



Early detection of subclinical oxidative stress and its correlation with biochemical and haematological indices

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Abstract

Introduction: There is increasing evidence suggesting that oxidative stress can be considered one of the first events in biology, which may happen in healthy people even before the appearance of any signs of disease. Purpose: The present study was designed to investigate subclinical oxidative stress and the correlation between it and some hematological and biochemical markers in apparently healthy subjects.

Methods: In the present study, 70 apparently healthy subjects were involved and were divided into groups based on their oxidative stress level. MDA and TAC were measured as indices of oxidative stress. Other variables measured were hemoglobin, total leukocyte count, NLR, along with biochemical parameters such as glucose and lipids. All tests were done by routine laboratory methods.

Results: Subjects having higher oxidative stress showed significantly higher MDA levels and significantly lower TAC levels compared to those with low oxidative stress ($p < 0.001$). Higher levels of NLR and CRP indicated mild inflammation. Additionally, there was a significant increase in the fasting glucose, total cholesterol, triglyceride, and LDL cholesterol levels and a significant reduction in the HDL cholesterol level ($p < 0.001$). Significant correlations were seen among the markers of oxidative stress, inflammation, and metabolism.

Conclusion: There is subclinical oxidative stress in apparently healthy subjects associated with early changes in hematological status, inflammation, and metabolism. This implies that oxidative stress markers could be useful early biomarkers of physiological dysfunction.

Keywords: Oxidative stress; Malondialdehyde; Subclinical inflammation; Metabolic biomarkers; Lipid profile; Glucose.

1 Introduction

Oxidative stress represents a biological condition arising from an imbalance between the production of reactive oxygen species (ROS) and the capacity of antioxidant defense systems to neutralize them [1]. Under physiological conditions, ROS play important roles in cellular signaling and homeostasis; however, excessive accumulation can lead to damage of lipids, proteins, and nucleic acids, ultimately contributing to cellular dysfunction [2].

As a matter of fact, the latest studies have shown that the presence of oxidative stress is not necessarily indicative of pathologies but may be subclinical and found in healthy people [3]. As a rule, such cases of oxidative stress go unnoticed and can actually precede various metabolic, cardiovascular and inflammatory diseases. At this point, it becomes quite clear that oxidative stress should be treated as a biomarker rather than a pathological phenomenon [4].

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MDA serves as an effective indicator of lipid peroxidation or oxidation of lipids, which can be seen as cell membrane damage, while TAC is useful in assessing the activity of antioxidants throughout the entire body system [5]. Moreover, hematological factors such as NLR along with biochemical parameters including glucose and lipid levels have been proposed as potential indicators of oxidative and inflammatory states [6].

There exists an intricate link and interaction between oxidative stress, inflammation, and metabolism disorders. Inflammation can be stimulated by oxidative stress while at the same time oxidative stress can be promoted by inflammation [7], thus forming a vicious circle which may result in the development of diseases [8].

While more research is being done on oxidative stress, not much information can be found about the relation of oxidative stress and hemato-biochemical variables of healthy subjects [9]. Thus, the purpose of the present study was to determine sub-clinical oxidative stress and its relationship with specific hemato-biochemical parameters of healthy adult subjects [10]. The results obtained from the study will be helpful to understand any changes taking place in the body at an early stage.

2 Material and methods

2.1 Study Framework

The current study was done with the aim of examining changes that occur in oxidative equilibrium in people who appear clinically healthy and whether these changes are related to changes in certain blood and biochemical parameters. The experiment involved an observational design where serum samples were obtained from individuals who appeared healthy and had no signs of disease.

2.2 Participants and Sample Size

A total of seventy adults who appeared to be healthy were used for the present research work with age ranges between 19 and 56 years. They were all volunteers from various backgrounds of society in order to achieve a wide variety of physiological profiles in the selected individuals. It should be mentioned that selection of the individuals was done cautiously in order to exclude any clinical disease.

In order to reduce the effect that potential confounders may have on measurements for oxidative stress, certain criteria for exclusion were used for selecting participants. People who had been previously diagnosed with chronic diseases, or had any infectious or inflammatory disorders, had recently undergone surgery, hospitalization or treatment involving corticosteroids or any other antioxidants, along with people who consumed alcohol or were smokers were excluded from the study. Moreover, individuals who had been involved in intense physical exercises one week before their sample collection were also excluded because such activities affect the oxidative stress markers.

Once the samples were processed in the laboratory, the subjects were classified based on their oxidative stress biomarker values.

2.3 Questionnaire and Preliminary Assessment

Prior to the blood samples' collection, each subject filled out a standardized information form aimed at gaining demographic and other general information that could potentially affect oxidative balance. The acquired information covered age, sex, body weight, height, sleep duration, amount of water consumed daily, exercise frequency, and diet. Moreover, information about the history of metabolic diseases in the family was solicited in order to reveal possible genetic background of biochemical and oxidative processes.

The body mass index was determined in each subject in order to evaluate his/her body composition and nutrition. The calculation of BMI was carried out using the formula [11]:

$$\text{BMI} = \text{Weight} / \text{Height}^2$$

The purpose of this preliminary assessment was to investigate the possible indirect contribution of lifestyle and physiological variables to oxidative balance and antioxidant status among apparently healthy individuals.

2.4 Blood Collection and Serum Preparation

Blood samples were drawn from the subjects in the morning session from 8:00 AM to 10:00 AM after overnight fasting to minimize the effect of fluctuations on the measured variables. About 6 mL venous blood sample was taken from each participant using sterile disposable syringes in an aseptic laboratory setting[12].

The blood samples that were obtained were classified into two categories depending on the nature of the analysis that had to be performed. The first set of blood was kept in a tube containing the anticoagulant EDTA for use in performing hematological tests, while the second category was kept in gel tubes for serum separation and biochemical tests.[13]

These serum samples were left to coagulate at room temperature for almost 25 minutes before being spun at 3500 rpm for 12 minutes. The serum obtained from this process was transferred to sterile microtubes and frozen at -20°C [14].

The issue of sample preservation was paid special attention to, and repeated freeze-thaw procedures were avoided due to the possible influence of temperature changes on the stability of oxidative stress biomarkers.

2.5 Hematological Measurements

Evaluation of the selected hemogram indices was conducted with the help of an automatic blood analyzer, which was calibrated before performing the measurements according to standard laboratory quality control measures. Hemogram indices chosen for the analysis were known for their correlation with the immune state, inflammation level, and overall physiological condition.

The studied hematological variables were Hb concentration, packed cell volume, total number of leukocytes, neutrophils percent, lymphocytes percent, and thrombocyte number. These indices were chosen due to their connection with inflammation processes and oxidation level in people who appear healthy.

In addition, the neutrophil-to-lymphocyte ratio (NLR), which is considered an indicator of systemic inflammatory balance, was calculated mathematically using the following equation[15]:

$$\text{NLR} = \text{Neutrophils} / \text{Lymphocytes}$$

The calculated NLR values were later used for correlation analysis with oxidative stress and biochemical markers.

2.6 Biochemical Measurements

Biochemical analysis was carried out using enzyme-linked colorimetric technique, employing laboratory test kits. Laboratory analysis of all test parameters was done in standardized laboratory conditions as per manufacturer's guidelines.

The biochemistry tests done included serum glucose levels during fasting, total cholesterol levels, triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL), alanine aminotransferase (ALT) activity and aspartate aminotransferase (AST) activity. Such parameters were chosen because they were indicators of metabolic and lipid metabolism status and liver functions, which could be affected by the imbalance of oxidation.

The absorbance values for the various biochemical tests conducted were measured via a UV-Vis spectrophotometer at different specific wavelengths [16]. The quality control samples as well as the calibration standards were incorporated in the test process to ensure the validity of the results obtained.

2.7 Evaluation of Oxidative Stress Profile

2.7.1 Serum Lipid Peroxidation Index

The process of lipid peroxidation was analyzed based on the measurement of malondialdehyde levels via the reaction of malondialdehyde with thiobarbituric acid.

Malondialdehyde will react with thiobarbituric acid in acidic medium, resulting in color formation, which can be measured at 532 nm. Levels were quantified in micromoles per liter [17].

2.7.2 Serum Antioxidant Defense Capacity

Serum samples were examined for total antioxidant efficiency. This technique is based on the ability of serum antioxidants to reduce oxidized intermediates to colored products.

The process involves monitoring optical density at a wavelength of 593 nm[18].

2.7.3 Inflammatory-Oxidative Interaction Marker

The presence of C-reactive protein concentration was used as an additional inflammatory marker to investigate the relationship between inflammation and oxidative stress in clinically healthy subjects [19].

C-reactive protein concentrations were assayed by immunologic agglutination technique.

2.7.4 Laboratory Quality Assurance

In order to assure accurate and reliable results from the analysis, stringent quality control protocols were applied during all lab analysis. Each assay was done in duplicates and means of the readings were taken to ensure accurate data entry for statistical analyses. New calibration standards were prepared before the analyses to ensure high level of accuracy and precision during measurement process. Control serum samples with known concentration were incorporated in each analysis on daily basis.

The laboratory glassware and micropipettes used were also calibrated before the study was carried out to avoid inaccuracies in the volume measured. Additionally, all hemolyzed sera, which might have caused interference in measuring biochemical parameters and oxidative stress, were excluded from the study.

2.8 Statistical Processing

2.8.1 Statistical Analysis

Statistical analyses were conducted via SPSS computer program. Data presentation is given in the format of Mean $\pm$ SEM. Normality check was done prior to statistical analysis. Group comparisons were made via independent sample t test. Pearson correlation and multiple regression analysis were carried out for assessing relationship and prediction, respectively. P value less than 0.05 was taken as statistically significant.

2.8.2 Ethical Considerations

The participation of all those in the experiment was completely on a voluntary basis. All the participants were made aware of the purpose of the experiment prior to sampling. No personal data was disclosed except for scientific research.

3 Results

3.1 Demographic and Baseline Characteristics

As can be seen from Table 1 below, a total of 70 seemingly healthy participants were used in this study, and they were divided into two groups depending on their level of oxidative stress (group with lower oxidative stress and group with higher oxidative stress).

There were no statistically significant differences found in terms of the participants' age and gender distributions ($p > 0.05$), indicating balanced baseline demographics for the two groups. However, body mass index was significantly higher among those in the group with higher oxidative stress ($p < 0.05$) as is evident from.

Table 1 Demographic characteristics of study population

Parameter	Lower OS Group	Higher OS Group	p-value
Age (years)	39.6 $\pm$ 9.8	41.3 $\pm$ 10.2	>0.05
Male/Female	18/17	19/16	>0.05
BMI (kg/m ²)	24.4 $\pm$ 3.0	27.1 $\pm$ 3.6	<0.05

3.2 Hematological and Inflammatory Profile

As presented in Table 2 and Figure 1, considerable changes have been seen in terms of hematological indicators among groups studied.

For the high oxidative stress group, there is a considerable decrease in the levels of hemoglobin along with an increase in the levels of total leukocytes. There is a remarkable increase in neutrophil-lymphocyte ratio (NLR) level ($p < 0.001$), as depicted in Figure 1, indicative of heightened inflammatory response.

Table 2 Hematological and inflammatory parameters

Parameter	Lower OS Group	Higher OS Group	p-value	Interpretation
Hb (g/dL)	13.6 ± 1.1	12.8 ± 1.2	<0.05	↓ oxygen transport capacity
WBC ($\times 10^3/\mu\text{L}$)	6.3 ± 1.0	8.2 ± 1.6	<0.01	Immune activation
Platelets ($\times 10^3/\mu\text{L}$)	250 ± 39	231 ± 43	<0.05	Hematological shift
NLR	1.9 ± 0.4	3.3 ± 0.7	<0.001	Systemic inflammation
CRP (mg/L)	2.7 ± 0.6	6.5 ± 1.1	<0.001	Inflammatory status

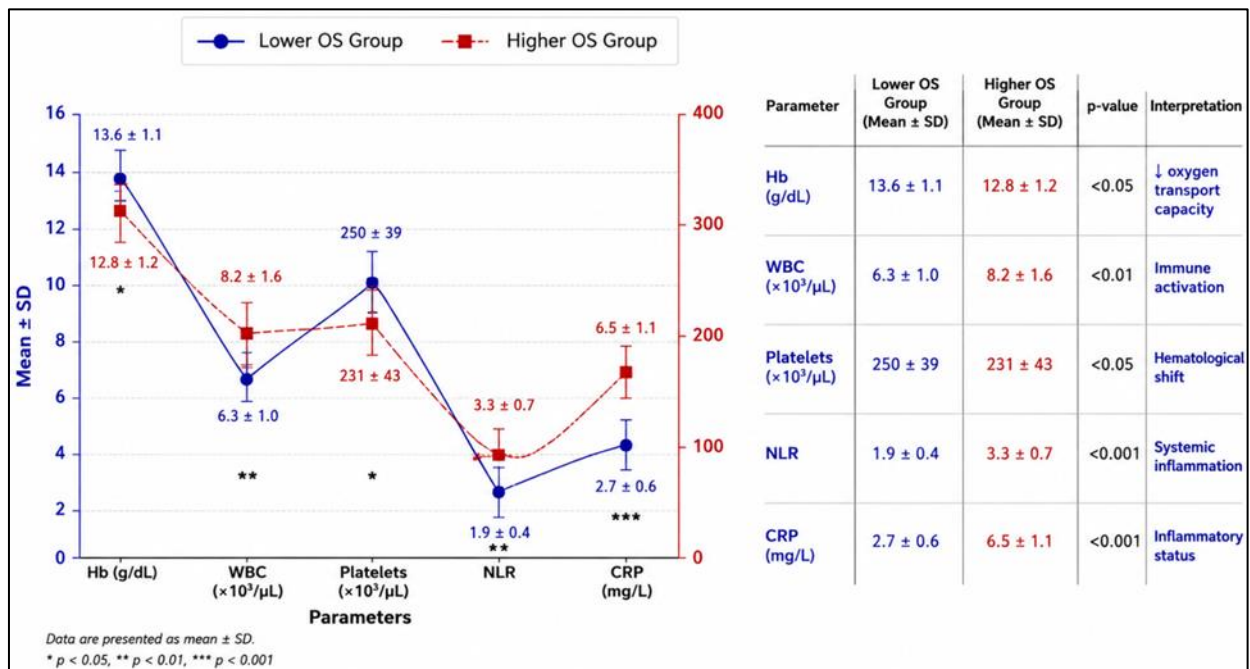


Figure 1 Association of Oxidative Stress Status with Hematological and Inflammatory Biomarkers in Adults

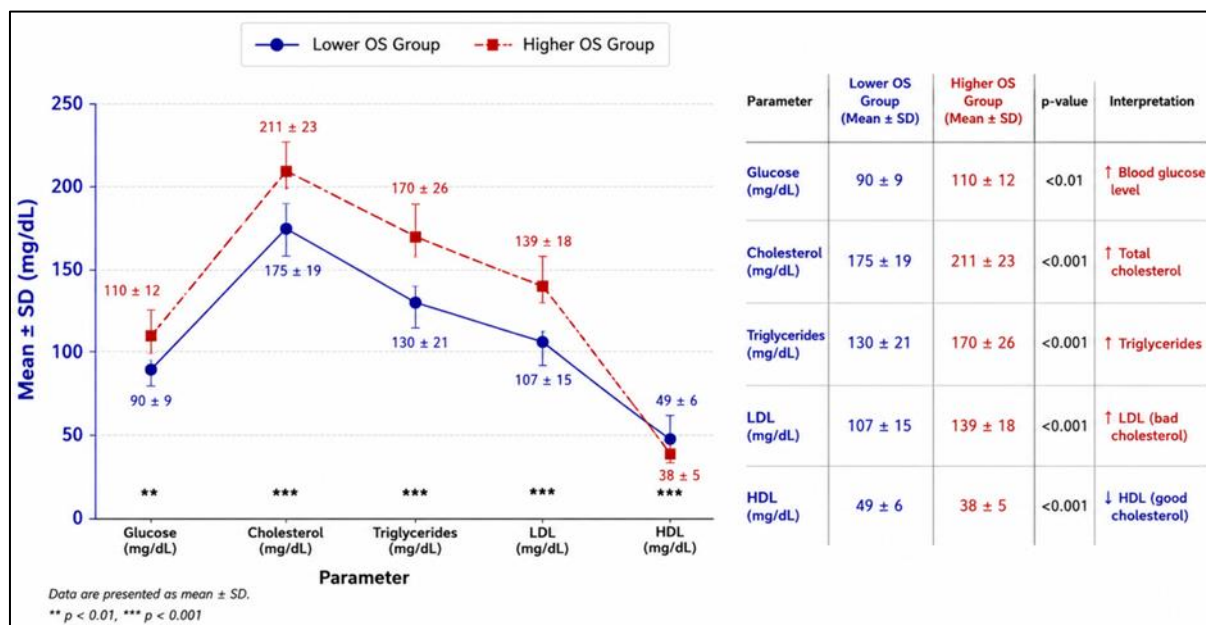
3.3 Metabolic and Biochemical Profile

As shown from Table 3 and Figure 2 below, subjects with elevated oxidative stress showed marked metabolic changes.

Marked elevation in fasting glucose levels, total cholesterol, triglycerides, and LDL cholesterol, together with a marked reduction in HDL cholesterol levels, were observed ($p < 0.001$). This is evident from Figure 2 below.

Table 3 Metabolic biochemical profile

Parameter	Lower OS Group	Higher OS Group	p-value
Glucose (mg/dL)	90 ± 9	110 ± 12	<0.01
Cholesterol (mg/dL)	175 ± 19	211 ± 23	<0.001
Triglycerides (mg/dL)	130 ± 21	170 ± 26	<0.001
LDL (mg/dL)	107 ± 15	139 ± 18	<0.001
HDL (mg/dL)	49 ± 6	38 ± 5	<0.001

**Figure 2** Association Between Oxidative Stress Status and Metabolic Biomarkers in Apparently Healthy Adults

3.4 Oxidative Stress Biomarkers

As can be seen from Table 4 and Figure 3, there was a notable imbalance between the production of oxidants and antioxidants.

It was evident that there was an increase in MDA levels in the high oxidative stress condition, thereby suggesting high levels of lipid peroxidation. On the other hand, TAC was decreased significantly ($P < 0.001$).

Table 4 Oxidative stress markers

Parameter	Lower OS Group	Higher OS Group	p-value	Interpretation
MDA (nmol/mL)	2.6 ± 0.4	4.9 ± 0.6	<0.001	Increased lipid peroxidation
TAC (mmol/L)	2.9 ± 0.5	1.8 ± 0.3	<0.001	Antioxidant depletion

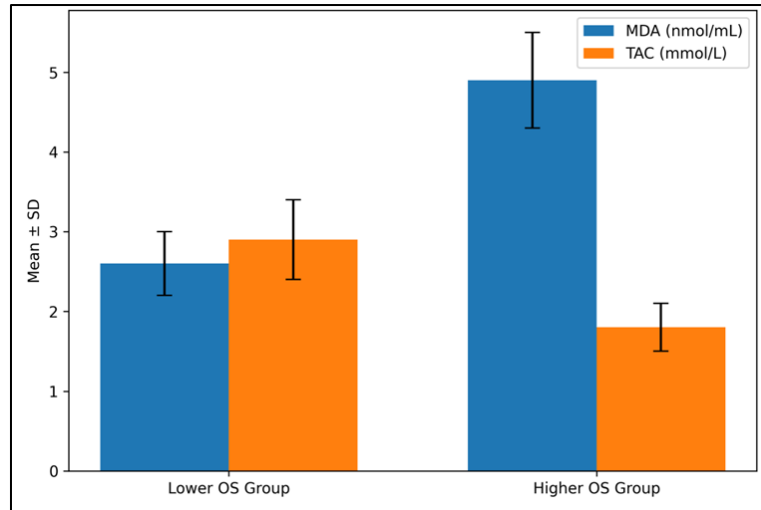


Figure 3 Oxidative Stress Profile in the Study Groups

3.5 Correlation Analysis

According to Table 5 and Figure 4, correlation analyses have demonstrated that there were statistically significant correlations between markers of oxidative stress and other biochemical and inflammatory factors.

There were very strong positive correlations found between MDA and CRP, NLR, and blood glucose, and on the other hand, there were negative correlations between TAC and these parameters, as can be seen from Figure 4.

Table 5 Correlation matrix of key parameters

Parameters	CRP	MDA	TAC	NLR	Glucose
CRP	1	+0.71*	-0.66*	+0.69*	+0.53*
MDA	+0.71*	1	-0.70*	+0.62*	+0.57*
TAC	-0.66*	-0.70*	1	-0.61*	-0.49*
NLR	+0.69*	+0.62*	-0.61*	1	+0.45*

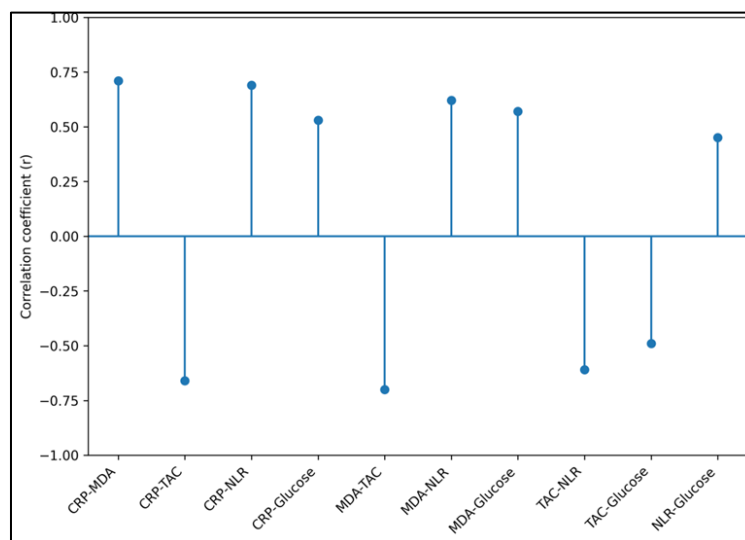


Figure 4 Correlation Matrix of Inflammatory, Oxidative Stress, and Metabolic Biomarkers

4 Discussion

It should be mentioned that the current research has revealed that apparently healthy persons can show distinct changes at the biochemical, hematological, and oxidative levels regardless of any visible disease.[20] This evidence suggests that oxidative stress can be viewed not only as the result of existing diseases, but as an early and silent biological phenomenon [21].

Among the key findings in this research include the increased level of malondialdehyde (MDA) along with lower total antioxidant capacity (TAC) found in subjects with higher oxidative stress level [23]. This finding is suggestive of high levels of lipid peroxidation with corresponding decrease in the efficiency of the body's own antioxidants [24]. The fact that this occurs could mean that oxidative reactions occur even at a subclinical level prior to any manifestation of metabolic disease [25]. There have also been observations of such oxidative changes at an early stage of metabolic disequilibrium [26].

Hematologic results also contribute to support the proposed hypothesis. An increase in neutrophil/lymphocyte ratio (NLR) as well as an increase in the leukocyte count signify the activation of the low-grade inflammatory process.[27] Inflammation and its activation have been demonstrated by numerous studies to be associated with increased NLR levels in various types of cancers.[28] For this particular case, however, the increase in NLR in healthy subjects denotes that the processes of oxidative stress and inflammation are very much correlated from the early stages of physiological changes.[29] An increase in CRP level further supports the presence of the inflammatory process [30].

The parameters related to metabolic function indicated a common trend in the development of dysfunction as manifested by elevated fasting glucose, total cholesterol, triglycerides, and LDL, in addition to low HDL concentrations [31]. While these changes were not outside the normal range, they pointed towards early metabolic disruption that could lead to conditions such as insulin resistance or dyslipidemia if left unresolved [32]. The correlation between oxidative stress markers and metabolic parameters suggests the involvement of oxidative processes in developing metabolic dysfunction through influencing cellular signaling and insulin sensitivity among other mechanisms [33].

The correlations found between the levels of MDA, TAC, NLR, CRP, and glucose confirm the complex relationship existing between oxidative stress, inflammation, and metabolism [34]. In particular, the positive correlation between MDA levels and inflammatory indicators shows that the process of lipid peroxidation is not only an effect but also a cause of activation of the inflammatory process [35]. In contrast, the negative correlation between TAC levels and other factors demonstrates the role of antioxidants' exhaustion in the enhancement of oxidative and inflammatory processes [36].

Another significant finding is that of the connection between high BMI values and oxidative stress [37]. It is possible to explain this connection due to the active nature of adipose tissue as an endocrine organ capable of generating inflammatory substances such as cytokines and oxygen radicals [38]. Even slight increments in BMI values outside the obese spectrum can cause metabolic disorders [39].

In summary, the findings from this investigation indicate that there is a close relationship between oxidative stress, inflammation, and metabolism even at a stage prior to the onset of any clinical disease [40]. This finding is in line with the hypothesis that oxidative dysbalance could be an early predictor of subsequent cardiometabolic risk [41].

In terms of its clinical significance, the results suggest that oxidative stress indicators, including MDA and TAC, along with basic inflammation markers such as NLR and CRP [42], should be considered for early diagnosis. This could lead to important conclusions regarding physiological abnormalities and preventive action [43].

Despite its strengths, the current study has some limitations. It is difficult to make any causal inference because of the cross-sectional design used in the study. Additionally, due to the smaller sample size, the findings may not be applicable to all groups of patients.

5 Conclusion

Based on the findings of this study, it can be observed that even individuals who seem healthy could be experiencing imbalances in oxidative stress, specifically higher levels of MDA and lower levels of TAC, as well as some other slight changes that could be considered indications of inflammation and metabolism.

In summary, the evidence of oxidative stress seems to be associated with inflammation and other biochemical factors.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The study was carried out in compliance with ethical guidelines for animal use and the ethical and official approval was obtained from the local animal use and care committee.

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