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Rat PKU Model Display Gender-Based Neuroinflammatory Changes: Proinflamatuary Cytokines and Lipid Peroxidation

Sıçan PKU Modeli Cinsiyete Dayalı Nöroinflamatuar Değişiklikler Gösterir: Proinflamatuar Sitokinler ve Lipid Peroksidasyonu

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Öz

Fenilketonüri (PKU), amino asit metabolizmasının konjenital kusurlarından kaynaklanır. Birikmiş fenilalanin kan-beyin bariyerini geçer ve kalıcı beyin hasarına neden olur, ancak fenilketonürinin altında yatan nöro-patofizyoloji tam olarak anlaşılamamıştır. Prefrontal kortekste inflamatuar yanıtın, lipit peroksidasyonunun ve oksidatif stresin rolünü incelemek için her iki cinsiyete ait kimyasal olarak indüklenmiş sıçan Fenilketonüri modeli oluşturuldu. Sonuçlarımız Fenilketonüride kontrollere kıyasla lipit peroksidasyonunda artış olduğunu gösterdi; bu artış sadece erkeklerde anlamlı derecede farklıydı ($p<0.001$). Erkek sıçan PKU gruplarında serum triptofan ($p<0.001$) ve interlökin-1β düzeylerinde ($p=0.014$) erkek kontrollere göre anlamlı farklılıklar gözlendi. Bu çalışma ile cinsiyete ilk kez bir PKU modelinde cinsiyete bağlı nöroinflamasyon ve lipid peroksidasyonunda değişiklikler rapor edilmiştir.

Anahtar Kelimeler: Cinsiyete Temelli, Fenilketonüri, Nöroinflamasyon, Oksidatif Stres, Proinflamatuar Sitokinler

Abstract

Phenylketonuria (PKU) results from congenital defects of amino acid metabolism. Accumulated phenylalanine crosses the blood-brain barrier and causes permanent brain damage, but the neuro-pathophysiology underlying phenylketonuria is not fully understood. Chemically-induced rat phenylketonuria model of both genders was generated to examine the role of inflammatory response, lipid peroxidation and oxidative stress in the prefrontal cortex. Our results showed that in phenylketonuria there was an increase in lipid peroxidation compared to controls, which was significantly different only in males ($p<0.001$). In male rat PKU groups, statistically significant differences were also observed in serum tryptophan ($p<0.001$) and interleukin-1β levels ($p=0.014$) as compared to male controls. In this study, gender-based changes in neuroinflammation and lipid peroxidation were reported for the first time in a PKU model.

Keywords: Gender Based, Phenylketonuria, Neuro-inflammation, Oxidative Stress, Proinflammatory Cytokines

Introduction

Phenylketonuria (PKU, MIM 261600) is characterized by toxic levels of phenylalanine (Phe) accumulation due to the mutation of Phe hydroxylase and dihydropteridine reductase deficiency (PAH, EC 1.14.16.1) (1,2). In patients with PKU, phenylalanine that cannot be converted to tyrosine accumulates in blood and other tissues. Crossing the blood-brain barrier, the accumulated phenylalanine causes irreversible progressive brain damage, mental retardation, behavioral problems and neuro-inflammation (3). Many researches have been published to elucidate the mechanisms underlying brain damage caused by PKU; however, no single factor has been identified as being directly responsible for it.

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In recent years, many studies have reported alterations in oxidative stress parameters in many congenital disorders of mediator metabolism, including PKU, both in animal models and in patients (4,5). It has been suggested that biomolecules such as lipids, proteins and DNA are affected by oxidative damage (6,7). In this context, data from in vitro and in vivo studies have shown that accumulation of Phe and its metabolites leads to decreased antioxidant defenses and increased oxidative damage in the rat brain (8,9). All these findings on oxidative damage suggest that it might contribute to the neurological symptoms observed in PKU patients (10). Oxidative stress is commonly observed in some inborn errors of intermediary metabolism. Although the reason for this oxidative stress is not completely understood, it may be caused by the accumulation of toxic metabolites that lead to the excessive production of free radicals. It may also be that an unusual increase in metabolic by-products will directly, or indirectly, depletes a cell's antioxidant capacity (4).

Cytokines are signaling molecules released from peripheral immune cells (monocytes, macrophages and lymphocytes). They act as intercellular messengers and are associated with activation of the immune system, cell differentiation and cell death

(11). Cytokines can be divided into pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, which activate inflammation (12). Inflammatory cytokines are released from glial cells in the brain. Pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α mediate microglial activation. By increasing inflammation, it contributes to the inflammation-mediated progression of neurodegenerative diseases (13).

Monoamine oxidase (MAO) (14) catalyzes the oxidation of monoamines which inactivates monoamine neurotransmitters such as serotonin, dopamine, adrenaline, and noradrenaline. A number of psychiatric and neurological disorders are thought to be caused by abnormal monoamine oxidase expression. Many studies have also linked increased MAO activity to increased oxidative stress and inflammation as potential deleterious by-products of oxidative deamination, aldehydes, hydrogen peroxide, and ammonia are constantly produced (15). Hence, MAO enzyme has been implicated with neuro-inflammation in several studies. Furthermore, MAO activation promotes cognitive impairment (16), cholinergic neuron destruction, and cholinergic system abnormalities (17).

In this study, an animal model of chemically-induced PKU was generated and proinflammatory cytokines, oxidative stress related parameters, and lipid peroxidation in the prefrontal cortex of rat brain were analyzed. Since the prefrontal cortex (PFC) has been reported to contribute to cognitive deficits (18), this study focused on the PFC. Our results display that while PKU rats had higher lipid peroxidation than controls, the significance was more pronounced in males. All observed parameters displayed a difference between control and PKU groups with a bias to gender. These findings suggest that IL-1β-proinflammatory cytokine-, and, MDA-lipid peroxidation-, in phenylketonuria differ by gender.

Material and Method

Reagents

Unless otherwise stated in the text, all chemicals were purchased from Millipore Sigma (St. Louis, MO, USA). Both of the stock solutions containing 152 µmol/ml phenylalanine and 26 µmol/ml p-chloro-phenylalanine (p-Cl-Phe - a PAH inhibitor), were prepared in 0.9% sodium chloride solution (saline) by heating at 37 °C for 1 h on the day of the experiments and had its pH adjusted to 7.4.

Animals

20 female and 20 male Sprague-Dawley rat pups (6 days old, weight 5 ± 2 grams) were used. In total, the pups of three mothers were randomly divided into male and female groups before sacrification. Animals were maintained under 12 h light-dark-cycle (lights on at 7:00 AM), at constant temperature (22 ± 1 °C) and with free access to food and water. All experimental procedures were designed to minimize the number of animals used and their suffering. The procedures were approved by Hacettepe University Committee on Animal Ethics (Protocol No 2022/07-16).

Generation of rat PKU model

Rat PKU model was generated as previously described (19). A total of 20 female and 20 male Sprague-Dawley rat pups (6 days old, weight 5 ± 2 g) were used in the study. Briefly, animals were divided into two groups: control animals received subcutaneous administration of 0.9% sodium chloride solution (saline) daily. PKU animals received phenylalanine (5.2 mmol/g of body weight) injection daily while the phenylalanine hydroxylase inhibitor and tyrosine hydroxylase inhibitor (20), p-Cl-Phe (0.9 mmol/g of body weight) injection was administered every other day. For all groups, injections started on the postnatal 6th day and continued until the postnatal 20th day (19,21,22). Animals were euthanized by decapitation without anesthesia and the brain was rapidly excised on a Petri dish placed on ice. The prefrontal cortex was dissected and stored at -80°C until used for the experiments. All injections were performed between 7:00-9:00 AM for the duration of the experiment.

Prefrontal cortex and hippocampus homogenate preparation

The prefrontal cortex was removed for biochemical analysis. Prefrontal cortex was homogenized %10 (w/v) by Ultra-Turrax ® (S8N-5 g, IKA-Werke GmbH) on ice for 3 x 10 seconds in 50 mM Tris pH 7.4 buffer containing 2 mM EDTA, 0.5% Triton X-100, and protease inhibitor cocktail. All treatments were done on ice to prevent protein denaturation. The solutions used were also kept on ice. Homogenates were centrifuged for 15 minutes at 13,000 g at +4°C. The supernatant was removed and used in the determination of selected parameters. Total protein was measured by the method of Lowry et al. (23) using bovine serum albumin as a standard.

Serum tryptophan determination

Blood samples were collected in EDTA tubes in order to obtain serum by centrifugation at 10,000 g for 10 min at +4°C and then tryptophan level was measured by HPLC (Shimadzu DGU-20A3). All results were calculated as mg/dl.

Determination of Lipid Peroxidation

Malondialdehyde levels were used to determine lipid peroxidation using a Cayman Chemical TBARS assay kit (Catalog No. 10009055, Ann Arbor, MI, USA). In a summary, 100 µL of the supernatant were prepared by centrifuging the homogenate at 1600 g for 10 minutes at 4°C, then mixing it with 100 µL of sodium lauryl sulfate lysis solution in glass tubes. The mixture was boiled for 45 minutes at 95°C after being incubated with thiobarbituric acid (TBA). After cooling the tubes on ice for 5 minutes, they were centrifuged at 10,000 g for 15 minutes, collecting 200 µL of supernatant fluid and measuring absorbance at 532 nm with a Molecular Devices SpectraMax M2 microplate reader (San Jose, CA, USA). The results were calculated using standard graphics. MDA concentration was reported in µM/mg protein.

Monoamine Oxidase Activity

BioVision Total Monoamine oxidase activity kit (catalog number: K795-100) was used to measure total MAO activity. The test is based on the fluorometric detection of H_2O_2, one of the by-products produced during oxidative deamination of the MAO substrate. Total MAO activity was measured kinetically through fluorescence (Ex/Em = 535/587 nm) at 25°C on a SpectraMax M2 microplate reader (Molecular Devices, CA, USA) at 25°C. The results were determined by considering the amount of peroxide formed. All activity results were calculated as µU/mg protein.

Oxidative Stress Related Enzyme Activity

Glutathione Peroxidase Activity

Glutathione peroxidase (GPx) activity was measured using the method set forth by Flohe and Günzler (24). The final activity medium contained 100 mM potassium phosphate buffer, 0.2 mM NADPH, 1 mM reduced glutathione (GSH), 1 mM EDTA, 4 mM sodium azide, 100 U/ml glutathione reductase enzyme, 0.1 mM hydrogen peroxide. Enzyme activity was determined by monitoring the decrease in absorbance at 340 nm for 10 minutes on the SpectraMax M2 microplate reader. The GPx activity unit was defined as the amount of enzyme that catalyzed the oxidation of 1 µmol of NADPH in 1 minute under these conditions, and the results of the GPx activity were given as µU/mg protein.

Glutathione Reductase Activity

Glutathione reductase (GR) activity was determined according to the Stall method (25) with a slight modification. The activity medium contained 100 mM Sodium phosphate buffer pH = 7.4, 0.2 mM NADPH, 1 mM oxidized glutathione (GSSG) and sample in the final. The results were immediately read kinetically for 10 minutes at a wavelength of 340 nm on a Spectramax M2 microplate reader from Molecular Devices. The results were calculated on the absorbance decrease of NADPH at 340 nm. A unit of activity (U) was defined as the amount of enzyme that catalyzes the oxidation of 1 µmol of NADPH in 1 min under these conditions. GR activity was given as µU/mg protein.

Superoxide dismutase Activity

Superoxide dismutase (SOD) activity was measured using the BioVision SOD activity kit (Catalog no: K335-100). The principle of method is based on WST-1, which forms a water-soluble formazan dye when reduced with the superoxide anion, is used in the sensitive SOD assay kit. The reduction rate with a superoxide anion is proportional to the activity of Xanthine Oxidase (XO) suppressed by SOD. All results were given as µU/mg protein.

Determination of proinflammatory cytokines: IL-1β, IL-6, TNF-α

Bioassay Technology Laboratory IL-1β Elisa kit (catalog number: E0119Ra), Bioassay Technology Laboratory TNF-α ELISA kit (catalog number: E0764Ra) and Bioassay Technology Laboratory IL-6 ELISA kit (catalog number: E0135Ra) were used to measure IL-1β, TNF-α and IL-6, respectively. 50 µl of standard and 40 µl of sample was added to the antibody-coated wells. 10 µL of biotinylated TNF-α, IL-1β and IL-6 was added to the sample wells. Then, 50 µl of streptavidin-HRP was added to all wells and incubated at 37°C for 1 hour. Following incubation, the wells were washed four times with 200 µL of wash buffer solution. After removal of the liquid, 100 µL of chromogenic substrate was added to each well and incubated for 10 minutes at 37°C in the dark. 50 µL of stop solution was added per well to prevent color formation reaction and the results were quickly measured at 450 nm with a Molecular Devices SpectraMax M2 microplate reader. The results were obtained from the standard chart and given as pg/mg protein.

Statistical analysis

Since the Shapiro-Wilk test is a more appropriate method for small sample sizes (<50 samples), the Shapiro-Wilk test was used to test whether the samples showed normal distribution (26). Data with normal distribution were analyzed by the Student's *t*-test for unpaired samples and expressed as mean ± standard deviation (SD) (n=7). Values of $p<0.05$ were considered significant. All analyses were performed in GraphPad Prism Software Version 9.0 (San Diego, CA, USA).

Results

To assess the involvement of neuro-inflammation and oxidative stress in PKU, a chemically-induced PKU model in male and female rats was established as performed previously (22)

and the selected parameters were analyzed in PFC homogenates. Neurotransmitter systems were assessed through monoaminergic neurotransmitter activities, while the lipid peroxidation products, anti-oxidant enzyme activities, and cytokines were analyzed to evaluate oxidative stress response and neuro-inflammation.

Neuro-inflammation-related cytokine levels

To evaluate neuro-inflammation, IL-1β, IL-6, and TNF-α from the prefrontal cortex were analyzed by ELISA. There was no significant difference in, IL-6 and TNF-α levels in neither group. The cytokine levels displayed a steady state. Briefly, median of IL-1β was found as 112.30 pg/mg vs 117.9 pg/mg in control males and PKU males, respectively (Fig 1B, Control male: 112.30±4.19, PKU male: 117.9±4.18 (p=0.014). IL-6 levels in control females were 1.54±0.35 pg/mg, and 1.41±0.29 pg/mg in PKU females (p=0.962) (Table 1).

Serum Trp levels

The serum Trp levels stayed at a constant level in female rat groups. Meanwhile, in PKU male rats statistically significant difference (p<0.001; Fig. 2C). In serum tryptophan levels were observed. Median of Trp in PKU male group was found 1.88 mg/dl while median of Trp in control male group was 2.56 mg/dl. These results display that Trp level differ with bias to gender in PKU (Table 1, Control male: 2.56±0.49 PKU male: 1.88±0.37).

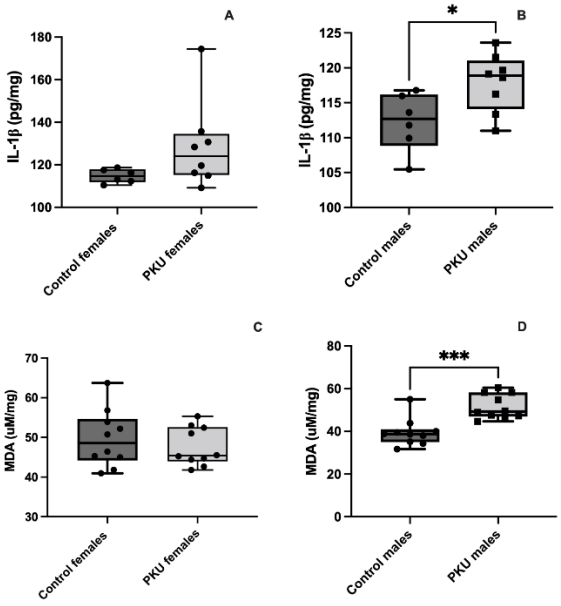

Figure 1. IL-1β (pg/mg) and MDA (uM/mg) levels in the PFC of phenylketonuria rat pups. P-Cl-Phe injection started on the 6th postnatal day and continued until the 20th postnatal day. **A)** IL-1β (pg/mg); Control Female: 114.7±3.20; PKU female: 128.7±20.50 **B)** IL-1β (pg/mg); Control male: 112.30±4.19. PKU male: 117.90±4.18 **C)** MDA (uM/mg); Control Female: 49.65±7.17; PKU female: 47.61±4.85 **D)** MDA (uM/mg); Control male: 39.51±6.42. PKU male: 51.56±5.82. Statistical significance was determined by One-tailed unpaired t test. The data were expressed as the as mean±standard deviation. *p≤0.05. **p≤0.01. ***p<0.001.

Table 1. Descriptive Statistics Table

	PKU		Control		p*
	Mean	Std. Deviation	Mean	Std. Deviation	
IL-1 β female	128.70	20.50	114.70	3.20	0.064
IL-1 β male	117.90	4.18	112.30	4.19	0.014
IL-6 female	1.41	0.29	1.54	0.35	0.962
IL-6 male	1.46	0.29	1.53	0.38	0.982
TNF-α female	27.21	14.97	22.81	7.39	0.234
TNF-α male	22.79	11.94	18.98	3.58	0.216
SOD female	94.03	0.94	96.86	1.52	<0.001
SOD male	93.80	5.93	95.40	2.46	0.248
MDA female	47.61	4.85	49.65	7.17	0.232
MDA male	51.56	5.82	39.51	6.42	<0.001
GR female	24.05	20.96	37.86	22.03	0.191
GR male	18.94	15.30	20.96	9.19	0.900
GPx female	24.51	21.86	17.43	6.16	0.169
GPx male	13.95	9.91	11.54	6.34	0.270
MAO female	3.02	2.81	10.97	13.61	0.077
MAO male	3.63	3.98	2.29	2.90	0.269
Trp female	2.18	0.40	2.15	0.63	0.456
Trp male	1.88	0.37	2.56	0.40	<0.001

*One-tailed unpaired t test result. The mean difference is significant at the 0.05.

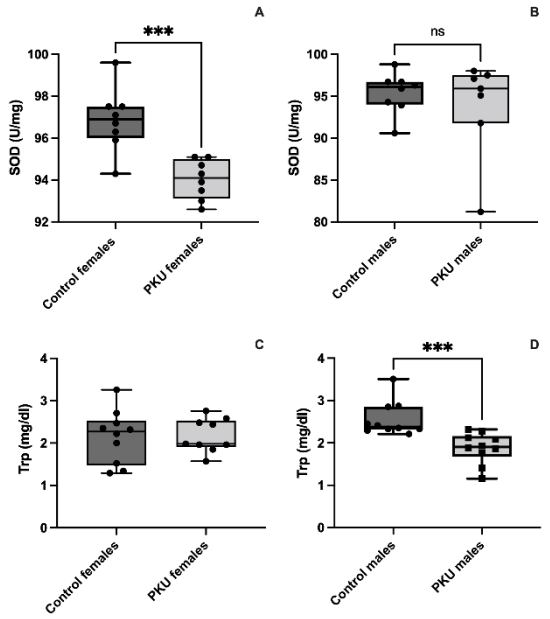

Figure 2. SOD (U/mg) and Trp (mg/dl). in the PFC of phenylketonuria rat pups. P-Cl-Phe injection started on the 6th postnatal day and continued until the 20th postnatal day. **A)** SOD (U/mg); Control Female: 96.86±1.52; PKU female: 94.03±0.94 **B)** SOD (U/mg); Control male: 95.4±2.46. PKU male: 93.80±5.93 **C)** Trp (mg/dl); Control Female: 2.15±0.63; PKU female: 2.18±0.40 **D)** Trp (mg/dl); Control male: 2.56±0.40. PKU male: 1.88±0.37. Statistical significance was determined by One-tailed unpaired t test. The data were expressed as the as mean±standard deviation. *p≤0.05. **p≤0.01. ***p <0.001.

Measurements for oxidative stress and lipid peroxidation

Superoxide dismutase (SOD) enzyme activity reduced in female PKU rats compared to female rats in control group (p<0.001; Fig 2A; 96.86±1.52 µU/mg protein vs 94.03±0.94 µU/mg protein in control and PKU groups, respectively). SOD activity did not display significant difference in male PKU rats compared to control group male rats (Table 1). The lipid peroxidation in the PFC revealed differences between genders in this rat PKU model. In the PFC mean of MDA levels in female groups, 49.65±7.17 µM/mg vs 47.61±4.85 µM/mg MDA in control and PKU females respectively, it was not statistically significant. However, in PKU male groups (51.56±5.81 µM/mg), MDA levels were significantly increased compare to their control (39.51±6.42 µM/mg) (p<0.001) (Figure 1D). These results showed that in this PKU model, males were more susceptible to the effects of lipid peroxidation as opposed to females.

A large variance in individual MAO activities it was not reflected in statistical results (Table 1). Mean of PKU female group was 10.97 µU/mg while mean of control female group was found as 3.92 µU/mg (Control Female: 10.97±13.61, PKU female: 3.02±2.81. Mean of PKU male group was 3.63 µU/mg while mean of control male group was 2.29 µU/mg (Control male: 2.94±2.90, PKU male: 3.63±3.98).

Oxidative stress was assessed through several parameters. The actual damage caused was measured through TBARs assay, which measured the major lipid oxidation product malondialdehyde, is a good index of the level of oxidative stress whereas the response was evaluated through the antioxidant enzymes glutathione peroxidase (GPx), and Glutathione reductase (GR) (Table 1).

Glutathione peroxidase (GPx) activity was not found significant as opposed to control group (Table 1). In males, mean of PKU group GPx activity was 13.95 µU/mg protein in their controls versus 11.54 µU/mg PKU group (Control male: 11.54±6.34, PKU male: 13.95±9.91). In female mean of PKU group GPx activity was 24.51 µU/mg protein while mean of their control was 17.43 µU/mg protein in PKU (Control Female: 17.43±6.16; PKU female: 24.51±21.86. The activity of GR, the anti-oxidant enzyme, not changed according to groups (Table 1).

Post hoc power analysis was performed according to the 5% type 1 error (p<0.05) threshold based on the IL-1β values of the male PKU and male control groups. In G*power 3.1.9.6 software, two independent means comparison was selected for t tests and by entering the mean values of the groups (male pku vs male control), the effect size was found to be 1.33. With this data and the number of cases per group, the power (1-beta) was found to be 0.75 (75%). Likewise, post hoc power analysis was performed according to the 5% type 1 error (p<0.05) threshold, based on the MDA values of the male PKU and male control groups. In G*power 3.1.9.6 software, two independent means comparison was selected for t tests and by entering the mean values of the groups (male pku vs male control), the effect size was found to be 1.96. With this data and the number of cases per group, the power (1-beta) was found to be 0.99 (99%).

Discussion

MDA is a marker of lipid peroxidation associated with increases in patients with traumatic and non-traumatic brain injury and PKU. However, molecular studies identifying individual lipids and their oxidatively altered molecules are needed to understand how these relate to the development of chronic complications in PKU. Studies have reported that PKU causes an increase in oxidative stress, lipid peroxidation and inflammation (27). In our study, while there was no change in the PKU female group, lipid peroxidation increased along with inflammation in the PKU male group. Our study is the first to reveal the relationship between inflammation and lipid peroxidation in PKU on a

gender basis. In a study comparing the MDA levels of male PKU patients and the male control group, it was reported that the MDA level was statistically higher in the PKU group (28). In our study, lipid peroxidation in the PFC revealed gender differences in the rat PKU model. Oxidative stress has been identified as an important pathophysiological feature of various inborn errors of metabolism, including phenylketonuria. In the reported study, proinflammatory cytokines IL-1β and IL-6 were significantly increased in PKU. This indicates that inflammation has occurred and provides evidence that it has occurred (29). These results are consistent with our results.

Currently, the relationship between MAO and inflammation has not been fully explained. For decades, researchers have systematically supported the role of MAO-related oxidative stress in various metabolic pathologies and cardiovascular diseases. The induced inflammatory load has been associated with the contribution of the MAO enzyme to hypertension, metabolic disorders, chronic kidney disease, and vascular oxidative stress (30). It was recently reported that mitochondrial MAO enzymes contribute to inflammation associated with endothelial dysfunction in mice (31). Type A and B monoamine oxidases (MAO-A, MAO-B) mediate and modulate intracellular signaling pathways for neuronal cell survival or death. Although the effect of MAO activity on BDNF expression has not yet been reported, the use of MAO-B inhibitors has been reported to increase BDNF expression (32). In our study, we predicted that MAO activity would increase in the PKU model, but MAO activity did not change. MAO enzymes have been linked to neuro-inflammation and oxidative stress as a result of their activity which generates hydrogen peroxide and ammonia as byproducts. It has been reported that newborns have low MAO-B activity and act as a modifying gene in phenylketonuria (33). Discussion has been limited because measurement of MAO activity in the PKU model has not been previously reported. In our study, no difference between control and PKU groups in total MAO activity is found. A recent paper reported that tryptophan levels decreased in PKU patients compared to the control group (34) but the authors did not define the findings with regards to gender. In comparison, we reported a decrease in tryptophan level only in the male PKU group as compared to the male controls. There was no change in tryptophan level between PKU and control groups in the females. According to the results, SOD enzyme activity did not make a significant difference between the groups.

Our findings did not show a significant difference in PFC GPx activity when the control and PKU rat groups were compared according to gender. There is already a debate about whether GPx is impaired in PKU. GR activity in the PFC was not change PKU groups of both genders as compared to their controls. In the literature, it has been shown in previous study that GR activity decreased in the PKU group (35). However, in this study, the results were not published according to gender.

Superoxide free radicals are converted by SOD into hydrogen peroxide, a less reactive molecule. In the hippocampus, it has been reported that the PKU group showed lower SOD activity than the control group (36). Although no change was observed in the male PKU group. TNF-α level was unchanged in both groups. When the literature was searched, it was determined that neither brain nor serum levels of TNF-α were measured in PKU. There is no scientific study related to IL -1β and PKU. However, we report for the first time statistically increased IL-1β in the male PKU group compared to control males. In literature blood IL-6 levels in PKU state no difference between controls and PKU. However, there was no statistically significant difference between the blood levels of PKU and IL-6 (37). According to our results, no change was observed in IL-6 levels.

There is substantial evidence that the dorsolateral prefrontal cortex serves critical cognitive abilities even in early infancy. It has been shown that in adult monkeys these cognitive abilities are critically dependent on dopaminergic projection to the prefrontal cortex, and there is a change in the distribution of dopamine axons in the dorsolateral prefrontal cortex (38). In a four-year longitudinal study, it was shown that these deficits are in working memory and inhibitory control functions related to the dorsolateral prefrontal cortex in children with PKU whose plasma Phe levels are 3-5 times normal. In another study involving PKU and prefrontal cortex reports that the behavioral disorder in PKU is caused by changes in the dopamine system in the frontal cortex (39). Although the relationship between the prefrontal cortex and cognitive competencies was revealed in the PKU animal model created, the relationship between oxidative stress and prefrontal cortex in PKU was not revealed (40). Considering that the prefrontal cortex has an effect on cognitive functions, it can be thought that neuro-inflammation in the prefrontal cortex also has an effect on the pathophysiology of PKU.

It is known that cognitive abilities are weakened in phenylketonuria. There is substantial evidence that the prefrontal cortex serves critical cognitive abilities even in early infancy. In our study, high dose of Phe on the prefrontal increased lipid peroxidation and IL-1β level in male gender. Significant gender differences were found in phenylketonuria. Adjuvant agents/treatments affecting the prognosis of the disease on a gender basis can be considered. The increase in Phe in the brain increase the possible inflammation, but in the results, we found, it is clear that inflammation markers differ according to gender.

Conclusion

Lipid peroxidation parameters, proinflammatory cytokines and, oxidative stress parameters were analyzed in the induced PKU rat model. In the study conducted with male and female groups, the male PKU group was found to have more abnormalities than the female PKU group.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

Ethics Committee Approval: The procedures were approved by Hacettepe University Committee on Animal Ethics (Protocol No 2022/07-16).

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