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Increased Oxidative/Nitrosative Stress in Common Metabolic Diseases in Gaziantep Region

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Abstract

The balance between oxidative/nitrosative stress and antioxidant activity has an important role in oxidative stress associated diseases such as phenylketonuria (PKU) and maple syrup urine disease (MSUD). We aimed in this study to evaluate the possible association between oxidative/nitrosative balance and clinical features of PKU and MSUD patients. The study included 22 controls, 40 PKU and 18 MSUP patients. Serum malondialdehyde (MDA), peroxynitrite ($ONOO^-$), 8-OH deoxyguanosine (8-OHdG), oxidative DNA damage marker, levels and some amino acid in plasma of patients were measured. Serum MDA and $ONOO^-$ levels were significantly higher in PKU and MSUD patients compared to the control group. Plasma valine leucine, isoleucine and phenylalanine levels were significantly higher in MSUD patients than in the control and PKU groups. Similarly, plasma phenylalanine levels were significantly higher in PKU patients compared to control and MSUP group. There was no difference between 8-OHdG levels when all groups were compared. Consequently, the results of this study clearly show that oxidative / nitrosative stress increases in patients with PKU and MSUD. Antioxidant supplementation may be beneficial in the treatment of these patients.

Keywords: Oxidative stress, nitrosative stress, peroxynitrite, malondialdehyde, DNA damage, amino acid.

1.0 Introduction

The deterioration of normal metabolic processes because of energy and redox imbalance leads to the emergence of many pathological conditions commonly known as metabolic. Maple syrup urine disease (MSUD) and phenylketonuria (PKU) are autosomal recessive metabolic disorders caused by a lack of branched chain α-ketoacid dehydrogenase complex and phenylalanine hydroxylase activity, respectively (Stepien, et al., 2017).

Reactive oxygen species (ROS), reactive nitrogen species (RNS), under normal conditions, production is controlled in all living systems. When a decrease in the antioxidant defense system or an increase in the production of free radicals and / or disturbances in the mechanisms of their harmlessness causes stress. Increases in ROS and RNS production are called as oxidative and nitrosative stress, respectively (Taysi, Cikman, et al., 2008; Taysi, Tascan, Ugur, & Demir, 2019).

To neutralize oxidative damage caused by free radicals produced in the body, antioxidant defense systems are found in various organs and tissues, as well as in serum and erythrocytes (Akyuz, et al., 2017; Celik, et al., 2019). Given the antioxidant defense system in living cells, many therapeutic strategies have been used in oxidative stress-related diseases to neutralize ROS / RNS in the pathophysiology of the disease. Although successful in some patients, these adjuvant therapies have not yet been included in the clinical management of patients (Aksoy, et al., 2003; Stepien, et al., 2017) . To our knowledge, both oxidative and nitrosative stress in these metabolic diseases have not been studied at the same time. Therefore, we aimed to evaluate simultaneously oxidative/nitrosative stress and some amino acids associated with these diseases.

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2.0 Material and Methods

The study was carried out at the Departments of Pediatrics and Medical Biochemistry of Gaziantep University. Patients and healthy control subjects were recruited into the study after obtaining their informed consent. The study protocol conformed to the principles of the Helsinki Declaration, and the Medical Ethics Committee of Gaziantep University approved the study. The study included 22 controls, 40 PKU and 18 MSUP patients. The patiens and healthy control subjects were recruited into the study after obtaining their informed consent. Venous blood was collected in blood tubes with and without additive, and centrifuged at 3000xg for 5 min to separate serum and plasma. The serum and plasma aliquots were stored at - 80 °C until the analysis date.

2.1 Biochemical analysis

2.1.1 Determination of serum peroxynitrite ($ONOO^-$), malondialdehyde (MDA), 8-hydroxy deoxyguanosine (8-OHdG) and plasma amino acid levels

Serum $ONOO^-$, MDA levels were performed as previously described (Al-Nimer, Al-Ani, & Ali, 2012; Jain, McVie, Duett, & Herbst, 1989; Vanuffelen, Van Der Zee, De Koster, Vansteveninck, & Elferink, 1998), which express as nmol/ml and μmol/ml, respectively. Serum 8-OHdG measurement was made using the Elx 800 instrument (Bio Tek Instruments, Winooski, VT, USA) with Northwest kit (Northwest, NWLSS 8-OHdG ELISA High Sensitivity Kit, Vancouver, Canada), which express as ng/ml. Plasma valine, leucine, isoleucine, tyrosine and phenylalanine levels were measured by Amino Acid Analyzer (PMA GmbH, 2011, Germany). The results were expressed as μmol/L.

3.0 Statistical analyses

Kolmogorov Smirnov test was used to test for normal distribution. One-way variance analysis and Least significant difference (LSD) multiple comparison tests were used to compare the variables with normal distribution.

4.0 Results

Parameters measured in all groups are shown in Table 1, Figures 1-3. Serum MDA (Figure 1) and $ONOO^-$ (Figure 2) levels were significantly higher in PKU and MSUD patients compared to the control group. Plasma valine leucine, isoleucine and phenylalanine levels were significantly higher in MSUD patients than in the control and PKU groups. Similarly, plasma phenylalanine levels were significantly higher in PKU patients compared to control and MSUP group. There was no difference between 8-OHdG levels when all groups were compared (Figure 3).

Table 1. Mean ± SD values of all parameters measured in this study.

	Groups		
	PKU (n=40)	MSUD (n=18)	Control (n=22)
Valine (μmol/L)	274.45 ± 151.98[e]	430.30 ± 208.24[c]	201.44 ± 43.78
Isoleucine (μmol/L)	60.96 ± 34.86[e]	129.46 ± 106.87[c]	40.11 ± 13.16
Leucine (μmol/L)	111.90 ± 67.16[e]	297.52 ± 287.05[c]	65.27 ± 22.05
Tyrosine (μmol/L)	56.73 ± 36.89[a,d]	90.98 ± 105.40	53.92 ± 28.97
Phenylalanine (μmol/L)	483.49 ± 405.01[c,e]	70.29 ± 22.43	55.63 ± 25.37

a: $p< 0.05$, b: $p< 0.01$, c: $p< 0.001$ vs. Control group,
d: $p< 0.05$, e: $p< 0.001$ vs. MSUD group.

Figure 1: Mean ± SD of malondialdehyde levels measured from patient and control groups.

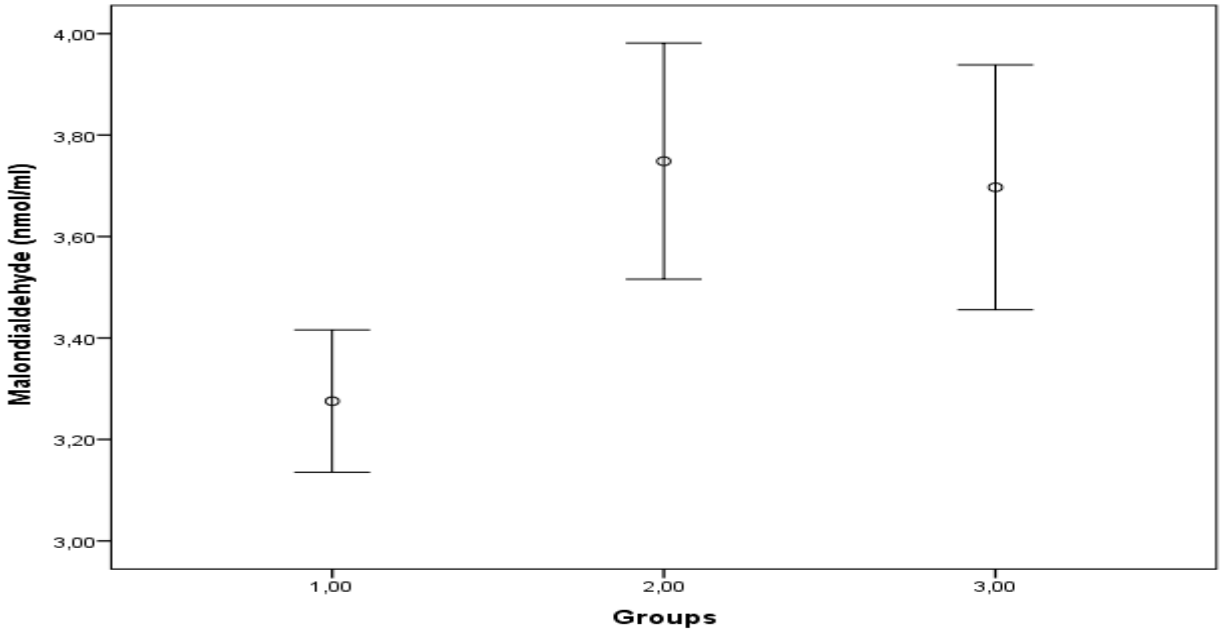


Figure 2: Mean ± SD of peroxynitrite levels measured from patient and control groups.

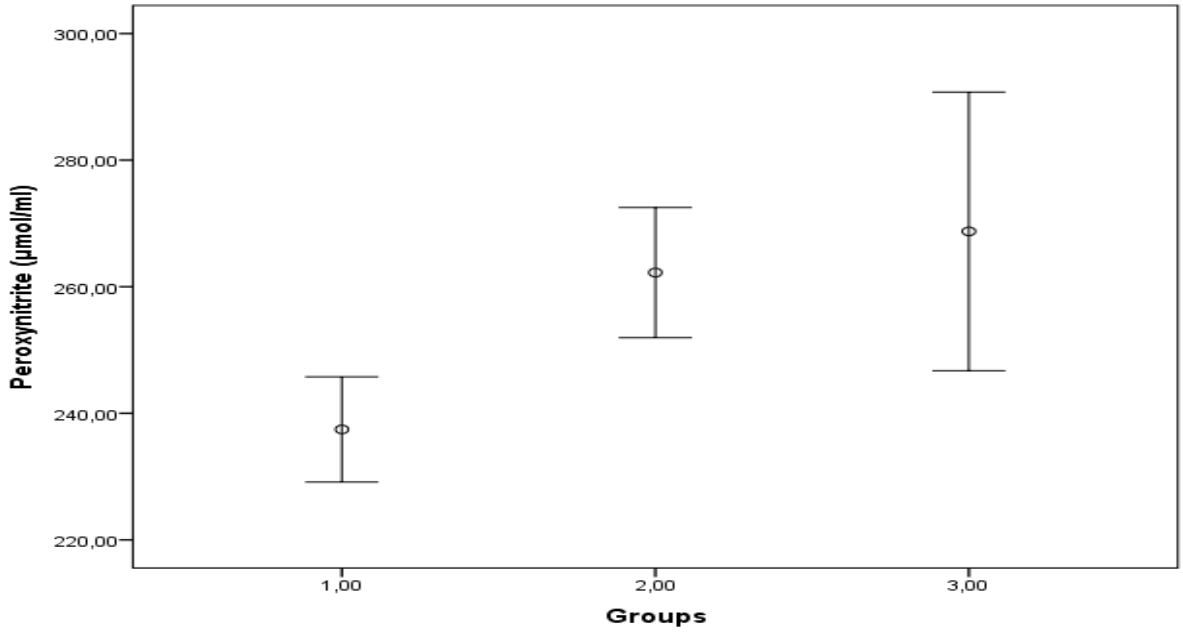
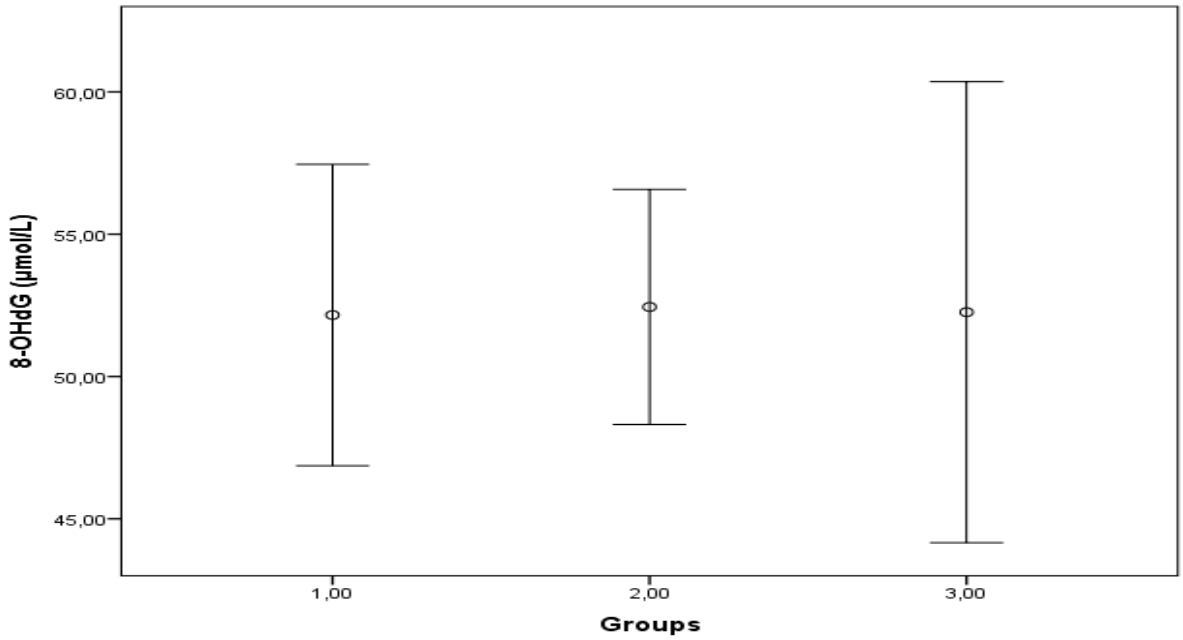


Figure 3: Mean ± SD of 8-OH deoxyguanosine levels measured from patient and control groups.



5.0 Discussion

The current study includes the parameters that determine DNA damage by oxidative, nitrosative stress, and PKU and MSUD. Plasma valine leucine, isoleucine and phenylalanine levels were significantly higher in MSUD patients compared to control and PKU groups. Likewise, plasma phenylalanine levels were significantly higher in patients with PKU compared to the control and MSUP group. Our results showed that MDA, which is an important marker of oxidative stress, and ONOO- levels, which are indicative of nitrosative stress, were significantly higher in patients with PKU and MSUD compared to the control group. This is consistent with the hypothesis that oxidative / nitrosative stress may increase in these diseases.

Severe mental retardation, microcephaly, developmental delay, epilepsy, behavioral changes, cerebral whiteness abnormalities and progressive supranuclear motor disorders are present in untreated PKU patients **(Pietz, 1998).** Although the pathophysiology underlying brain injury has not yet been fully described, oxidative / nitosative stress occupies an important place. The presence of oxidative stress in PKU appears to be already present at diagnosis and persists even in the presence of dietary compliance. Sistori et al. measured some parameters of oxidative stress in plasma of patients with PKU (Stepien, et al., 2017). They have reported that the increased oxidative stress markers and the decreased total antioxidant capacity in erythrocytes of these patients. These findings were consistent with our results of increased oxidative stress.

Maple syrup urine disease (MSUD) is a congenital metabolism error characterized by a defect in the tiamine-dependent enzyme branched-chain α-keto acid dehydrogenase. Affected patients show severe brain dysfunction, manifested as convulsions, coma, psychomotor delay, and mental retardation. It has been reported that oxidative stress contributes significantly to brain damage in neurodegenerative diseases, seizures, and demyelination, since the brain has relatively low levels of antioxidant defense, as well as high lipid content, particularly unsaturated fatty acids, and iron that stimulates the Fenton reaction being therefore highly susceptible to reactive species attack (Barschak, et al., 2006). Barschak et al. (Barschak, et al., 2008) have reported that TBARS and total antioxidant reactivity measurements was significantly increased in plasma from MSUD patients both with low and high Leu levels, as compared to the control group. These findings were consistent with the results of our study.

Many evidence suggests that oxidative / nitrosative stress is involved in many physiological functions, such as vascular tone regulation, oxygen sensing, and host defense mechanisms. However, it should be emphasized that the electrophilicity of free radicals is highly sensitive to reactions that may alter the functionality of molecules such as lipids, proteins, and DNA.

Therefore, the cellular concentration of free radicals needs to be tightly controlled to avoid such harmful effects. The balance between free radical-producing enzymes and detersive enzyme systems may be impaired in pathological conditions of various diseases (Stepien, et al., 2017; Taysi, et al., 2019) . ROS and RNS have been implicated in the pathogenesis of a number of pathologic conditions, such as systemic lupus erythematosus, diabetes mellitus, otitis media, rheumatoid arthritis, Behçet's disease(Aktan, Taysi, Gumustekin, Bakan, & Sutbeyaz, 2003; Memisogullari, Taysi, Bakan, & Capoglu, 2003; Taysi, Gul, Sari, Akcay, & Bakan, 2002; Taysi, Polat, Gul, Sari, & Bakan, 2002; Taysi, Sari, et al., 2008), hypertension, acute and chronic pancreatitis(Leung & Chan, 2009), and various metabolic diseases (Stepien, et al., 2017). Researchers have indicated that ROS/RNS formation processes are intimately linked to the development of the inflammatory disorders. The detrimental effects of highly reactive ROS/RNS are mediated by their direct actions on macromolecules such as lipids, proteins, and nucleic acids, and activation of proinflammatory signal cascades, which subsequently lead to activation of immune responses (Cikman, et al., 2015; Leung & Chan, 2009).

Although oxidative damage and activation of proinflammatory/apoptotic pathways appear to take place simultaneously during various metabolic and others diseases, the threshold ROS/RNS levels may differ. Activation of proinflammatory / apoptotic pathways may require only a small amount of ROS / RNS, which can be obtained in a limited cellular compartment. In contrast, oxidative damage to macromolecules probably requires extensive ROS/RNS production that suppresses defense mechanisms. The amount of ROS/RNS required to trigger stress-activated pathways is probably much lower than that required for oxidative damage.

Since oxidative stress occurs as a central player in chronic metabolic diseases such as diabetes, obesity, cancer and CVDs, it is necessity to investigate mechanisms that disrupt the normal balance between oxidative and antioxidative processes. Excessive ROS and RNS release, as discussed above, leads to the oxidation of all-important macromolecules of life, including lipid, proteins and nucleic acids. Damaged macro-biomolecules disrupt normal cellular physiology leading to diseases associated with metabolic disorders. DNA damage from persistent oxidative stress may not only lead to genomic instability, but can also activate transcription factors and induce the expression of proto-oncogenes (Rani, Deep, Singh, Palle, & Yadav, 2016; Stepien, et al., 2017).

As a results, to our knowledge this is the first report searching peroxynitrite, a important marker of nitrosative stress, in patients affected by MSUD and PKU. We found that oxidative/nitrosative stress was significantly increased in PKU and MSUP patients. Uncontrolled production of free radicals leads to oxidative / nitrosative damage and activation of reactive signal cascades (Cikman, et al., 2015; Leung & Chan, 2009) and therefore antioxidant treatment may be recommended as a potential treatment for disorders associated with overproduction of free radicals. Future research should focus on understanding disease mechanisms and identifying common goals to prevent or treat oxidative/nitrosative stress-induced pathologies in people with metabolic disorders.

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