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Oxidative Stress Marker Malondialdehyde and Glutathione Antioxidant in Hypertensive Patients

Ayat A. Hassan and Sahera G. Sayyah

ABSTRACT

The study included measuring the average concentration of Malondialdehyde (MDA) and glutathione for hypertensive patients in Iraq, specifically Basra Governorate, where the study group reached 50 patients (26males and 24 females), and the patients were divided according to age, gender, family history, period of illness, medication, and diet in comparison. With 40 healthy cases (19males and 21 females) as a control group. The results of the study showed a significant increase in lipid peroxide measured by (MDA) in the serum of hypertensive patients at a significant level (P<0.0001) compared with the control group. Also, its level increased significantly at (P<0.0001) with advancing age and according to the sex factor among the study group. And its level increased significantly at the level of significance (P<0.05) with the length of the disease period. The results also did not show significant differences for patients who have a family history, patients who are being treated with antihypertensive drugs, and patients who adhere to a healthy diet. The results also showed a significant decrease in the average concentration of (GSH) as an antioxidant at a level (P<0.0001) in the blood of hypertensive patients compared to the control group. This decline increases with age and in both sexes, males, and females. While the results did not show significant differences in the level of (GSH) in patients who have a family history, length of illness, medication, and adherence to a healthy diet. Moreover, a negative correlation was observed between the level of (GSH) and the level of (MDA) in the study group .We conclude through the results of the study that the presence of a defect in patients in the rate of concentration of (GSH) indicates an increase in free radicals, and that an increase in the level of (MDA) indicates an increase in the active types of (ROS), and this increase accompanies a decrease in antioxidants such as (GSH).

Keywords: GSH, Glutathione, MDA, Malondialdehyde, reactive oxygen species, ROS.

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I. Introduction

Blood pressure represents the force of blood in the blood vessels from the heart to the parts of the body by a process called circulation. As for high blood pressure, it represents a rise in the driving force of blood inside the blood vessels due to narrowing in the walls of the arteries [1]. Hypertension is affected by three factors: the amount of blood in the arteries, the number of heartbeats, the expansion and softness of the arteries. The normal average value for blood pressure in adults is 140l90 mmHg, and if the value is higher than that and continuously, the person is considered to have hypertension [2]. Among the most serious complications caused by this disease is damage to the arteries (Engiopathy) [3], the heart (Cardiopathy) [4], [5], the kidneys (Nephropathy) [6], the brain Encephalopathy [4], the eyes (Retinopathy) [7] and impaired sexual function [8], the hypertension is also a risk factor for pregnant women. [9] Since this disease is considered fatal and dangerous, the World Health Organization has taken care of educating people and making organized programs to reduce this disease. [10] The severity of this disease increases with age [11], race [12], family history [13], obesity [14], laziness and lethargy [15], smoking [16], drinking alcohol [17], a rich diet. With sodium and poor in potassium [18], and the presence of other chronic diseases that may exacerbate the disease. [19]. As for recent studies in 2021, they indicate that exposure to polluted air with metal pollutants affects immune genes and increases the risk of high blood pressure. [20] Recently it has been found that free radicals are the cause of most chronic diseases and the active free radicals in the body are produced as a result of metabolic reactions. [21] As an active free electron remains looking for another electron to settle, in the absence of another electron, it will attack the cell and the attack force will reach the genes. [22] In the natural state, free radicals are beneficial to the body. But when it exceeds the normal limit, it will be very harmful. They are of two types, ROS,

RNS, and some do not contain a root but have the ability to react and destroy the cell from hydrogen peroxide. In order to reduce the damage of free radicals, the presence of antioxidants is necessary, which are two types of enzymatic antioxidants and non-enzymatic antioxidants. [23] One of the most powerful antioxidants is glutathione. [24] Which is a water-soluble tripeptide that protects the body from deterioration, damage, and disease. Glutathione will work to fight free radicals and (Fig. 1) [25] shows the reaction mechanism. But it is not correct for GSSG to remain in the body, as it causes what is called oxidative stress. Therefore, NAPH plays a role in returning glutathione to its natural state in the body. As for oxidative stress, it can be measured from the ratio of GSH / GSSG, as well as from the MDA analysis and from the protein carbon analysis. And it was adopted in my studies to measure oxidative stress by analyzing MDA [26], which is a toxic compound, and its presence is a marker for lipid peroxide that has the ability to interact with proteins and DNA, which are chain reactions of oxidative decomposition of fats as in (Fig. 2). [27]

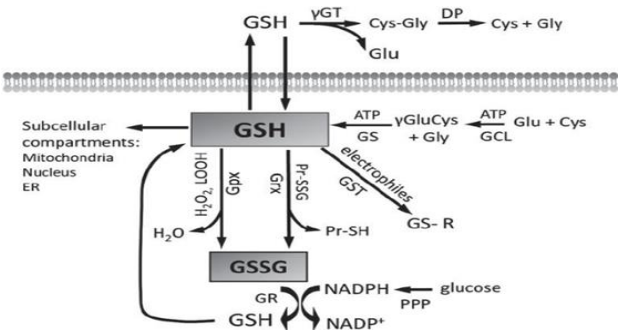

Fig. 1. shows the synthesis of extracellular glutathione in the presence of the three nucleic acids glutamine, glycine, and cysteine. This two-step reaction occurs based on ATP and the enzyme GCL that splits the action of glutathione in the mitochondrial, endoplasmic reticulum, and nucleus in separate and regulated redox reactions as part of the antioxidant defense. Glutathione also participates in two reactions in the presence of the catalytic enzymes Gpx and Grx and GSH converters after giving it two molecules of hydrogen to GSSG and then in the presence of NADPH works to give glutathione reduced to two molecules of glutathione and the cycle is repeated again.

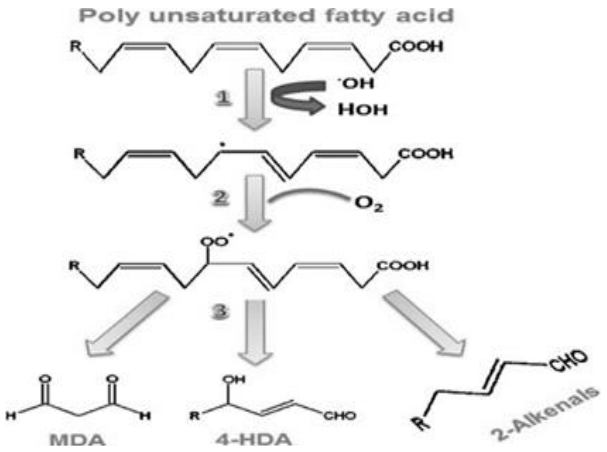

Fig. 2. Step 1: The free radicals remove the hydrogen ion from the unsaturated fatty acid to form a fatty free radical. In Step 2: the formed fatty radical tends to settle and rearrange itself into a diene. 3: The resulting compound decomposes symmetrically to form a group of products such as alkanes, alkenes, and Malondialdehyde. The current study was conducted to estimate the average concentration of MDA and GSH in the serum of hypertensive patients in several variables (age, sex, family history, period of illness, medication, diet) in Basrah Governorate–Iraq.

II. Materials and Methods

A. Samples

The study was conducted on 50 patients (26 males and 24 females) and 40 apparently healthy patients, representing the control group (19 males and 21 females), and their ages ranged from 20 to 60 years. Divided into three groups (20–35 years old, 36–50 years old, and (>50) years old). The information was recorded for patients with blood pressure and the study groups were divided according to males and females. According to family history: history (+ve) and history (-ve). According to the period of the disease: (1–5) years, (6–10) years and (>10) years, and according to medication: it is treated (+ve), it is not treated (-ve) and according to the type of diet: If the diet contains salt (+ve) does salt free (-ve). 5 ml of the blood of patients and healthy people was withdrawn through a syringe from the vein and transferred to test tubes containing the gel and left for 10 minutes. The test tubes were placed in a centrifuge at 3000 revolutions per minute for 10 minutes. After the serum separated from the blood components, the serum was divided into Micro tubes and kept in deep freeze until the examination.

B. Instruments

1) Elisys Uno, Human (Germany)
2) UV/VIS spectrophotometer APEL Japan
3) Centrifuge, Hattic EBA 20, Germany
4) Vortex stirrer, Galen Kamp, Germany
5) Water bath, Galen Kamp, Germany.

C. Determination of the concentration of (MDA)

The concentration rate of Malondialdehyde in blood serums was examined using a German kit from (Zell Bio GmbH) [28]–[30] and the measurement method was based on the formation of a complex of Malondialdehyde and Thioboric acid in an acidic medium under a high temperature of 90°C–100°C and the pink color formed at a wavelength of 535 nm is measured. The following equations show the formation of the MDA-TBA complex [31] as in (Fig. 3).

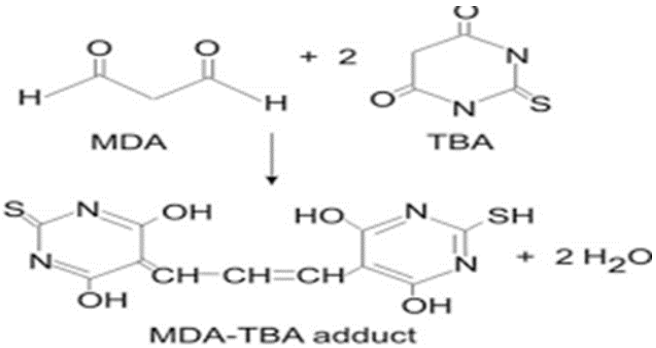

Fig. 3. Formation of the MDA-TBA complex.

D. Procedure

At the beginning of the work the reagents and samples are equilibrated at room temperature, the halogen sample is shaken and then 50 µ L of sample or standard solution is added to a marked test tube, 50 µ L of reagent R4 is added and heated if it is cloudy to become a clear solution. The test

is in an ice bath. The tubes are placed in a centrifuge at 4000 rpm for 10 minutes. The pink filter is carefully withdrawn and transferred to the microscopic plate. The absorbance is read in the ELISA machine at a wavelength of 535 nm [31].

E. Calculation

The unknown MDA level is calculated through the standard curve, which was drawn using the absorptive points of the standers.

1) Standards preparation [31]

The standard of the standard solution is 5000 µ mole, at the beginning it is diluted in a ratio of 1:50, then 100 µ mole is prepared and by serial dilution the following solutions are prepared 100 µ M, 50 µM, 25 µM, 12.5 µM , 6.25 µM , 3.12 µM , 1.56 µM, 0 µM . Dilute each time with 100 µL of distilled water. As in Fig. 4.

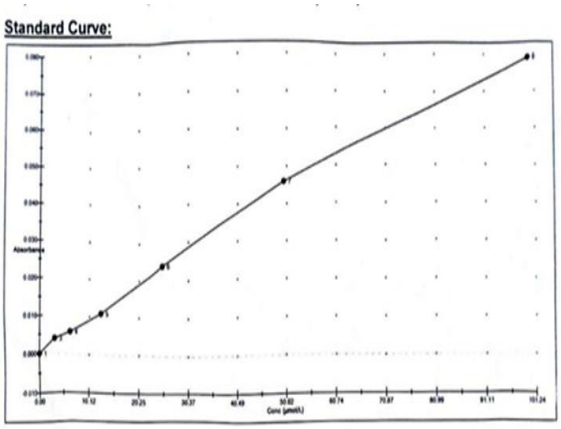

Fig.4. Stander carves off the Elisys kit (MDA).

F. Determination of Glutathione Concentration in Serum

The determination of glutathione in serum is done by the modified method used by researchers (Sedlak & Lindsay; Tietz) [32]–[34], and the method is based on the use of Elman's solution [5,5-dithio bis (2-Nitrobenzoic acid)] DTNB Elman's reagent, as it reacts quickly with glutathione and is reduced by the sulfhydryl group (SH group) of glutathione to form a colored product whose absorbance is read at 412 nm. As in Fig. 5, which illustrates the Mechanism of glutathione quantification. The resulting concentration depends on the concentration of glutathione present in the blood serum.

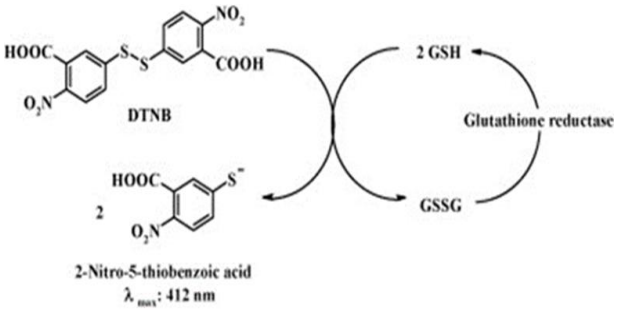

Fig.5. Mechanism of glutathione quantification [35].

G. Preparation of Reagent

4% (S.S.A) Sulfosalicylic acid. A (0.1mM) (0.1mM) Elman's reagent is prepared by taking 0.00396 g of DTNB and dissolving in 100 ml of the buffer (8 pH) prepared by mixing (0.6M) KH2PO4 and (0.08M) Na2HPO4 and neutralizing it with a NaOH base using a pH meter. Ph. Meter.

H. Procedure

The test is carried out for the determination of glutathione by adding 150 µl and 150 µl of 4% Sulfosalicylic acid, mixed for two minutes, and then placed in a centrifuge at a speed of 2000 rpm for 5 minutes. 150µl is taken from the formed filtrate, 4.5ml of German reagent is added and shaken for 5 minutes, then the absorbance is read at 412 nm. The blank is treated the same and distilled water is added instead of serum.

I. Calculation

The concentration of glutathione in the blood serum is estimated based on the following equation:

$$GSH\ (\mu mole/L) = (A\ teat - A\ blank)/(Eo \times L) * 10^6 \quad (1)$$

Where:
Eo – Extinction coefficient 13600 M^{-1} cm^{-1}
L – light bath 1 cm

III. Statistical Analysis

The results and data were analyzed through the statistical program SPSS version No. 25 using a one-way analysis (ANOVA) and calculating the concentration rate, standard deviation (SD) and the probability value and it was less than 0.0001 and this value is considered highly statistically significant and also represented the probability of the lowest value at a significance level at 0. 05.

IV. Results and Discussion

The study showed in Table I that there was a statistically significant increase in the average concentration of MDA at the significance level of $P<0.0001$ among the study group according to the divided age groups. An increase in oxidative stress is also observed with age. This study agrees with another study in which it shows that as long as oxidative stress was associated with weak immunity and the aging process, it also increases blood pressure. [36] That is, patients who have high oxidative stress are expected to experience the aging process early, as it is expected that the high pressure will worsen as a result of the increase in free radicals in the attack on the cell. The study also showed in Table I that there was a significant increase in the level of MDA between male patients and healthy males and between sick females and healthy females at the significance level of $P<0.0001$. Not calculated statistically. However, some studies indicated that there are differences between the sexes [37]. The study showed in Table I that there were no significant differences in the rate of MDA concentration according to family history among the study groups of patients, meaning that hypertension may be genetic, but the rate of oxidative stress concentration does not depend on it. While some studies indicated that mitochondria are the main source of NADPH generation, it is believed that seeking treatment for this imbalance reduces oxidative stress and

thus reduces high blood pressure [38]. The study showed in Table II that the increase in the duration of the disease increases the oxidative stress. And that there were significant statistically significant differences at the level of $P<0.05$ among the study groups. There are studies that agree with this study. It also showed in Table II that there are no statistically significant differences between patients undergoing treatment and patients who are not undergoing treatment, meaning that oxidative stress is not affected by pressure medications, but rather reduces blood pressure only. This study agreed with another study conducted on 41 patients who were divided into three groups. They took three types of antihypertensive drugs and noted that the drugs did not reduce oxidative stress [39]. The results in Table II showed that there were no significant differences between patients who adhere to a sodium-free diet and patients who eat all kinds of foods. This means that oxidative stress is not affected by sodium in the body, unlike blood pressure, which rises with high sodium in the body. The results of the study in Table I for the measurements of the average GSH concentration between study groups of patients and healthy subjects according to age groups and gender differences showed that there were differences at the level of significance $P<0.0001$ and these results came simultaneously with the increase in oxidative stress in patients, as GSH depletion indicates There is a defect in the work of mitochondria and an imbalance between antioxidants and oxidants [38]. And since GSH is considered a major antioxidant, its deficiency indicates weak immunity in patients [40]. The results also showed in Table II that there were no statistical differences in the average GSH concentration in each of the variables (family history, period of illness, medication, and diet), and it can be explained that as long as GSH was considered a protective system against lipid peroxide [41]. The deficiency of GSH is offset by an increase in MDA, and it is worth noting that the factors that are not affected by oxidative stress, are also not affected by the antioxidants.

V. Abbreviations Used

GPx – GSH peroxidase
GR – GSSG-reductase
Grx – glutaredoxin
GSH – glutathione
GSSG – oxidized GSH
GST – GSH-S-transferase
ROS – reactive oxygen species
NADPH – Nicotinamide adenine dinucleotide phosphate

TABLE I: Concentration Rate Data MDA and GSH in the Serum of the Study Groups

Variable		Hypertensive patients (50) MDA µmole/L		Control (40) MDA µmole/L		Probability
		No	Mean±SD	No	Mean±SD	
Age/Year	20–35	12	285.99±122.69	13	50.13±18.87	*P* <0.0001
	36–50	14	330.84±135.17	20	52.08±21.12	*P* <0.0001
	>50	14	331.30±161.00	17	55.76±30.66	*P* <0.0001
Sex	Male	26	289.39±161.88	19	56.31±23.59	*P* <0.0001
	Female	24	319.27±128.95	21	47.18±17.27	*P* <0.0001
Variable		Hypertensive patients (50) GSH µmole/L		Control (40) GSH µmole/L		Probability
		No	Mean±SD	No	Mean±SD	
Age/Year	20–35	12	0.01±0.00	13	1.32±0.23	*P* <0.0001
	36–50	14	0.01±0.00	20	1.29±0.33	*P* <0.0001
	>50	14	0.01±0.01	17	1.49±0.15	*P* <0.0001
Sex	Male	26	0.016±0.01	19	1.29±0.18	*P* <0.0001
	Female	24	0.01±0.00	21	1.38±0.36	*P* <0.0001

TABLE II: Concentration Rate Data MDA and GSH in the Serum of the Study Groups

Variable		Hypertensive patients (50) MDA µmole/L		Probability
		No	Mean±SD	
Family history	Positive	32	306.25±151.43	*P* >0.05
	Negative	18	292.25±127.46	
Sickness period/Year	1–5	28	247.47±157.35	*P* >0.05
	6–10	13	356.76±111.85	
	>10	12	361.73±142.40	
Medication	Positive	19	299.21±149.63	*P* >0.05
	Negative	31	306.51±146.64	
Diet	Positive	34	306.54±150.21	*P*>0.05
	Negative	16	289.01±131.77	
Variable		Hypertensive patients (50) GSH µmole/L		Probability
		No	Mean±SD	
Family history	Positive	32	0.01±0.00	*P* >0.05
	Negative	18	0.01±0.01	
Sickness period/Year	1–5	28	0.01±0.01	*P* >0.05
	6–10	13	0.01±0.01	
	>10	12	0.01±0.00	
Medication	Positive	19	0.01±0.00	*P* >0.05
	Negative	31	0.01±0.01	
Diet	Positive	34	0.13±0.01	*P* >0.05
	Negative	16	0.01±0.05	

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A. Authors' Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ayat Am. Hassan. The first draft of the manuscript was written by Sahera Gh. Sayyah commented on previous versions of the manuscript. All authors read and approved the final manuscript.

B. Ethics Approval

This is an observational study. The Research Ethics Committee has confirmed that no ethical approval is required.

C. Consent to Participate.

Informed consent was obtained from all individual participants included in the study.

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E. Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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