23.8	26	Non	Non	Non	Middle	Mixed
S 13	57	26	15	Non	Non	Percent (Covid-19)		Mixed
S 14	45	27.8	20	Non	Non	Percent (Covid-19)	Little	Mixed

Continue to Table 3.5

S 15	18	21	2	Non	Non	Percent (Covid-19)	Middle	Mixed
S 16	22	24.2	3	Non	Non	Percent (Covid-19)	Middle	Mixed
S 17	26	31.4	6	Non	Non	Percent (Covid-19)	Middle	Mixed
S 18	50	20.9	10	Present	Non	Non	Little	Mixed
S 19	44	26.7	18	Non	Non	Non	Little	Mixed
S 20	67	23.7	35	Present	Non	Percent (Covid-19)	Little	Mixed
S 21	49	20.7	2	Non	Non	Non	Little	Mixed
S 22	35	29.3	7	Non	Non	Non	Middle	Mixed
S 23	37	27.3	17	Non	Non	Non	Little	Vegetable and fruit-based diet
S 24	20	21.8	4	Non	Non	Percent (Covid-19)	Middle	Mixed
S 25	46	25.5	12	Non	Non	Percent (Covid-19)	Middle	Mixed
S 26	57	25.4	14	Present	Non	Percent (Covid-19)	Middle	Vegetable and fruit-based diet
S 27	19	21	2	Non	Non	Non	Many	Mixed
S 28	25	25.9	6	Non	Non	Non	Middle	Mixed

Continue to Table 3.5

S 29	27	22.6	6	Non	Non	Non	Little	Vegetable and fruit-based diet
S 30	67	28.5	27	Non	Non	Percent (Covid-19)	Middle	Mixed
S 31	28	26.3	5	Non	Non	Percent (Covid-19)	Middle	Mixed
S 32	42	28.4	15	Non	Non	Percent (Covid-19)	Little	Mixed
S 33	26	28.7	8	Non	Non	Percent (Covid-19)	Middle	Mixed
S 34	32	24.2	4	Non	Non	Percent (Covid-19)	Little	Mixed
S 35	40	30.2	20	Non	Non	Percent (Covid-19)	Little	Mixed
S 36	4	31.1	24	Non	Non	Percent (Covid-19)	Little	Mixed
S 37	35	25.8	10	Non	Non	Non	Middle	Mixed
S 38	51	22	30	Non	Non	Non	Little	Mixed
S 39	25	22.6	3	Non	Non	Non	Middle	Mixed
S 40	42	25.2	13	Non	Non	Non	Little	Mixed

3.6 BIOSTATISTICS

SPSS 21 statistical package program was used in the analysis of the study data. (SPSS Inc, Chicago, Illinois, USA). Descriptive statistics were applied to all variables. The simple liner regration were used to analysis the creatinine and the 8-OHdG concentrations. The status of the data according to different variables was shown with various graphic analyzes. The conformity of the data to the normal distribution was analyzed with the Kolmogorov Smirnov test. Comparison of 8-OHdG concentration values between the greengrocer workers and the samples belonging to the control group was performed using the independent t test. Univariate relationships (age, body mass index [BMI], eating habits, viral infection, and family history of cancer) and 8-OHdG concentration levels among participants were analyzed using the independent samples t test. Multivariate relationships between participants (Physical activity and years of employment) were evaluated with the ONE-WAY ANOVA TEST.

4. RESULTS

This study was carried out with male individuals in the greengrocer profession living in Istanbul. Accordingly, the entire control group was composed of male volunteers living in Istanbul, who were not exposed to the chemicals that greengrocers are exposed to.

The calibration graph of the measurement of the standards made using the validated Creatinine ELISA analysis kit for detecting Creatinine levels in urine samples is given in Figure (4.1). The graph of the calibration of the measurement of the standards made using the validated oxidative DNA/RNA ELISA kit for detecting 8-OHdG levels in urine samples is given in Figure (4.2). The R^2 values of the calibration charts were found to be 0.979 and 0.9505, respectively, including Creatinine and DNA-Oxidative Damage (Figures 4.1 and 4.2). The control and participant groups ' DNA oxidation and creatinine concentrations were calculated according to calibration curves. (Figures 4.1 and 4.2).

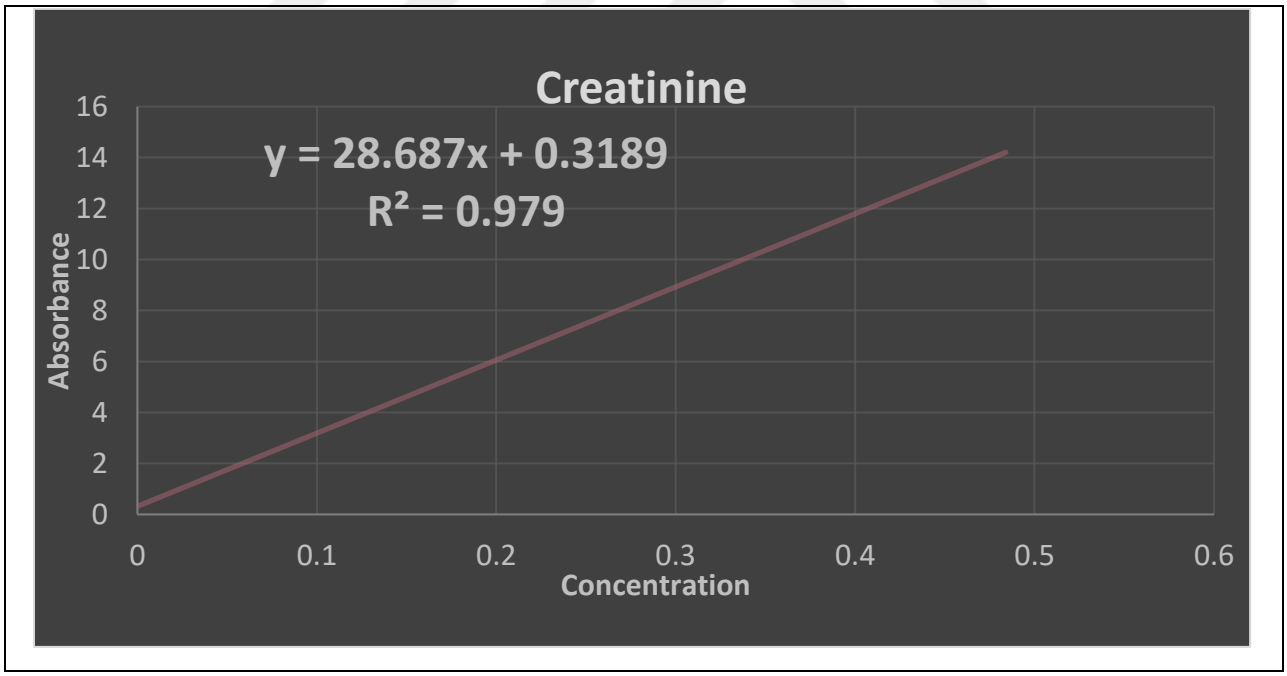

Figure 4.1: Creatinine Calibration Curve

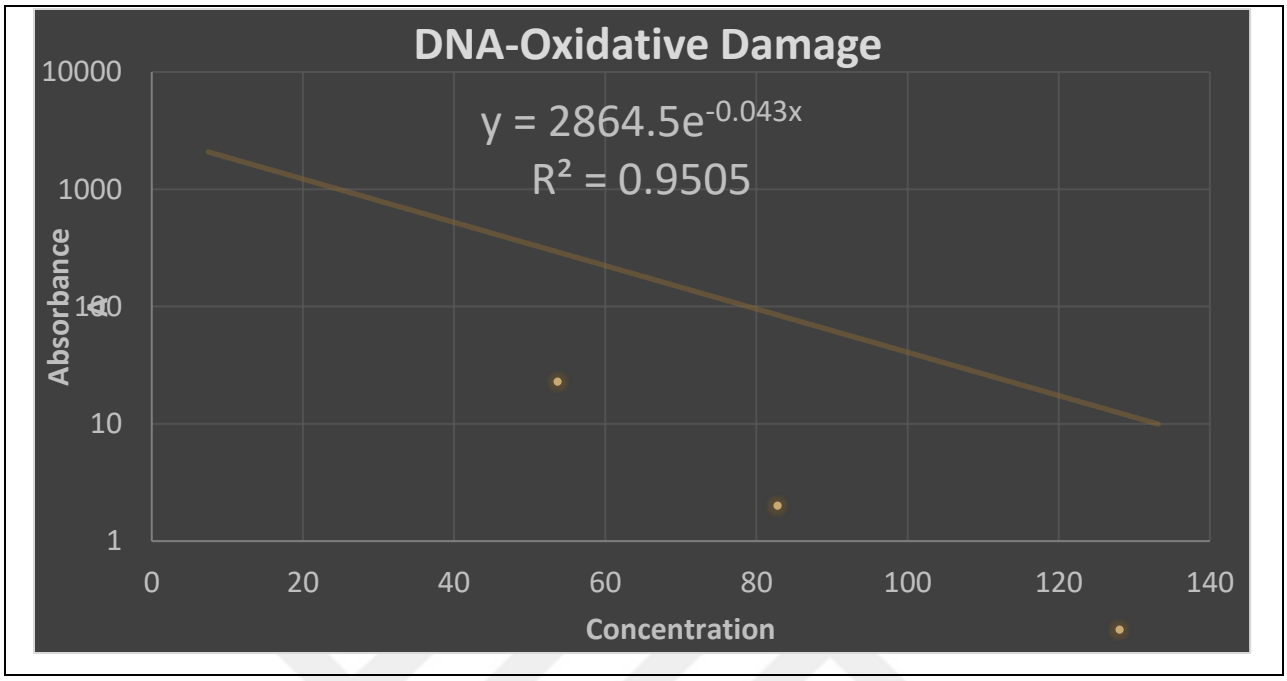

Figure 4.2: DNA-Oxidative Damage calibration curve

Then, correction of the 8-OHdG values calculated according to the calibration curve with creatinine was performed for both the control group and the participants. Thus, 8-OHdG/Creatinine concentrations (ng/mg) were calculated for both the control group and the participants (Table 4.1and 4.2).

The results show that the age range of the control group is 22-73 years, and the age range of our sample group is 18-60 years. All of our samples consisted of male individuals, since those working in the greengrocer sector were generally men. The 8-OHdG concentration range of the control group was 0.16 - 3.65 ng/mg, and the mean was 1.67 ng/mg (Table 4.3). The 8-OHdG concentration range values of our study sample group are 0.30- 8.83 ng/mg, and the mean of the group was 2.55 ng/mg (Table 4.2-4.3), as shown in (Figure 4.3). The result was determined that the data of both the control group and our sample group showed normal distribution according to the Kolmogorov Smirnov test. There was no significant difference at the level of $p \leq 0.05$ in terms of 8-OHdG concentration values between the greengrocer workers and the samples belonging to the control group. However, the fact that the obtained P-value ($p=0.06$) is very close to the significance limits is important information (Table 4.4).

Table 4.1: The 8-OHdG/Creatinine Concentration of Control Group

Control sample number	**8-OHdG/Creatinine(ng/mg)**	**Control sample number**	**8-OHdG/Creatinine(ng/mg)**
CONTROL 1	O.68	CONTROL 11	3.11
CONTROL 2	O.16	CONTROL 12	O.60
CONTROL 3	O.90	CONTROL 13	1.85
CONTROL 4	O.71	CONTROL 14	2.53
CONTROL 5	O.40	CONTROL 15	2.82
CONTROL 6	O.53	CONTROL 16	2.43
CONTROL 7	2.74	CONTROL 17	1.78
CONTROL 8	3.65	CONTROL 18	2.46
CONTROL 9	1.54	CONTROL 19	2.11
CONTROL 10	1.26	CONTROL 20	1.20

Table 4.2: The 8-OHdG/Creatinine Concentration of Sample Group.

Sample number	8-OHdG/ Creatinine (ng/mg)	Sample number	8-OHdG/ Creatinine (ng/mg)	Sample number	8-OHdG/ Creatinine (ng/mg)	Sample number	8-OHdG/ Creatinine(ng/mg)
S1	1.58	S 11	2.77	S 21	8.42	S 31	1.15
S2	2.26	S 12	2.83	S 22	5.81	S 32	2.82
S3	4.60	S 13	4.70	S 23	1.10	S 34	1.19
S4	1.45	S 14	1.50	S 24	2.46	S 35	0.89
S5	2.80	S 15	2.65	S 25	8.83	S 36	6.75
S6	1.93	S 16	2.29	S 26	1.83	S 37	1.17
S7	0.59	S 17	1.66	S 27	0.30	S 38	1.63
S8	3.53	S 18	3.20	S 28	0.84	S 39	0.32
S9	1.83	S 19	2.67	S 29	2.24	S 40	1.65
S10	2.82	S 20	2.15	S 30	0.69		

Table 4.3: Comparison between Control group samples and study group samples for descriptive statistics of samples and normalized urinary 8-OHdG/creatinine (ng/mg) concentration values.

Groups	Mean	SD	Minimum	Maximum
Samples (n=40)	2,55	1,96	0,30	8,83
Control (n=20)	1,67	1,02	0,16	3,65
-P value	0,06			

$p \leq 0,05$

Table 4.4: Urinary 8-OHdG (ng/mg) creatinine defined by various factors.

Variables		**N**	**Mean**	**SD**	**P-value**
BMI (Body Mass Index) (kg/m^2)	30 ≥ 30 <	33 7	2,61 2,31	1,98 2,03	0,72
Age (years)	40 ≥ 40 <	19 21	2,12 2,95	1,35 2,35	0,18
Eating habits	Vegetable and Fruits Mixed	6 34	3,18 2,44	2,87 1,79	0,40
Physical activity	Little Middle Many	35 21 4	2,31 2,42 1,00	1,72 1,88 0,74	0,32
Years of employment	Control 1-10 11-20 21+	20 17 10 13	1,67 2,21 3,14 2,56	1,02 1,38 2,24 2,40	0,15
Virial Infection	Non Present	18 22	2,44 2,65	2,06 1,92	0,73
Family history of cancer	Non Present	35 5	2,58 2,35	2,09 0,60	0,80

$p \leq 0,05$

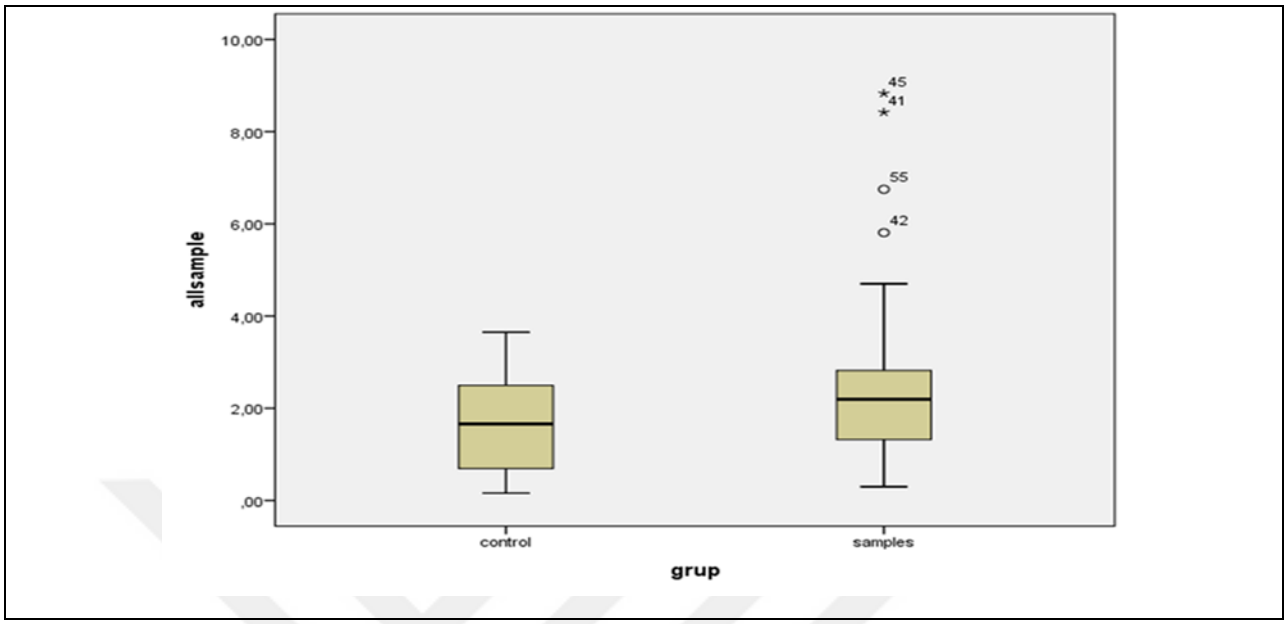

Figure 4.3: Box-plot Graphic of Mean the 8-OHdG concentrations of the participants and the control group

Average of 8-OHdG concentrations of the sample group was higher than that of the control group (Figure 4.3).

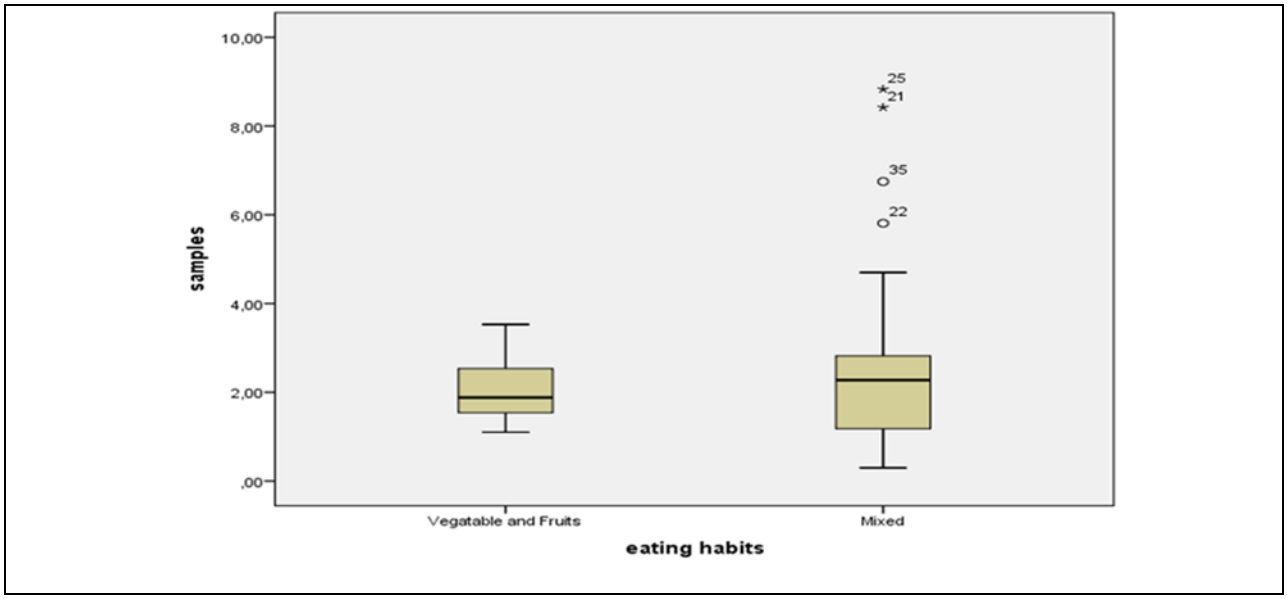

Figure 4.4: Box-plot Graphic of Sample groups in terms of eating habits.

A tremendous piece of the sample group (34 individuals) is fed with mixed. There was no monstrous differentiation at p≤0.05 level between dietary examples and 8-OHdG center levels among the individuals (Figure 4.4).

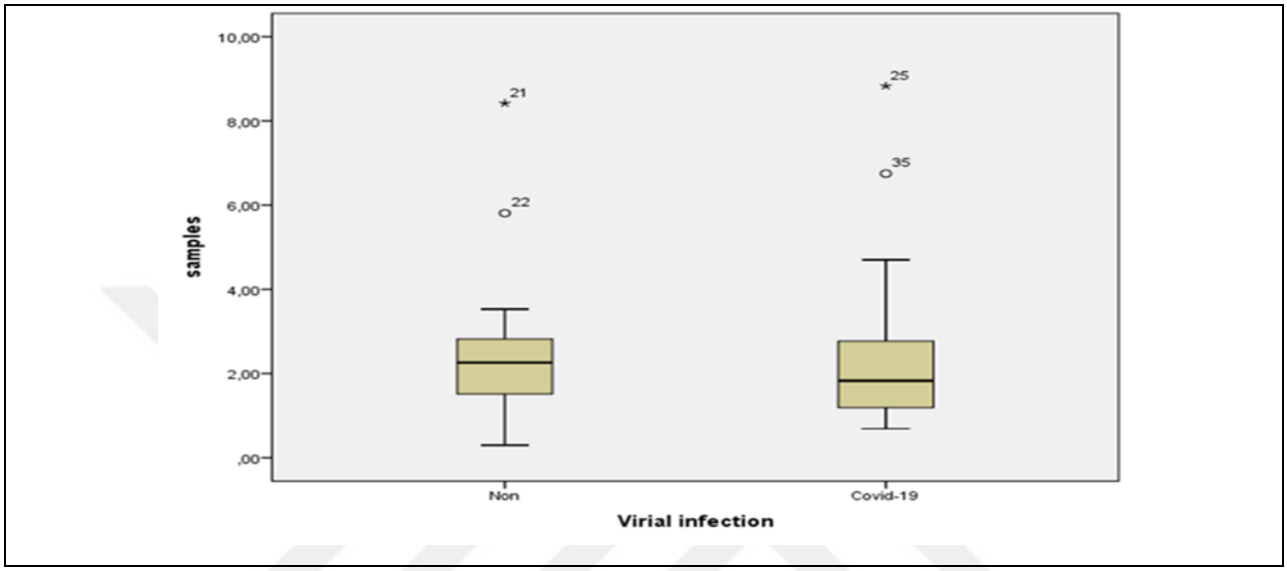

Figure 4.5: Box-plot Graphic of Sample groups with and without viral infection (Covid-19).

The greater part of the members (22 members) had a viral disease (Table 4.4). There was no massive contrast between the 8-OHdG concentration levels at p≤0.05 between the members who had a viral disease (Covid-19) and the individuals who didn't (Figure 4.5).

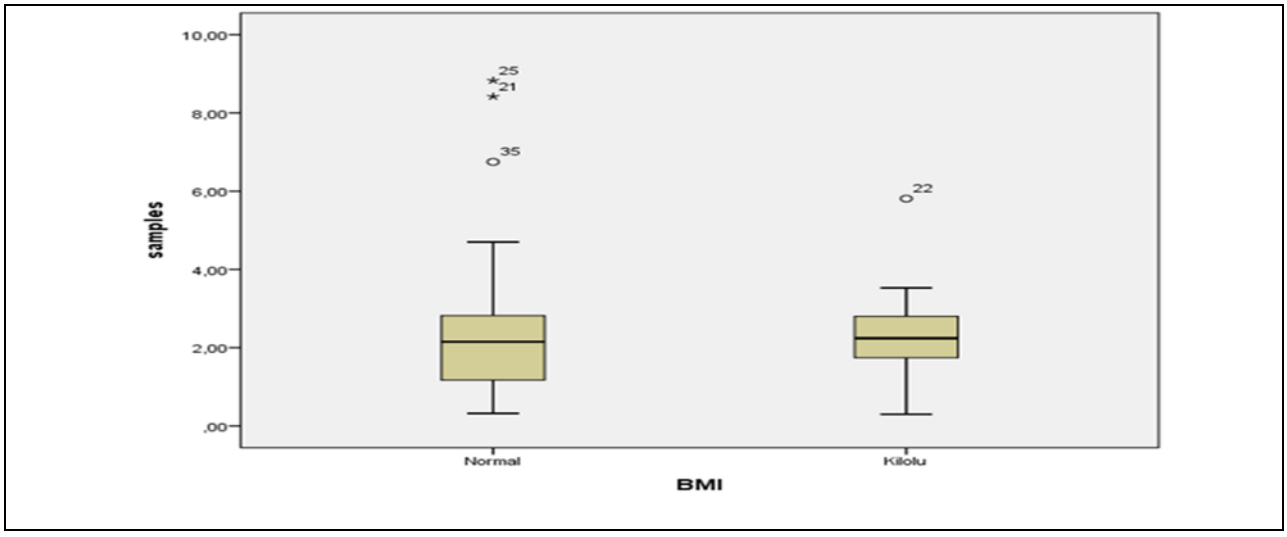

Figure 4.6: Box-plot Graph of Sample groups with and without normal Body Mass Index.

The average 8-OHdG concentration of the individuals was 2.61 ng/mg, and the majority of them had a body mass index of above 30 (Table 4.4). There was no discernible difference between the participant groups with and without normal body mass index and 8-OhdG concentration levels when the box-plot graph was assessed at the $p \leq 0.05$ level (Figure 4.6).

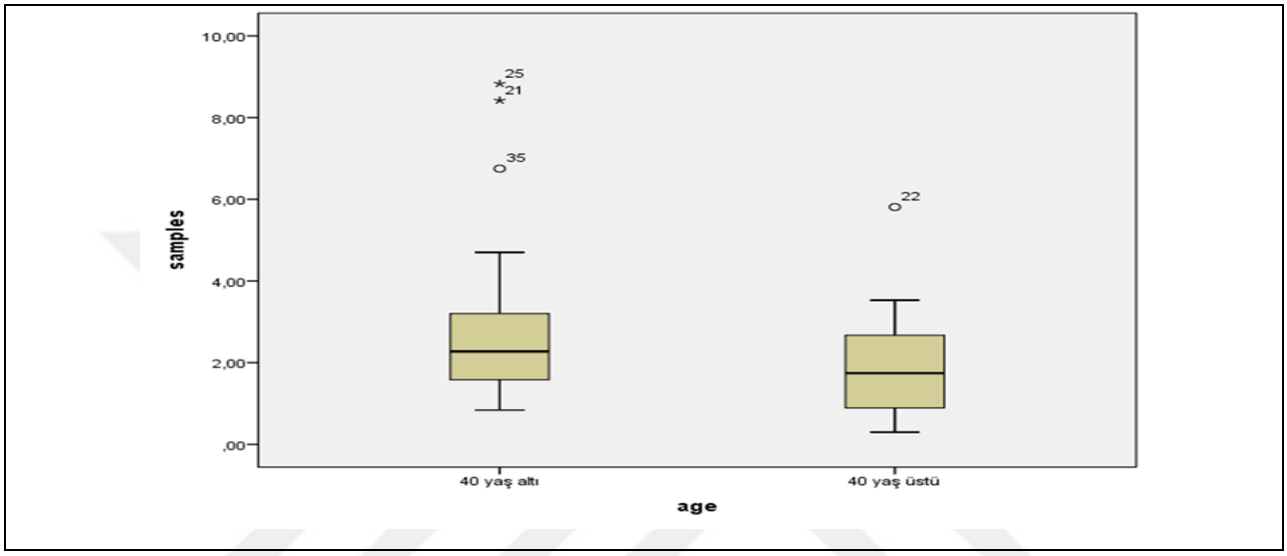

Figure 4.7: Box-plot Graphic of Sample Groups under 40 and over 40 years old.

Members younger than 40 had somewhat higher 8-OHdG concentrations (Table 4.4). There was no significant difference (at $p \leq 0.05$ level) in 8-OHdG Concentrations between those under 40 years old and those more than 40 years old (Figure 4.7).

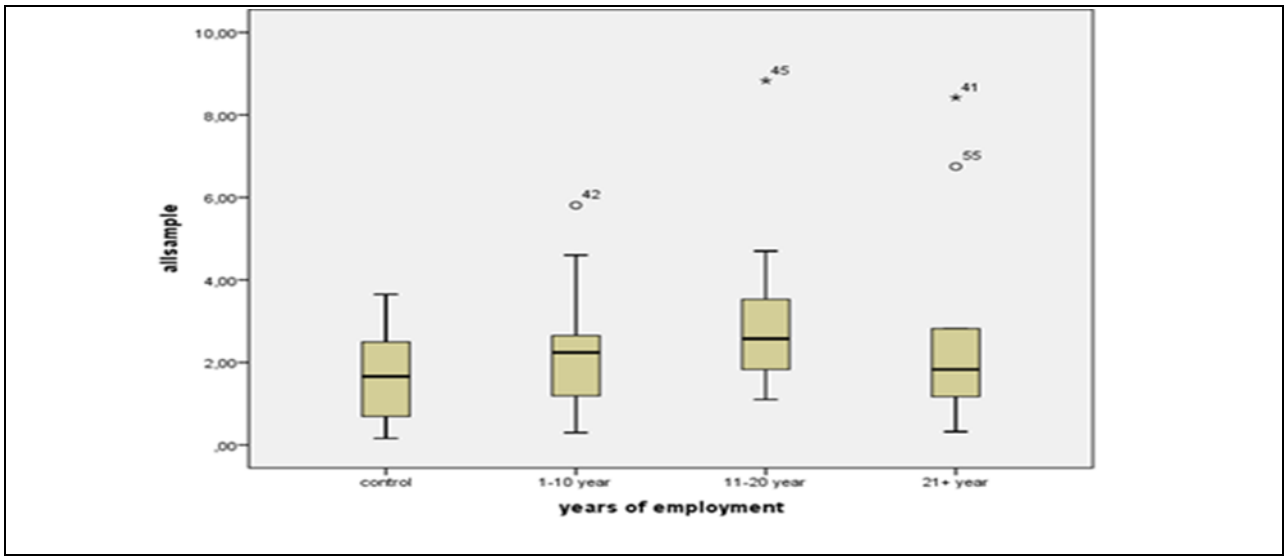

Figure 4.8: Box-plot Graphic of Sample groups in terms of years of employment.

Compared to those whom not working in greengrocery (control group)

The averages concentration of all working-year intervals were found to be higher (Table 4.4). There was no huge contrast at the p≤0.05 level concerning multivariate relationships as years of employment among the participants (Figure 4.8).

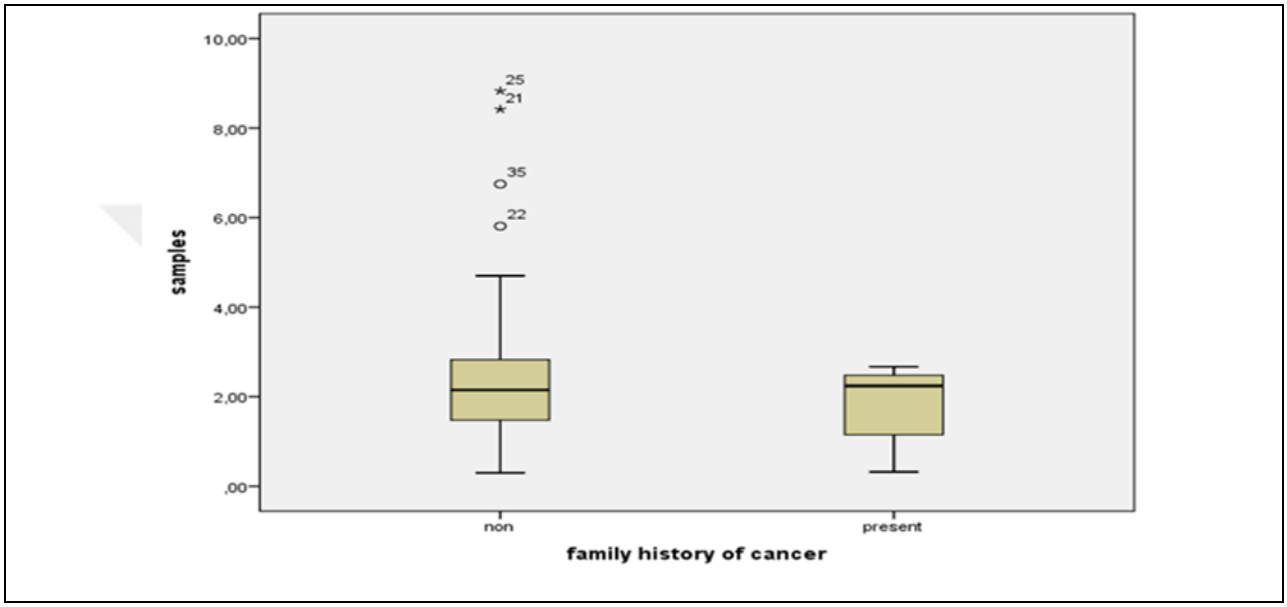

Figure 4.9: Box-plot Graphic of Sample groups in terms of family history of cancer.

35 members had no family background of cancer (Table 4.4). There was no tremendous distinction at p≤0.05 level between univariate connections as family background of disease and 8-OHdG concentration levels among the members (Figure 4.9).

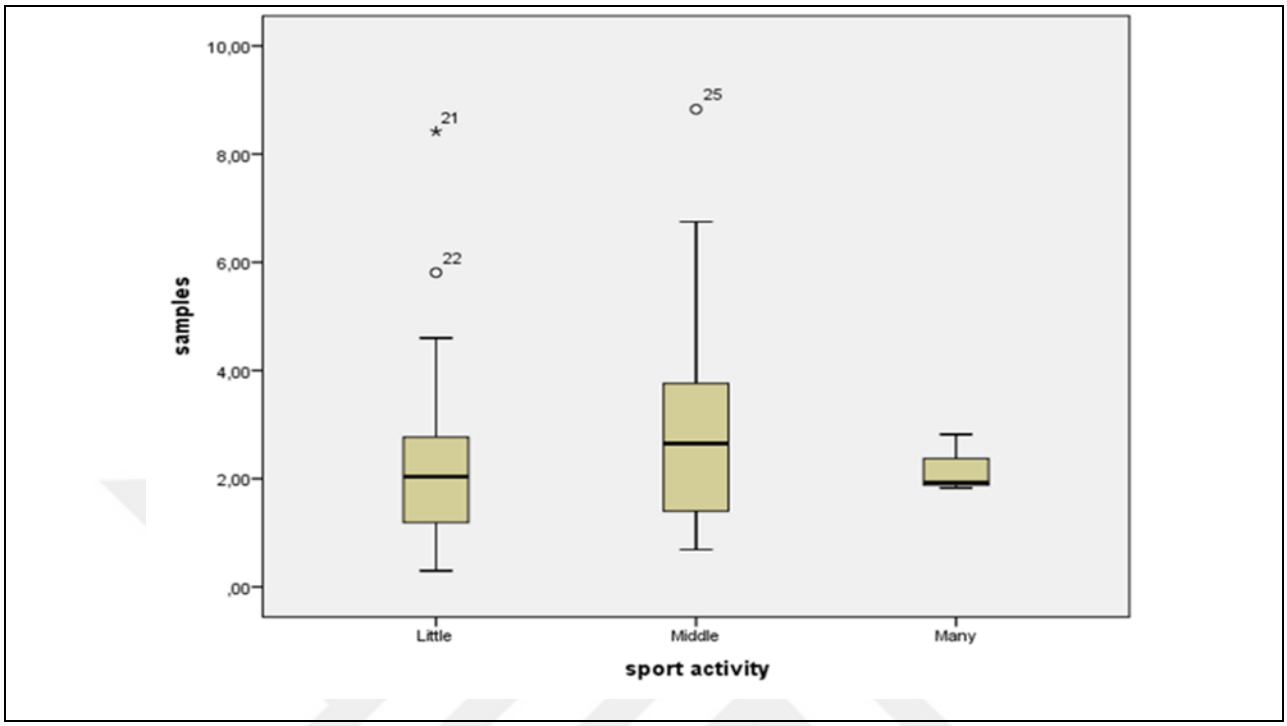

Figure 4.10: Box-plot Plot of Sample groups in terms of sports activity intensity.

Most of the sample groups did very little physical activity (Table 11). There was no significant difference at the $p \leq 0.05$ level between multivariate relationships as physical activity among the participants (Figure 4.10).

5. DISCUSSION AND CONCLUSION

Many studies confirm the apparent effect of chemical pesticides on the DNA oxidative damage of workers in agriculture, such as farmers who work in direct contact with pesticides [101,102]. In this study, we tried to find if there were also effects on greengrocers who deal with pesticides indirectly by dealing with vegetables and fruits, which leads to an increase in their oxidative stress and thus leads to many diseases such as cancer and sabotage the functions of body cell.

To lead this examination, we determined the degree of urinary 8-OHdG/Creatinine (ng/mg) as a biomarker for oxidative Stress in the human body Using ELISA method. Unlike chromatographic and mass spectrometry examination methods, this method was chosen because it is considered the most useful method because it requires less time, is less complex, and is less costly. [102]. ELISA analysis methods are frequently used to analyze 8-OHdG in urine samples [103]. In epidemiological assessments, this can be a to some degree precarious situation considering the way that finding any relationship with potential determinants is more fundamental than choosing the veritable worth of a substance, concerning what is going on [104].

The research results did not show a significant relationship between the concentrations of 8-OHdG/creatinine (ng/mg) between the sample group working in the field of selling greengroceries and the control group working in various other fields. However, the relationship was very close to the significant limits (0.06), where the concentrations level of 8-OHdG/creatinine(ng/mg) for the sample were higher and ranged between (0.30-8.83) while for the control, they were lower, as their levels ranged between (0.16-3.65).

On the level of $p \leq 0.05$, there was no significant or close to a significant relationship between the two groups, where their p-value was (0.72) On the other hand, a very high proportion of the sample group (33 samples) had a body mass index above 30 and had higher 8-OHdG levels than those with a low BMI., and this is what Mizoue et al. [105] agreed with, while Van Zeeland et al. [104] differed, where they noticed this inverse relationship only in smokers and not in non-smokers, as for our research sample was from non-smokers only.

Concerning the relationship between the age variable and its relationship with oxidative stress, we found that there is a slight increase in the levels of 8-OHdG/creatinine (ng/mg) for people who are

less than 40 years compared with those more than 40 years old, and this is what Sakano et al. [106] agreed on. However, Miwa et al. [107] disagreed with us, stating that there is no relationship. We did not find "significant differences at the level of $p \leq 0.05$", where it was (0.18).

Although we did not find any correlation between (family history of cancer or viral infection) and oxidative stress at the level of $p \leq 0.05$, where the p-values were (0.80) for family history of cancer and (0.73) for viral infection, we must note that there is a slight increase in 8-OHdG/creatinine (ng/mg) for people who had a Covid-19 infection compared to the people who did not have a Covid-19 in the past year.

In our study, we did not find any significant change on the level of $p \leq 0.05$ with the variable of eating habits, where it was only (0.40). While on the type of food consumed, we found an inversed relationship between vegetable and fruit-based diets and the concentrations of 8-OHdG/creatinine (ng/mg), which is what Tamae et al. [108] also agree on. However, Zalion et al. [104] did not. "There was no significant difference" at the $p \leq 0.05$ level between multivariate relationships as physical activity among the participants. The p-value was (0.32), which Zalion et al [104] also agree on.

For our last multivariable years of employment as a greengrocer, we found a big difference in the concentrations of 8-OHdG/creatinine (ng/mg) between the sample and control group especially for those who work from 11 to 20 years.

In this study, we wanted to determine the oxidative damage that greengrocers in Istanbul are exposed to as a result of their handling of vegetables and fruits containing pesticide residues. As a result we have found that oxidative stress is relatively higher than workers in other fields, where the research results are very close to the significant limits.

In this study, despite our limited sample number, with the help of biostatistic studies, 8-OHdG concentrations were determined found to be higher in greengrocer workers than in the control group. Our results provide a framework for further studies with clinical and epidemiological purposes in investigating the factors that examine the effects of pesticide exposure in greengrocery workers. The information presented in this study has the potential to be used in examining the effects of pesticide exposure, provided that it is supported by more comprehensive studies.

REFERENCES

[1] Yardım, N. Çipil, Z., Vardar, C., & Mollahaliloğlu, S. (2007). Occupational accidents and occupational diseases in Turkey: death rates between 2000-2005. Dicle Tip Journal, 34(4), 264-271.

[2] Costa, LG: "Pasitisitlerin Toksik Etkisi.", Casarett & Doull's Toxicology. The basic Science of Poisons, 7. Baskı: Klaassen C. D. Editör, The McGraw-hill Companies Inc., ABD (2007), 883-931.

[3] Agrawal D, Sultana P ve Gupta GSD (1991).

[4] Oxidative damage and changes in the glutathione redox system in erythrocytes from rats treated with hexachlorocyclohexane Food and Chemical Toxicology 29: 459-462 6.

[5] Kisby, G.E., Muniz, J.F., Scherer, J., Lasarev. M. R. Koshy, M. Kow, Y. W & McCauley, L. (2009) Oxidative stress and DNA damage in farm workers Joumal of agromedicine, 14(2), 206-214.

[6] Benz CC, Yau C. Cancer paradigms in aging, oxidative stress, and parallax Nat Rev. Cancer 2008.8.875-879.

[7] London & Meyers' General Patterns of Agricultural Chemical Usage in the Southern Regions of South Africa' (1995) 91 South African Journal of Science 508. 2 Silent Spring (Houghton Mifflin 1962).

[8] Unsworth 2010 - History of pesticide use. International Union of pure and applied chemistry (IUPAC). 2010. http://agrochemicals.iupac.org/index.php?option=com_so-bi2&sobi2Task=sobi2Details&catid=3&sobi2Id=31 (Accessed 2 September 2014).

[9] K.H. Buchel, Chemistry of Pesticides, John Wiley & Sons, Inc., New York, USA, 1983.

[10] Aktar, Md. Wasim, Dwaipayan Sengupta, and Ashim Chowdhury. "Impact of Pesticides Use in Agriculture: Their Benefits and Hazards." Interdisciplinary Toxicology 2.1 (2009): 1-12. PMC.

[11] Cooper, Jerry and Dobson, Hans. "The Benefits of Pesticides to Mankind and the Environment." Crop Protection 26 (2007): 1337-1348.

[12] Arias-Estévez, Manuel, et al. "The Mobility and Degradation of Pesticides in Soils and the Pollution of Groundwater Resources." Agriculture, Ecosystems & Environment 123.4 (2008): 247-260.

[13] 13- Corsini, E., et al. "Pesticide Induced Immunotoxicity in Humans: A Comprehensive Review of the Existing Evidence." Toxicology 307 (2013): 123-135.

[14] Arias-Estévez, Manuel, et al. "The Mobility and Degradation of Pesticides in Soils and the Pollution of Groundwater Resources." Agriculture, Ecosystems & Environment 123.4 (2008): 247-260.

[15] Pesticide Management division, NIPHM; Pesticide classification on use, chemical nature, formulation toxicity and action, etc, Hyderabad p.1-17 [Cited on 2018 May 5] available from http://www.niphm.gov.in.

[16] Algimantas Mieldazys, Ramunas Mieldyzys, Gedimanas Vilkevicius, Aleksandaras; Agriculture use of pesticides/ plant protection products, Forestry operations and OSH issues OSHWiki. [Cited on 2018 April 24]. Available from https://oshwiki.eu./wiki/Agriculture.

[17] World Health Organization; Pesticides: Children Health and the Environment 2008. [Cited on 2018 April 7]; available from: http://www.who.int.ceh/pesticides.pdf.

[18] WHO- World Health Organization, the WHO recommended classification of pesticides by hazard and guidelines to classification: 2009.2010 [cited on 2018 may 6] available from ttp://www.who.int/ipcs/publications/pesticides_2009.pdf

[19] WHO- World Health Organization, the WHO recommended classification of pesticides by hazard and guidelines to classification: 2009.2010 [cited on 2018 may 6] available from http://www.who.int/ipcs/publications/pesticides _2009.pdf.

[20] Halliwell B: Free radicals, antioxidants and human disease: Curiosity, cause or consequence. Lancet 344:721-724, 1994Davis, Jesse & Goadrich, Mark. (2006)" The Relationship Between PrecisionRecall and ROC Curves", Proceedings of the 23rd International Conference on Machine Learning, ACM 2006.

[21] Zarkovic N. 4-Hydroxynonenal as a bioactive marker of pathological processes.Mol Aspect med, 2003;24:231-91.

[22] Halliwell B. Free radicals antioxidants and human disease: curiosity, cause, or consequence.Lancet, 1994; 344:721-724.

[23] Halliwell B: Free radicals and antioxidants: A personal view. Neut Rev, 1994; 52: 235–65.

[24] Rumley AG, Paterson JR: Analytical aspects of antioxidants and free radical activity in clinical biochemistry. Ann Clin Biochem, 1998;35: 181–200.

[25] Lioi MB, Scarfi MR, Santoro A et al: Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro. Mutat Res, 1998; 403: 13–20.

[26] Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol 190; 186: 1-85.

[27] Saran M, Michel C, Bors W. Reactions of NO with O2-. Implications for the action of endothelium- derived relaxing factor. Free Radic Res Commun 1989; 83: 1705-1715.

[28] K. H. Cheeseman, T. F. Slater.An introduction to free radical biochemistry.British Medical Bidltttn 1993 Vol 49, No 3, pp. 481-493

[29] Slater TF. Necrogenic action of carbon tetrachloride in the rat: a speculative mechanism based on activation. Nature 1966; 209: 36-40.

[30] Cheeseman KH. Lipid peroxidation in biological systems. In: Halliwell B, Aruoma OL ed. DNA and Free Radicals. London: Ellis Horwood, 1993 (In press).

[31] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. Free Radic Biol Med 1991; 11: 81-128.

[32] Marx G, Chevion M. Site-specific modification of albumin by free radicals. Reaction with copper (II) and ascorbate. Biochem J 1986; 236: 397-400.

[33] Cooke MS, Lunec J, Evans MD. Progress in the analysis of urinary oxidative DNA damage. Free Radic Biol Med 2002;33: 1601 – 14.

[34] Kasai H. Analysis of a form of oxidative DNA damage, 8- hydroxy-2V-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutat Res 1997;387:147 – 63.

[35] Chiou C-C, Chang P-Y, Chan E-C, Wu T-L, Tsao K-C, Wu TJ, Urinary 8-hydroxydeoxyguanosine and its analogues as DNA marker of oxidative stress: development of an ELISA and measurement in both bladder and prostate cancers. Clin. Chim. Acta [in press].

[36] Lily L. Wua,b,c, Chiuan-Chian Chioud , Pi-Yueh Change , James T. Wua,c , Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics,Clinica Chimica Acta 339 (2004) 1–9.

[37] P. O'Neill, Pulse radiolytic study of the interaction of thiols and ascorbate with OH adducts of dGMP and dG: implications for DNA repair processes, Radiat. Res. 96 (1983) 198–210.

[38] Steenken S, Jovanovic SV. How easily oxidizable is DNA? One-electron reduction potentials of adenosine and guanosine radicals in aqueous solution. J Am Chem Soc 1997;119:617 – 618.

[39] Steenken S, Jovanovic SV, Bietti M, Bernhard K. The trap depth (in DNA) of 8-oxo-7,8-dihydro-2' -deoxyguanosine as derived from electron-transfer equilibria in aqeous solution. J Am Chem Soc 2000;122:2373 – 284.

[40] Steenken S. Purine bases, nucleosides, and nucleotides: aqueous solution redox chemistry and transformation reactions of their radical cations and e -and OH adducts. Chem Rev 1989;89:503 – 520.

[41] O'Neill P. Pulse radiolytic study of the interaction of thiols and ascorbate with OH adducts of dGMP and dG: implications for DNA repair processes. Radiat Res 1983;96:198 – 210.

[42] Vieira AJSC, Steenken S. Pattern of OH radical reaction with Adenine and its nucleosides and nucleotides. Characterization of two types of isomeric OH adduct and their unimolecular transformation reactions. J Am Chem Soc 1990;112:6986 – 6994.

[43] Vieira AJSC, Steenken S. Pattern of OH radical reactions with N6,N6- dimethyladenosine. Production of three isomeric OH adducts and their dehydration and ring-opening reactions. J Amer Chem Soc 1987;109:7441 – 7448.

[44] Von Sonntag C. Free-radical-induced DNA damage and its repair. Hiedelberg: Springer; 2006.

[45] Hazra DK, Steenken S. Pattern of OH radical addition to cytosine and 1-, 3-, 5-, and 6-substituted cytosines. Electron tranfer and dehydration reactions of OH adducts. J Am Chem Soc 1983;105:4380 – 436.

[46] Buxton GV, Greenstock CL, Helman WP, Ross AB. Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms, and hydroxyl radicals (˙OH/ ˙O2) in aqueous solution. J Phys Chem Ref Data 1988;17:513 – 886.

[47] R.A. Beckman, L.A. Loeb, Genetic instability in cancer: theory and experiment, Semin. Cancer Biol. 15 (2005) 423–435.

[48] E.C. Friedberg, G.C. Walker, W. Siede, R.D. Wood, R.A. Schultz, T. Ellenberger, DNA Repair and Mutagenesis, ASM Press, Washington, D.C., 2006.

[49] C. von Sonntag, Free-radical-induced DNA Damage and its Repair, Springer, Hiedelberg, 2006.

[50] M. Dizdaroglu, Base-excision repair of oxidative DNA damage by DNA glycosylases, Mutat. Res. 591 (2005) 45–59.

[51] M. Dizdaroglu, S.M. Burgess, P. Jaruga, T.K. Hazra, H. Rodriguez, R.S. Lloyd, Substrate specificity and excision kinetics of Escherichia coli endonuclease VIII (Nei) for modified bases in DNA damaged by free radicals, Biochemistry 40 (2001) 12150–12156.

[52] M. Dizdaroglu, C. Bauche, H. Rodriguez, J. Laval, Novel substrates of Escherichia coli Nth protein and its kinetics for excision of modified bases from DNA damaged by free radicals, Biochemistry 39 (2000) 5586–5592.

[53] A. Sancar, Mechanisms of DNA excision repair, Science 266 (1994) 1954– 1956.

[54] E.C. Friedberg, Nucleotide excision repair of DNA: the very early history, DNA Repair (Amst) 10 (2011) 668–672.

[55] I. Mellon, P.C. Hanawalt, Induction of the Escherichia coli lactose operon selectively increases repair of its transcribed DNA strand, Nature 342 (1989) 95–98.

[56] M. Dizdaroglu, Free-radical-induced formation of an 8,50 -cyclo-20 - deoxyguanosine moiety in deoxyribonucleic acid, Biochem. J. 238 (1986) 247–254.

[57] I. Kuraoka, C. Bender, A. Romieu, J. Cadet, R.D. Wood, T. Lindahl, Removal of oxygen free-radical-induced 50 ,8-purine cyclodeoxynucleosides from DNA by the nucleotide excision-repair pathway in human cells, Proc. Natl. Acad. Sci. USA 97 (2000) 3832–3837.

[58] A.R. Parker, J.R. Eshleman, Human MutY: gene structure, protein functions and interactions, and role in carcinogenesis, Cell Mol. Life Sci. 60 (2003) 2064–2083.

[59] T. Ohtsubo, K. Nishioka, Y. Imaiso, S. Iwai, H. Shimokawa, H. Oda, T. Fujiwara, Y. Nakabeppu, Identification of human MutY homolog (hMYH) as a repair enzyme for 2-hydroxyadenine in DNA and detection of multiple forms of hMYH located in nuclei and mitochondria, Nucleic Acids Res. 28 (2000) 1355–1364.

[60] J.P. Cheadle, J.R. Sampson, MUTYH-associated polyposis – from defect in base excision repair to clinical genetic testing, DNA Repair (Amst) 6 (2007) 274– 279.

[61] H. Maki, M. Sekiguchi, MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis, Nature 355 (1992) 273–275.

[62] J.Y. Mo, H. Maki, M. Sekiguchi, Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kilodalton protein: sanitization of nucleotide pool, Proc. Natl. Acad. Sci. USA 89 (1992) 11021–11025.

[63] T. Tajiri, H. Maki, M. Sekiguchi, Functional cooperation of MutT, MutM and MutY proteins in preventing mutations caused by spontaneous oxidation of guanine nucleotide in Escherichia coli, Mutat. Res. DNA Repair 336 (1995) 257–267.

[64] Y. Sakai, M. Furuichi, M. Takahashi, M. Mishima, S. Iwai, M. Shirakawa, Y. Nakabeppu, A molecular basis for the selective recognition of 2-hydroxydATP and 8-oxo-dGTP by human MTH1, J. Biol. Chem. 277 (2002) 8579–8587.

[65] T. Tsuzuki, A. Egashira, H. Igarashi, T. Iwakuma, Y. Nakatsuru, Y. Tominaga, H. Kawate, K. Nakao, K. Nakamura, F. Ide, S. Kura, Y. Nakabeppu, M. Katsuki, T. Ishikawa, M. Sekiguchi, Spontaneous tumorigenesis in mice defective in the MTH1 gene encoding 8-oxo-dGTPase, Proc. Natl. Acad. Sci. USA 98 (2001) 11456–11461.

[66] M. Shrivastav, L.P. De Haro, J.A. Nickoloff, Regulation of DNA double-strand break repair pathway choice, Cell Res. 18 (2008) 134–147.

[67] J. Rouse, S.P. Jackson, Interfaces between the detection, signaling, and repair of DNA damage, Science 297 (2002) 547–551.

[68] S.P. Jackson, Sensing and repairing DNA double-strand breaks, Carcinogenesis 23 (2002) 687–696.

[69] Cipak A, Gasparovic A, Lovakovic T, Zarkovic N. Oxidative stress and antioxidants: biological response modifi ers of oxidative homeostasis in cancer. Period Biol 2010;in press.

[70] pak, R.; Özyürek, M.; Güçlü, K.; Çapanoğlu, E. Antioxidant Activity/Capacity Measurement. 1. Classification, Physicochemical Principles, Mechanisms, and Electron Transfer (ET)-Based Assays. J. Agric. Food Chem. 2016, 64, 997−1027, doi:10.1021/acs.jafc.5b04739.

[71] Zaitsev, V.G., Ostrovskii, O.V., and Zakrevskii, V.I., Relationships between Chemical Structure and Action Target as the Basis for Classification of Antioxidants of Direct Influence, Eksperim. Klin. Farmakol., 2003, vol. 66, pp. 66–70.

[72] O.M. Ighodaro, O.A.AkinloyeFirst line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid , Alexandria Journal of Medicine, xxx (2017) xxx–xxx.

[73] Niki E. Antioxidant defense in eukaryotic cells.In:Poli G, Albano E, Dianzani MU, eds. Free radicals: from basic science to medicine. Basel. Switzerland: Birkhauser Verlag; 1993:365-373.

[74] Fridovich I. Superoxide radical and superoxide dismutases. Ann Rev Biochem.1995;64:97-112.

[75] Dringen R, Pawlowski PG, Hirrlinger J. Peroxide detoxification by brain cells. J Neurosci Res. 2005; 79:157-165.

[76] Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 2010;48:909-930.

[77] Marklund SL. Extracellular superoxide dismutase and other superoxidedismutase isoenzymes in tissues from nine mammalian species. Biochem J.1984;222:649-655.

[78] Radi R, Turrens JF, Chang LY, Bush KM, Crapo JD, Freeman BA. Detection of catalase in rat heart mitochondria. J Biol Chem. 1991;266:22028–22034.

[79] Radi R, Turrens JF, Chang LY, Bush KM, Crapo JD, Freeman BA. Detection of catalase in rat heart mitochondria. J Biol Chem. 1991;266:22028-22034.

[80] Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 2010;48:909-930.

[81] Hissin PJ, Hilf R (1976) A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem 74(1):214–226.

[82] I. Mironczuk-Chodakowska, A. M. Witkowska, M. E.Zujko. Endogenous non-enzymatic antioxidants in the human body. Advances in Medical Sciences 63 (2018) 68–78.

[83] Marshall CW. Vitamins and Minerals: Help or Harm? George F. Stickley Company (1986).

[84] Ibrahim KS, El-Sayed EM. Potential role of nutrients on immunity; International Food Research Journal 23 (2015): 464-474.

[85] Shimizu H, Tsubola T, Kanki K, et al. All-trans retinoic acid ameliorates hepatic stellate cell activation via suppression of thioredoxin interacting protein expression. J Cell Physiol 233 (2018): 607-616.

[86] Gad A, Abu Hamed S, Khalifa M, et al. Retinoic acid improves maturation rate and upregulates the expression of antioxidant-related genes in vitro matured buffalo (Bubalus bubalis) oocytes. Int J Vet Sci Med 6 (2018): 279-285.

[87] Malivindi R, Rago V, De Rose D, et al. (2018). Influence of all-trans retinoic acid on sperm metabolism and oxidative stress: Its involvement in the physiopathology of varicocele-associated male infertility. J Cell Physiol 233 (2018): 9526-9537.

[88] Padayatty SJ, Katz A, Wang Y, et al. Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention. Journal of the American College of Nutrition 22 (2003): 18-35.

[89] Avci B, Bahadir A, Tuncel OK, et al. Influence of alpha-tocopherol and alpha-lipoic acid on bisphenol-A-induced oxidative damage in liver and ovarian tissue of rats. Toxicology and Industrial Health 32 (2016): 1381-1390.

[90] Ajila CM, Brar SK, Verma M, Tyagi RD, Godbout S, Valéro JR (2011) Extraction and analysis of polyphenols: recent trends. Crit Rev Biotechnol 31(3):227–249.

[91] Amit K, Priyadarsini KI (2011) Free radicals, oxidative stress and importance of antioxidants in human health. J Med Allied Sci 1(2):53–60.

[92] Biswas SK, McClure D, Jimenez LA, Megson IL, Rahman I (2005) Curcumin induces glutathione biosynthesis and inhibits NF-kappaB activation and interleukin-8 release in alveolar epithelial cells: mechanism of free radical scavenging activity. Antioxid Redox Signal 7(1–2):32–41.

[93] Papas AM (1998) Antioxidant status, diet, nutrition, and health. CRC Series, Boca Raton, FL

[94] Zelenka J, Dvorák A, Alán L, Zadinová M, Haluzík M, Vítek L. Hyperbilirubinemia protects against aging-associated inflammation and metabolic deterioration. Oxid Med Cell Longev 2016;2016:6190609.

[95] Ziberna L, Martelanc M, Franko M, Passamonti S. Bilirubin is an endogenous antioxidant in human vascular endothelial cells. Sci Rep 2016;6:29240

[96] Sautin YY, Nakagawa T, Zharikov S, Johnson RJ. Adverse effects of the classic antioxidant uric acid in adipocytes: NADPH oxidase-mediated oxidative/ nitrosative stress. Am J Physiol Cell Physiol 2007;293(2):C584–96.

[97] Stinefelt B, Leonard SS, Blemings KP, Shi X, Klandorf H. Free radical scavenging, DNA protection, and inhibition of lipid peroxidation mediated by uric acid. Ann Clin Lab Sci 2005;35(1):37–45.

[98] Gupta VK, Sharma SK (2006) Plant as natural antioxidant. Nat Product Radiance 5(4):326–334 Hägg M, Ylikoski S, Kumpulainen J (1995) Vitamin C content in fruits and berries consumed in Finland. J Food Compost Anal 8:12–20.

[99] Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM, Lawal A (2010) Antioxidants: its medicinal and pharmacological applications. Afr J Pure Appl Chem 4(8):142–151.

[100] Bolognesi C. Genotoxicity of pesticides: a review of human biomonitoring studies. Mutat Res.2003;543: 251–272.

[101] Sapbamrer, R., Khacha-ananda, S., Sittitoon, N. et al. A longitudinal follow-up study of oxidative stress and DNA damage among farmers exposed to pesticide mixtures. Environ Sci Pollut Res 26, 13185–13194 (2019).

[102] Fariba T,Gholamhassan V, Mohammad A, et al. Comparative Toxicological Study between Exposed and Non-Exposed Farmers to Organophosphorus Pesticides. 2016 Spring; 18(1): 89–96.

[103] regard L, Møller P, Henriksen T, et al. Human and methodological sources of variability in the measurement of urinary 8-oxo-7, 8-dihydro-2′-deoxyguanosine. Antioxid Redox Signal. 2013;18(18):2377-2391.

[104] M. Elisabetta Zanolin , Paolo Girardi , et al. Measurement of a urinary marker (8-hydroxydeoxyguanosine, 8-OHdG) of DNA oxidative stress in epidemiological surveys: a pilot study. Int J Biol Markers 2015; 30(3): e341-e345.

[105] Mizoue T, Tokunaga S, Kasai H, Kawai K, Sato M, Kubo T. Body mass index and oxidative DNA damage: a longitudinal study. Cancer Sci. 2007; 98(8): 1254-1258.

[106] Sakano N, Wang DH, Takahashi N, et al. Oxidative stress biomarkers and lifestyles in Japanese healthy people. J Clin Biochem Nutr. 2009;44(2):185-195.

[107] Miwa M, Matsumaru H, Akimoto Y, Naito S, Ochi H. Quantitative determination of urinary 8-hydroxy-2′-deoxyguanosine level in healthy Japanese volunteers. Biofactors. 2004;22(1-4): 249-253.

[108] Tamae K, Kawai K, Yamasaki S, et al. Effect of age, smoking and other lifestyle factors on urinary 7-methylguanine and 8-hydroxydeoxyguanosine. Cancer Sci. 2009;100(4):715-721.

APPENDIX A

a) QUESTIONNAIRE FORM (ANKET FORMU)

“Aşağıda adı geçen araştırmada kullanılacak materyal ile ilgili bilgilerin gizli tutulacağını ve belirtilen amaç dışında amaçlarla kullanılmayacağını taahhüt ederiz.”

“Anket Formundaki tüm açıklamaları okudum. Bana, yukarıda konusu ve amacı belirtilen araştırma ile ilgili yazılı ve sözlü açıklama aşağıda adı belirtilen doktor tarafından yapıldı. Araştırmaya gönüllü olarak katıldığımı, istediğim zaman gerekçeli veya gerekçesiz olarak araştırmadan ayrılabileceğimi biliyorum.”

“Söz konusu araştırmaya, hiçbir baskı ve zorlama olmaksızın kendi rızamla katılmayı kabul ediyorum.”

Araştırmanın başlığı: Manav Çalışanlarında Pestisit Maruziyeti ile Oksidatif Hasar Arasındaki İlişkinin Değerlendirilmesi

Anket formunda 11 adet soru yer almaktadır. Sorulara yanıt verme süreniz 20 dakikadır. Araştırmaya katılmak gönüllülük esasına dayalıdır. Araştırma sürerken herhangi bir zamanda istemeniz durumunda sorumlu araştırmacıyı bilgilendirmek koşulu ile araştırmadan ayrılabilirsiniz. Araştırma sırasında sizden alınan bilgiler araştırmacıda saklı kalacak ve toplanan veriler yalnızca bilimsel amaçla kullanılacaktır. Ankette bulunan sorulara doğru yanıt vermenizi rica eder, desteğiniz için teşekkür ederiz.

1. Cinsiyet:

[ ] Erkek [ ] Kadın

2. Yaş:

3. Kilo:

4. Boy:

5. Ne kadar süredir bu meslekte çalışmaktasınız?

6. Günde kaç saat çalışıyorsunuz?

7. Aile bireylerinizde ve/veya sizde kanser hastalığı var mı?

8. Düzenli kullandığınız herhangi bir ilaç var mı?

[ ] Evet [ ] Hayır Varsa adı:

9. Son 1 yılda herhangi bir viral enfeksiyon geçirdiniz mi?

(Covıd-19, sarılık, kızamık, kızamıkçık, Su çiçeği, kabakulak, menenjit)

[ ] Evet ise Nedir [ ] Hayır

10. Yaptığınız spor ve aktiviteler var mıdır?

[ ] Az enerji gerektiren spor ya da diğer aktivite (yürüme, bahçe işleri, ev işleri gibi)……..saat/gün

[ ] Orta derecede enerji gerektiren spor ya da diğer aktivite (ağır ev işi/ bahçe işleri, yüzme, bisiklet sürme gibi) ……..saat/gün

[ ] Fazla güç ve enerji gerektiren spor ya da diğer aktivite (koşu, hızlı yüzme /ya da bisiklet sürme, futbol gibi) ……..saat/gün

11. Beslenme alışkanlığında size en fazla uyan tanım aşağıdakilerden hangisidir?

[ ] Et ağırlıklı beslenme [ ] Sebze ve meyve ağırlıklı beslenme

[ ] Kuru bakliyat ağırlıklı beslenme

[ ] Balık ve diğer deniz ürünleri ağırlıklı beslenme [ ] Karışık

b) The Clinical Research Ethics Committee Acceptance Form

ALTINBAŞ ÜNİVERSİTESİ
KLİNİK ARAŞTIRMALAR ETİK KURULU

Sayı: 99 **Tarih:06.01.2022**
Konu: Dr. Öğr. Üyesi Şükriye Karadayı

Sayın Dr. Öğr. Üyesi Şükriye Karadayı
Altınbaş Üniversitesi SHMYO Müdürlüğü

İlgi: Altınbaş Üniversitesi SHMYO Müdürlüğünün 20.12.2021 tarihli yazısı ile Altınbaş Üniversitesi Klinik Araştırmalar Etik Kurulunun 2021/107 sayılı yazısı

Sorumlu araştırıcılığını üstlendiğiniz **2021/107** dosya numaralı **"Manav Çalışanlarında Pestisit Maruziyeti ile Oksidatif Hasar Arasındaki İlişkinin Değerlendirilmesi"** başlıklı çalışma, kurulumuzun **06 Ocak 2022 tarih ve 12 sayılı toplantısında** görüşülerek etik yönden uygun bulunmuş olup, tutanaklar ekte sunulmuştur.
Bilgilerinizi rica ederim.

Prof. Dr. Mustafa Aydın BARLAS
Altınbaş Üniversitesi Klinik Araştırmalar
Etik Kurul Başkanı
E-imzalıdır

Eki: Altınbaş Üniversitesi Klinik Araştırmaları Etik Kurulu Karar Formu

ETİK KURUL BİLGİLERİ	ETİK KURULUN ADI	**ALTINBAŞ ÜNİVERSİTESİ KLİNİK ARAŞTIRMALARI ETİK KURULU KARAR FORMU**
	AÇIK ADRESİ:	**Kartaltepe Mah. İncirli cad. No:11 Bakırköy / İstanbul**
	TELEFON	**(0 212) 709 45 28**
	FAKS	**(0 212) 445 81 71**
	E-POSTA	etikkurul@altinbas.edu.tr

BAŞVURU BİLGİLERİ	ARAŞTIRMANIN AÇIK ADI	**"Manav Çalışanlarında Pestisit Maruziyeti ile Oksidatif Hasar Arasındaki İlişkinin Değerlendirilmesi"**			
	ARAŞTIRMA PROTOKOL KODU	**2021/107**			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACI UNVANI/ADI/SOYADI	**Dr. Öğr. Üyesi Şükriye Karadayı**			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ UZMANLIK ALANI	**Adli Fen Bilimleri**			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ BULUNDUĞU MERKEZ	**Altınbaş Üniversitesi SHMYO/Tıbbi Laboratuvar Teknikleri**			
	DESTEKLEYİCİ	---			
	DESTEKLEYİCİNİN YASAL TEMSİLCİSİ	---			
	ARAŞTIRMANIN FAZI	FAZ 1	↑		
		FAZ 2	↑		
		FAZ 3	↑		
		FAZ 4	↑		
	ARAŞTIRMANIN TÜRÜ	Yeni Bir Endikasyon	↑		
		Yüksek Doz Araştırması	↑		
		Diğer ise belirtiniz: Bilimsel Araştırma			
	ARAŞTIRMAYA KATILAN MERKEZLER	TEK MERKEZ ↑	ÇOK MERKEZLİ ■	ULUSAL ■	ULUSLAR ARASI ↑