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Metabolic Infrastructure of Pregnant Women with Fetuses Having Nervous System Abnormalities; Metabolomic Analysis

Tuba Reçber [1,a], Emirhan Nemutlu [1,b,*], Emine Aydın [2,c], Murat Cagan [2,d], Hanife Guler Donmez [3,e], Sedef Kır [1,f], Mehmet Sinan Beksac [2,g]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, Hacettepe University, Ankara, Türkiye,
[2]Division of Perinatology, Department of Obstetrics and Gynecology, Faculty of Medicine, Hacettepe University, Ankara, Türkiye,
[3]Department of Biology, Faculty of Science, Hacettepe University, Ankara, Türkiye
*Corresponding author

Research Article

ABSTRACT

Central nervous system diseases are neurological disorders that affect the structure or function of the brain and spinal cord that make up the central nervous system. In this study, it was aimed to examine the impaired/altered metabolomic profiles of pregnant women carrying fetuses with nervous system abnormalities (NSA). The study group consisted of 30 normal pregnancies with normal fetuses (control group) and 8 pregnancies with fetuses having NSA (study group), as determined by prenatal screening and diagnosis as part of an antenatal care program. Metabolomic analyses were carried out using gas chromatography-mass spectrometry (GC-MS). GC-MS-based metabolomics analysis was able to identify 95 metabolites and 27 of them were statistically significant between the two groups (p<0.05). Moreover, the pathway analysis, performed with significantly altered metabolites, showed alteration in the alanine, aspartate, and glutamate metabolism, citrate cycle, aminoacyl t-RNA biosynthesis, and glutathione metabolism. Alanine, aspartate and glutamate metabolism, citrate cycle, aminoacyl t-RNA biosynthesis, and glutathione metabolism seem to be critical in the prenatal screening of NSAs. However, abnormality-specific studies are necessary for further recommendations.

Keywords: Pregnancy, Congenital nervous system abnormalities, Metabolomics, Prenatal screening, Prenatal diagnosis.

a 📧 tuba.recber@hacettepe.edu.tr   ID https://orcid.org/0000-0001-8257-7628
b 📧 enemutlu@hacettepe.edu.tr   ID https://orcid.org/0000-0002-7337-6215
c 📧 eminebaskurtaydin@gmail.com   ID https://orcid.org/0000-0001-8877-2803
d 📧 drmuratcagan@gmail.com   ID https://orcid.org/0000-0003-0629-4401
e 📧 hanifetanir@hacettepe.edu.tr   ID https://orcid.org/0000-0002-7413-4939
f 📧 sekir@hacettepe.edu.tr   ID https://orcid.org/0000-0003-1322-1665
g 📧 beksac@hacettepe.edu.tr   ID https://orcid.org/0000-0001-6362-787X

Introduction

Nervous system development involves 3 germinal layers and ectoderm is the key initiating player in the embryogenesis of the central nervous system (CNS) [1, 2]. Embryogenesis of CNS can be complicated and may result in mild, moderate or severe congenital defects [2, 3]. There is a wide spectrum of fetal nervous system abnormalities (NSA) such as anencephaly, craniorachischisis, iniencephaly, encephalocele, spina bifida, microcephaly, hydrocephaly etc., and these anomalies need to be prenatally diagnosed as early as possible [2-5]. Genetical, epigenetic and teratological factors are the main courses behind NSA and need to be investigated within the framework of antenatal care programs [6-12]. This necessitates the evaluation of maternal metabolism as well as other screening tests [6-15].

Developing better non-invasive tests is a necessity in perinatal medicine and the application of metabolomics could be extremely useful in the prenatal screening/diagnosis of congenital NSA [15-17]. Metabolomics is the identification and quantification of small-molecule metabolites (molecular weight <1000 Da) in tissues, cells and physiological fluids within a certain period of time [13-15]. Metabolites are intermediate products of various types of biochemical reactions that participate in bonding metabolic pathways [13, 15-17].

Metabolomics measures and analyses the products of cellular biochemistry and might be useful in the prenatal detection of NSA abnormalities. In this study, we aimed to configure impaired/altered metabolomic profiles of pregnant women carrying fetuses with NSA.

Materials and Methods

This study was consisted of 30 normal pregnancies with normal fetuses (control group) and 8 pregnancies with fetuses having NSAs with or without co-abnormalities (study group), as determined by second trimester ultrasonographic examination (14-22nd gestational weeks) as a part of antenatal care program. Antecubital venous bloods were withdrawn from the patients during the course of the examinations. All patients were delivered (or termination of pregnancy) at Hacettepe University. Hacettepe University Perinatology Registry was used for necessary clinical data collection. The study protocol was approved by the Ethical

Committee of Hacettepe University (GO 13/173), and informed consent was obtained from all patients.

Metabolomics Analysis

Metabolomic analyses were carried out using gas chromatography-mass spectrometry (GC-MS). A 100 µL of plasma was spiked with 900 µL methanol-water (8:1, v/v) mixture containing 5 µg/mL of internal standard (IS) myristic-d27 acid at ambient temperature. It was vortexed for 30 sec and centrifuged at 15000 rpm for 10 min. Afterward, 400 µL of supernatant was evaporated to dryness. The residues were methoxyaminated using methoxyamine hydrochloride (20 mg/mL in pyridine) and derivatized with MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide with 1% TMCS (trimethylchlorosilane). After derivatization, the samples were transferred into GC–MS vials and analysed using GC–MS (Shimadzu GCMS-QP2010 Ultra) with a DB-5MS stationary phase column (30 m + 10 m DuraGuard × 0.25 mm i.d. and 0.25-µm film thickness). The mass range was set from 50 to 650 Daltons. The run time was 37.5 min.

Data Analysis

Data deconvolution, peak alignment, normalization, and data matrix creation were carried out using MS-DIAL (ver. 4.0) software. Metabolite identification for GC-MS was done using a commercially available retention index library (Fiehn Retention Index Library). For metabolite identification, retention time tolerance was determined as 0.05 minutes, m/z tolerance 0.5 Da, EI similarity cut off 70% and identification score cut off 70%. The data matrix obtained from MS-DIAL was normalized using the internal standard and then transferred to an Excel work file. Any metabolite traits having more than 50% of the values missing were excluded from the data matrix. Missing values in the data matrix were filled with the half value of the smallest concentration in the metabolite group. The final data matrix was imported into the SIMCA-P+ (v13.0, Umetrics, Sweden) and Metaboanalyst software for multivariate analyses. Within the scope of multivariate analyzes, partial least squares differentiation analysis (PLS-DA), variable importance in projection (VIP) analysis, coefficient plots, heatmap analysis, and pathway analysis were performed.

Results

Study groups showed no statistically significant difference in terms of demographic findings ($p>0.05$, for all). Table 1 shows Fetal NSAs and co-abnormalities (if any) of the study group cases.

A GC–MS-based metabolomic profiling study has been performed in pregnant women with NSA (n=8) and the control group (n=30). As a result of these analyses, 95 of 277 metabolites (182 unknown) were identified (Table 2). Twenty-seven of the identified metabolites were statistically significant between the two groups ($p<0.05$) (Table 2). Among these metabolites, 3-aminopropionitrile, linoleic acid, cysteine, tartaric acid, porphine, oxalic acid, ornithine, 5-hydroxy-L-tryptophan, pyruvic acid, glucose-6-phosphoric acid, phosphoric acid, 3-aminoisobutyric acid, citric acid, alpha-ketoglutaric acid, glycolic acid, tyrosine, pyrophosphate, glycine and lactic acid plasma levels were statistically significantly lower in second-trimester pregnancies having fetuses with NSAs compared to control group pregnancies. On the other hand, palatinose, tryptophan, sorbitol, epsilon-caprolactam, lauric acid, lactobionic acid, glucosaminic acid and creatinine plasma levels were found to be higher in study group pregnancies compared to controls.

The multivariate statistical analysis of GC–MS metabolomics results was performed using PLS-DA and heatmap and pathway analysis methods (Figure 1 and 2). The statistical goodness and robustness of the models were evaluated using R^2 and Q^2 values, respectively. The pathway analysis, performed with significantly altered metabolites, showed alteration in the alanine, aspartate and glutamate metabolism, citrate cycle, aminoacyl t-RNA biosynthesis and glutathione metabolism (Figure 3).

Table 1. Fetal nervous system abnormalities and co-abnormalities (if any) of the study group cases

Case no	Neurological defects	Co-abnormalities
NSA-1	Occipital encephalocele	None
NSA-2	Anencephaly	Fetal megacystis
NSA-3	Spina bifida	None
NSA-4	Cerebellar vermian hypoplasia Lateral ventricle dilatation	Increased renal echogenicity Single umbilical artery Bilateral cleft lip and cleft palate Aortic stenosis
NSA-5	Bilateral ventriculomegaly Spina bifida	Hyperechogenic fetal bowel Oligohydramnios
NSA-6	Lobar holoprosencephaly Microcephaly	Cleft lip and cleft palate
NSA-7	Mega cisterna magna	Cleft lip and cleft palate Bilateral clench hand Aortic coarctation
NSA-8	Cerebellar hypoplasia	Hydrops fetalis

Table 2. Metabolites and relative amounts identified by GC-MS analysis

Metabolite name	NSA			Control			p value
	Mean±SE			Mean±SE			
2-butyne-1,4-diol	0.945	±	0.124	0.951	±	0.152	0.983
2-hydroxybutyric acid	1.149	±	0.149	0.942	±	0.060	0.155
2-ketoisocaproic acid	1.006	±	0.097	1.028	±	0.068	0.879
2-keto-L-gulonic acid	0.706	±	0.125	1.199	±	0.193	0.214
3-aminoisobutyric acid	0.924	±	0.064	1.121	±	0.028	0.005
3-aminopropionitrile	0.678	±	0.074	1.237	±	0.042	<0.000
3-indoleacetic acid	0.882	±	0.209	1.156	±	0.174	0.456
3-methyl-2-oxobutanoic acid	0.920	±	0.082	1.093	±	0.060	0.182
3-Phosphoglyceric acid	1.230	±	0.197	0.971	±	0.116	0.315
5-hydroxy-L-tryptophan	0.564	±	0.092	1.230	±	0.084	0.001
Alanine	1.014	±	0.060	1.030	±	0.041	0.851
Alpha ketoglutaric acid	0.580	±	0.055	1.093	±	0.062	<0.000
Asparagine	0.937	±	0.070	1.014	±	0.074	0.615
Aspartic acid	1.072	±	0.131	0.935	±	0.085	0.460
Benzoic acid	1.000	±	0.039	1.043	±	0.036	0.562
Biuret	0.799	±	0.106	0.727	±	0.104	0.739
Capric acid	0.777	±	0.108	0.837	±	0.114	0.800
Cholesterol	0.899	±	0.075	1.084	±	0.045	0.069
Citric acid	0.822	±	0.063	1.117	±	0.058	0.018
Creatinine	1.569	±	0.163	0.798	±	0.067	<0.000
Cycloleucine	0.958	±	0.212	1.041	±	0.091	0.697
Cysteine	0.831	±	0.076	1.160	±	0.042	0.001
Epsilon-caprolactam	1.416	±	0.155	0.667	±	0.072	<0.000
Fructose	1.732	±	0.978	0.831	±	0.086	0.108
Fucose	0.879	±	0.111	1.118	±	0.076	0.149
Glucoheptonic acid	0.988	±	0.103	1.020	±	0.046	0.768
Gluconic acid	0.792	±	0.351	1.238	±	0.389	0.578
Gluconic acid lactone	0.990	±	0.177	1.065	±	0.085	0.699
Glucosaminic acid	1.264	±	0.144	0.981	±	0.058	0.047
Glucose	0.946	±	0.090	1.075	±	0.065	0.358
Glucose-6-phosphoric acid	0.720	±	0.068	1.152	±	0.059	0.001
Glucuronic acid	1.060	±	0.099	1.054	±	0.153	0.985
Glutamic acid	0.956	±	0.105	0.872	±	0.082	0.630
Glutamine	1.237	±	0.118	0.969	±	0.103	0.220
Glyceric acid	0.924	±	0.074	1.026	±	0.068	0.476
Glycerol	0.967	±	0.051	1.075	±	0.036	0.172
Glycine	0.862	±	0.100	1.144	±	0.061	0.040
Glycolic acid	0.900	±	0.068	1.082	±	0.031	0.016
Heptadecanoic acid	1.055	±	0.097	0.987	±	0.034	0.425
Iminodiacetic acid	1.020	±	0.162	0.985	±	0.089	0.859
Indole-3-acetamide	0.793	±	0.291	1.209	±	0.178	0.289
Isoleucine	0.915	±	0.077	1.040	±	0.069	0.383
Isopropyl beta-D-1-thiogalactopyranoside	1.101	±	0.181	1.067	±	0.129	0.900
Lactamide	0.875	±	0.266	0.886	±	0.098	0.965
Lactic acid	0.714	±	0.089	1.172	±	0.110	0.049
Lactobionic acid	1.213	±	0.152	0.962	±	0.036	0.026
Lauric acid	1.276	±	0.126	1.019	±	0.040	0.018
Linoleic acid	0.804	±	0.106	1.098	±	0.059	0.030
Lysine	1.094	±	0.123	1.026	±	0.058	0.613
Malic acid	0.980	±	0.051	1.052	±	0.048	0.470
Malonic acid	0.875	±	0.266	0.886	±	0.098	0.965
Methionine	0.943	±	0.065	1.053	±	0.071	0.449
Methyl linolenate	0.632	±	0.117	1.399	±	0.309	0.223
Methyl Palmitate	0.830	±	0.177	1.319	±	0.254	0.347
Methyl Stearate	0.865	±	0.232	1.264	±	0.249	0.439
Mucic acid	1.017	±	0.120	1.065	±	0.063	0.732
Myristic acid	0.866	±	0.134	0.985	±	0.061	0.406
N-(2-hydroxyethyl)iminodiacetic acid	0.852	±	0.092	1.131	±	0.145	0.348
N-acetyl-D-glucosamine	1.107	±	0.097	1.068	±	0.062	0.772
N-carbamyl-L-glutamic acid	0.810	±	0.179	1.086	±	0.097	0.209
N-ethylglycine	1.090	±	0.079	0.963	±	0.063	0.342
Norvaline	0.964	±	0.065	1.036	±	0.059	0.566
Oleic acid	0.758	±	0.153	1.063	±	0.088	0.122

O-phosphocolamine	0.960	±	0.123	1.095	±	0.083	0.450
Ornithine	0.762	±	0.051	1.165	±	0.092	0.036
Oxalic acid	0.479	±	0.076	1.322	±	0.074	<0.000
Palatinose	2.388	±	1.058	0.583	±	0.069	0.004
Palmitic acid	0.990	±	0.083	1.026	±	0.044	0.716
Palmitoleic acid	0.986	±	0.389	0.967	±	0.120	0.952
Phenylalanine	1.054	±	0.081	1.026	±	0.042	0.772
Phosphoric acid	0.834	±	0.047	1.094	±	0.043	0.006
Pipecolic acid	1.279	±	0.204	0.925	±	0.098	0.121
Porphine	0.482	±	0.089	1.314	±	0.105	<0.000
Proline	0.902	±	0.097	1.026	±	0.089	0.505
Pyroglutamic acid	1.050	±	0.053	1.051	±	0.032	0.997
Pyrophosphate	0.860	±	0.083	1.125	±	0.046	0.013
Pyruvic acid	0.391	±	0.100	1.401	±	0.165	0.004
Ribitol	0.975	±	0.072	1.089	±	0.063	0.393
Ribose	0.869	±	0.265	1.245	±	0.371	0.620
Sedoheptulose anhydride monohydrate	0.862	±	0.194	1.175	±	0.188	0.426
Serine	0.907	±	0.055	1.041	±	0.055	0.249
Sorbitol	1.260	±	0.100	0.954	±	0.036	0.002
Stearic acid	1.005	±	0.072	1.029	±	0.049	0.827
Tartaric acid	0.725	±	0.130	1.155	±	0.085	0.024
Tartronic acid	0.880	±	0.139	1.118	±	0.089	0.223
Threitol	1.021	±	0.204	1.076	±	0.055	0.724
Threonine	0.968	±	0.082	1.037	±	0.048	0.517
Trans-4-hydroxy-L-proline	0.850	±	0.037	1.076	±	0.093	0.229
Trans-dehydroandrosterone	1.079	±	0.116	1.043	±	0.053	0.768
Trehalose	0.866	±	0.123	1.199	±	0.113	0.164
Tryptophan	1.627	±	0.188	0.764	±	0.100	<0.000
Tyrosine	0.783	±	0.089	1.133	±	0.067	0.017
Urea	0.958	±	0.094	1.047	±	0.057	0.473
Uric acid	0.800	±	0.139	1.116	±	0.106	0.166
Valine	0.913	±	0.073	1.084	±	0.050	0.121

Metabolites that differ between groups are written in bold.

*: $P < 0.05$ indicates that the difference is statistically significant.

**: Data value after normalization to Internal standard.
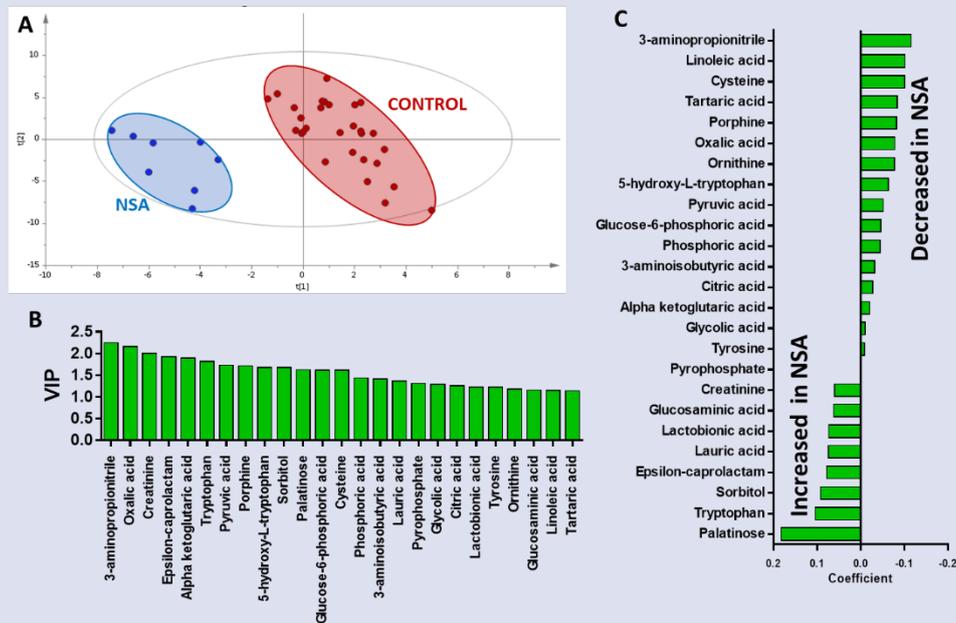
Figure 1. Comparison of plasma metabolic profiling of the control and nervous system abnormalities groups (A) Partial least squares differentiation analysis (PLS-DA) score plot showing a clear distinction between the groups (R^2: 0.614 and Q^2: 0.612); (B) Variable importance in projection (VIP) charts of metabolites that are effective in separating the groups; (C) Coefficient plots of the metabolic variables in the PLS-DA model.
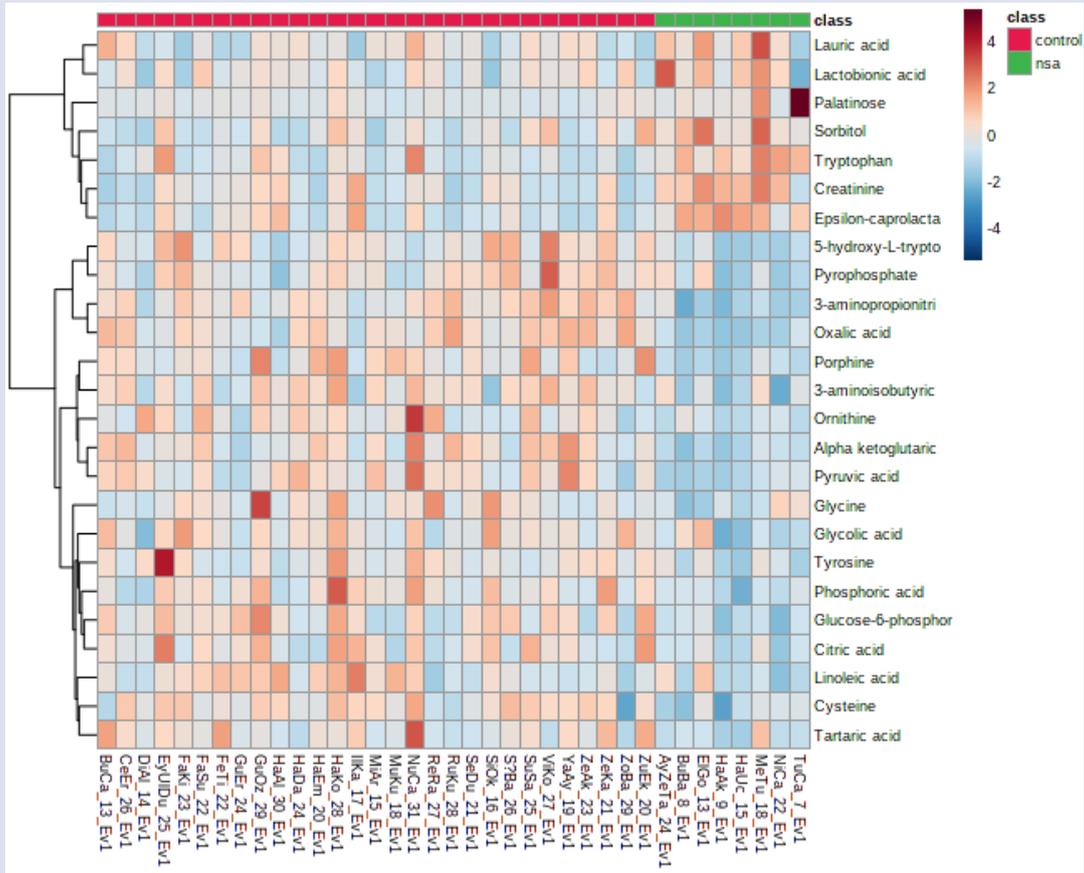
Figure 2. Heat color graphics of selected metabolites that are effective in the differentiation of metabolic profiles.
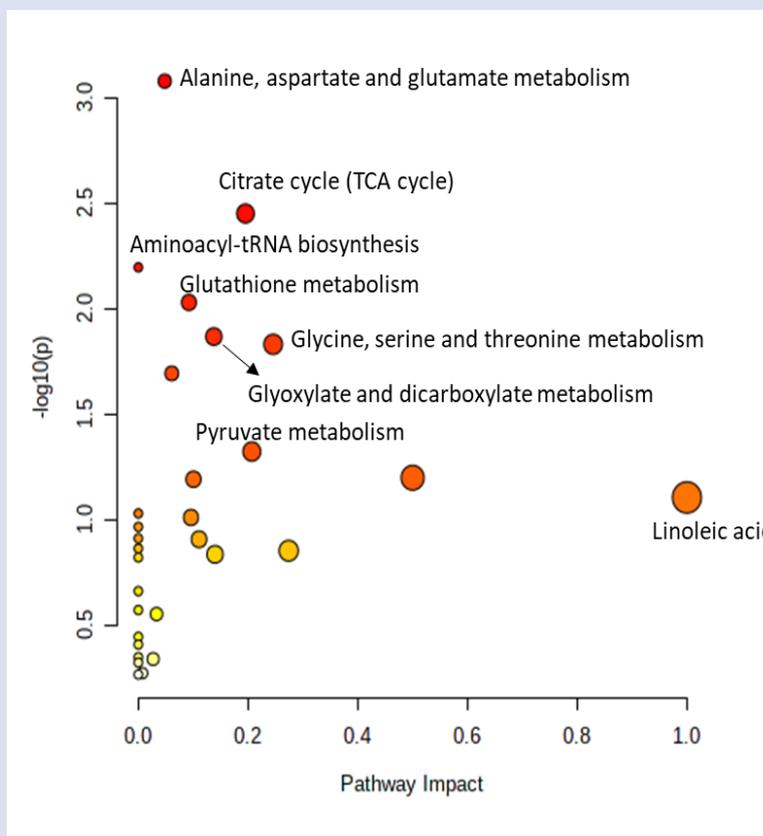
Figure 3. Pathway analysis.

Discussion

Numerous studies have revealed the involvement of the lysyl oxidase enzyme family in mouse models of tissue fibrosis in organs. Lsyl oxidase inhibitor 3 Aminopropionitrile inhibits LOX activity and inhibits the formation of collagen cross-links and fibrosis [18]. Linoleic acid is a long-chain polyunsaturated fatty acid. It is found in the brain's gray matter and makes up about 15% of the fatty acids in the human frontal cortex. Linoleic acid is of great importance for the development of the fetal brain and its deficiency may responsible for the development of neurological abnormalities [19-21]. Lauric acid, middle chain fatty acids, typically increased the mRNA expression of glial-derived neurotrophic factor (*GDNF*), interleukin-6 (*Il6*), and C–C motif chemokine 2 (*Ccl2*) in astrocytes and enhances the presynaptic maturation [22]. Cysteine, which plays a role in protein synthesis, also has critical importance in redox homeostasis due to its strong antioxidant properties. Redox-modulated events do not only occur in peripheral tissues, but it is better understood in the light of new information that these events also play critical roles in the brain. It is known that irregularities in cysteine metabolism are associated with various neurodegenerative disorders [23]. The dysregulation of lipid and redox homeostasis may be responsible for the development of neurological abnormalities.

The low level of intrinsic antioxidants such as tartaric acid and citric acid in the brain and the high level of polyunsaturated fatty acids pose a risk of oxidative stress-induced molecular damage [24]. Moreover high level of tartaric acid were found in autistic children [25]. The increase in the production of porphyrins and porphyrin precursors was found to result from partial enzyme blocks along the heme biosynthetic pathway, resulting in secondary depression of the enzyme Aminolevulinic acid synthase (ALA-synthase). Therefore, it is thought that neurological manifestations may be related to a decrease in essential heme proteins or other heme-containing compounds in the nervous system or to the toxic effect of overproduction of the porphyrin precursors ALA and porphobilinogen (PBG) [26]. The impact of the presence of oxalic acid was studied in the diseases of central nervous system, especially in schizophrenia [27, 28].

Ornithine carbamoyltransferase deficiency is the commonest of the inherited urea cycle disorders going together with disabling neurological complications [29]. L-5-hydroxytryptophan, which plays a role in various neurological and metabolic diseases, is a limiting step in the biosynthesis of serotonin and melatonin with its synthesis from tryptophan [30]. It has been reported that measurement of lactic acid and pyruvic acid in cerebrospinal fluid may be of help to detect various infectious diseases with central nervous system involvement [31, 32].

Glucose-6-phosphate (G6P) is a key metabolite in energy metabolism. The alteration of G6P may relate to either glucose-6-phosphate isomerase (GPI) or glucose-6-phosphate dehydrogenase (G6PD). Both deficiencies have an effect on neurological impairment and neuroprotection [33, 34]. In addition, muscle phosphate metabolism may be altered in various central nervous system disorders by different metabolic impairments [35]. β-hydroxybutyrate, a ketone body, is one of the main mediators of brain growth and myelin formation, as it is the main substrate of phospholipid and sphingolipid synthesis [36]. The alteration of the β-hydroxybutyrate in NSA may also evaluate inadequate nervous system development.

Alpha-ketoglutarate and citric acid are important molecules involved in the Krebs cycle. These molecules have critical roles in various metabolic pathways, especially in cellular energy metabolism. Moreover, alpha-ketoglutarate, which is a source of glutamate and glutamine, stimulates protein synthesis and prevents protein degradation in muscles, as it is a nitrogen scavenger [37].

The idea that the development of chronic metabolic diseases may be caused by excessive sugar consumption is increasing the use of sweeteners around the world. Palatinose is absorbed from the intestine and metabolized slowly by isomaltase, but the rate of insulin stimulation is very low. Different sweeteners are notable for human health because they affect the central nervous system, gut hormones, and gut microbiota [38]. Metabolism of tryptophan takes place along the kynurenine pathway, which has pronounced effects on neurons in the central nervous system. Changes in kynurenine levels are implicated in several central nervous system disorders such as AIDS-dementia complex and Huntington's disease [39]. It has been reported that sorbitol and myo-inositol metabolism are critical in the pathogenesis of peripheral neuropathy in patients with diabetes mellitus [40].

In this study, we have demonstrated that 3-aminopropionitrile, linoleic acid, cysteine, tartaric acid, porphine, oxalic acid, ornithine, 5-hydroxy-L-tryptophan, pyruvic acid, glucose-6-phosphoric acid, phosphoric acid, 3-aminoisobutyric acid, citric acid, alpha-ketoglutaric acid, glycolic acid, tyrosine, pyrophosphate, glycine and lactic acid plasma levels were statistically significantly lower in second-trimester pregnancies having fetuses with NSAs compared to control group pregnancies. On the other hand, palatinose, tryptophan, sorbitol, epsilon-caprolactam, lauric acid, lactobionic acid, glucosaminic acid and creatinine plasma levels were found to be higher in study group pregnancies compared to controls. Moreover, the pathway analysis, performed with significantly altered metabolites, showed alteration in the alanine, aspartate and glutamate metabolism, citrate cycle, aminoacyl t-RNA biosynthesis and glutathione metabolism. Thusly, this study identified altered pathways and suggested candidate biomarkers that could be used for the screening of NSA within the

framework of antenatal care programs. However, the finding must be validated with wider groups.

Our findings indicate a special type of altered metabolic status in pregnant women carrying fetuses with NSA(s) with or without co-abnormalities. It could be speculated that this altered metabolic status might influence both gametogenesis, embryogenesis and organogenesis at various levels. The main limitation of this study is the number of patients, heterogeneity of the NSAs and the presence co-abnormalities in 75% of the cases. On the other hand, this study will encourage prenatal screening/diagnosis programs to diversify their methodological approaches in this medical field.

In conclusion, "alanine, aspartate and glutamate metabolism", citrate cycle, aminoacyl t-RNA biosynthesis and glutathione metabolism seem to be critical in the prenatal screening of NSAs. However, abnormality specific studies are necessary for further recommendations.

Conflicts of interest

There are no conflicts of interest in this work.

References

[1] Joan S., Terry L., The basics of brain development, *Neuropsychol Rev,* 20 (2010) 327-348.

[2] Kaplan K.M,. Spivak J.M., Bendo J.A., Embryology of the spine and associated congenital abnormalities, *The Spine Journal,* 5 (5) (2005) 564-576.

[3] Spirt B., Oliphant M., Gordon L., Fetal central nervous system abnormalities, *Radiologic Clinics of North America,* 28 (1) (1990) 59-73.

[4] Aydın E., Tanacan A., Büyükeren M., Uçkan H., Yurdakök M., Beksaç M.S., Congenital central nervous system anomalies: Ten-year single center experience on a challenging issue in perinatal medicine, *Journal of the Turkish German Gynecological Association,* 20 (3) (2019) 170.

[5] Tanacan A., Ozgen B., Fadiloglu E,. Unal C., Oguz K.K., Beksac M.S., Prenatal diagnosis of central nervous system abnormalities: Neurosonography versus fetal magnetic resonance imaging, *European Journal of Obstetrics & Gynecology and Reproductive Biology,* 250 (2020) 195-202.

[6] Pulikkunnel S.T., Thomas S., Neural tube defects: pathogenesis and folate metabolism, *The Journal of the Association of Physicians of India,* 3 (2005) 127-135.

[7] Obeid R., Oexle K., Rißmann A., Pietrzik K., Koletzko B., Folate status and health: challenges and opportunities, *Journal of perinatal medicine,* 44 (3) (2016) 261-268.

[8] Greene N.D., Leung K.Y., Copp A.J., Inositol, neural tube closure and the prevention of neural tube defects, *Birth defects research,* 109 (2) (2017) 68-80.

[9] Beaudin A.E., Stover P.J., Folate-mediated one-carbon metabolism and neural tube defects: Balancing genome synthesis and gene expression, *Birth Defects Research Part C: Embryo Today: Reviews,* 81 (3) (2007) 183-203.

[10] Stokes B.A., Sabatino J.A., Zohn I.E., High levels of iron supplementation prevents neural tube defects in the Fpn1ffe mouse model, *Birth defects research,* 109 (2) (2017) 81-91.

[11] Berihu B.A., Welderufael A.L., Berhe Y., Magana T., Mulugeta A., Asfaw S., Gebreselassie K., Maternal risk factors associated with neural tube defects in Tigray regional state of Ethiopia, *Brain and Development,* 41 (1) (2019) 11-18.

[12] Welderufael A.L., Berihu B.A., Berhe Y., Magana T., Asfaw S., Gebreselassie K., Belay E., Kebede H., Mulugeta A., Nutritional status among women whose pregnancy outcome was afflicted with neural tube defects in Tigray region of Ethiopia, *Brain and Development,* 41 (5) (2019) 406-412.

[13] Recber T., Orgul G., Aydın E., Tanacan A., Nemutlu E., Kır S., Beksac M.S., Metabolic infrastructure of pregnant women with methylenetetrahydrofolate reductase polymorphisms: A metabolomic analysis, *Biomedical Chromatography,* 34 (8) (2020) e4842.

[14] Beksaç M.S., Durak B., Özkan Ö., Çakar A.N., Balci S., Karakaş Ü., Laleli Y., An artificial intelligent diagnostic system with neural networks to determine genetical disorders and fetal health by using maternal serum markers, *European Journal of Obstetrics & Gynecology and Reproductive Biology,* 59 (2) (1995) 131-136.

[15] Nemutlu E., Orgul G,. Recber T., Aydin E., Ozkan E., Turgal M., Alikasifoglu M., Kir S., Beksac M.S., Metabolic infrastructure of pregnant women with trisomy 21 fetuses; metabolomic analysis, *Zeitschrift für Geburtshilfe und Neonatologie,* 223 (05) (2019) 297-303.

[16] Monni G., Atzori L., Corda V., Dessolis F., Iuculano A., Hurt K.J., Murgia F. Metabolomics in prenatal medicine: A review, *Frontiers in Medicine,* 771 (2021).

[17] Özkan E., Nemutlu E., Beksac M.S., Kır S., GC–MS analysis of seven metabolites for the screening of pregnant women with Down Syndrome fetuses, *Journal of Pharmaceutical and Biomedical Analysis,* 188 (2020) 113427.

[18] González-Santamaría J., Villalba M., Busnadiego O., López-Olañeta M.M., Sandoval P., Snabel J., López-Cabrera M., Erler J.T., Hanemaaijer R., Lara-Pezzi E., Matrix cross-linking lysyl oxidases are induced in response to myocardial infarction and promote cardiac dysfunction, *Cardiovascular research,* 109 (1) (2016) 67-78.

[19] Holman R., Johnson S., Hatch T., A case of human linolenic acid deficiency involving neurological abnormalities, *The American journal of clinical nutrition,* 35 (3) (1982) 617-623.

[20] Meng H., A case of human linolenic acid deficiency involving neurological abnormalities, *The American journal of clinical nutrition,* 37 (1) (1983) 157-159.

[21] Basak S., Mallick R., Duttaroy A.K., Maternal docosahexaenoic acid status during pregnancy and its impact on infant neurodevelopment, *Nutrients,* 12 (12) (2020) 3615.

[22] Nakajima S., Kunugi H., Lauric acid promotes neuronal maturation mediated by astrocytes in primary cortical cultures, *Heliyon,* 6 (5) (2020) e03892.

[23] Paul B.D., Sbodio J.I., Snyder S.H., Cysteine metabolism in neuronal redox homeostasis, *Trends in pharmacological sciences,* 39 (5) (2018) 513-524.

[24] Gutteridge J., Oxidative stress in neurobiology: An important role for iron. In: *Oxidative Stress and Aging.* edn.: Springer, (1995) 287-302.

[25] Shaw W., Kassen E,. Chaves E., Increased urinary excretion of analogs of Krebs cycle metabolites and arabinose in two brothers with autistic features, *Clinical chemistry,* 41 (8) (1995) 1094-1104.

[26] Becker D.M., Kramer S., The neurological manifestations of porphyria: a review, *Medicine,* 56 (5) (1977) 411-423.

[27] Kuberski Z., Behavior of oxalic acid in diseases of the central nervous system with regard to investigation of cerebrospinal fluid, *Neurologia, neurochirurgia i psychiatria polska,* 6 (5) (1956) 521-526.

[28] Sciorta A., Blood and cerebrospinal fluid level of oxalic acid in schizophrenia, *Rivista sperimentale di freniatria e medicina legale delle alienazioni mentali,* 78 (2) (1954) 391-408.

[29] Nicolaides P., Liebsch D., Dale N., Leonard J., Surtees R., Neurological outcome of patients with ornithine carbamoyltransferase deficiency, *Archives of disease in childhood,* 86 (1) (2002) 54-56.

[30] Maffei M.E., 5-Hydroxytryptophan (5-HTP): Natural occurrence, analysis, biosynthesis, biotechnology, physiology and toxicology, *International journal of molecular sciences,* 22 (1) (2020) 181.

[31] Choremis K., Constantinides V., Nicolaides P., Pyruvic acid in the cerebrospinal fluid during various infectious diseases with central nervous system involvement. In: *Annales paediatrici International review of pediatrics,* (1953) 337-345.

[32] Duinkerke S., Gabreëls F., Boerbooms A.T., Kok J., Renier W., Can determination of lactic acid and pyruvic acid in cerebrospinal fluid help in diagnosing central nervous system involvement in systemic lupus erythematosus?, *Clinical neurology and neurosurgery,* 85(4) (1983) 225-230.

[33] Kugler W., Breme K., Laspe P., Muirhead H., Davies C., Winkler H., Schröter W., Lakomek M., Molecular basis of neurological dysfunction coupled with haemolytic anaemia in human glucose-6-phosphate isomerase (GPI) deficiency, *Human genetics,* 1998, 103 (4) (1998) 450-454.

[34] Tiwari M., Glucose 6 phosphatase dehydrogenase (G6PD) and neurodegenerative disorders: Mapping diagnostic and therapeutic opportunities, *Genes & diseases,* 4 (4) (2017) 196-203.

[35] Argov Z., De Stefano N., Arnold D., Muscle high-energy phosphates in central nervous system disorders. The phosphorus MRS experience, *The Italian Journal of Neurological Sciences,* 18 (6) (1997) 353-357.

[36] Tanianskii D.A., Jarzebska N., Birkenfeld A.L., O'Sullivan J.F., Rodionov R.N., Beta-aminoisobutyric acid as a novel regulator of carbohydrate and lipid metabolism, *Nutrients,* 11 (3) 2019) 524.

[37] Wu N., Yang M., Gaur U., Xu H., Yao Y., Li D., Alpha-ketoglutarate: physiological functions and applications, *Biomolecules & therapeutics,* 24 (1) (2016) 1.

[38] Moriconi E., Feraco A,. Marzolla V., Infante M., Lombardo M., Fabbri A., Caprio M., Neuroendocrine and metabolic effects of low-calorie and non-calorie sweeteners, *Frontiers in Endocrinology, (*2020) 444.

[39] Stone T.W., Mackay G.M., Forrest C.M., Clark C.J., Darlington L.G., Tryptophan metabolites and brain disorders, (2003).

[40] Mosiewicz J., Grzywa M., Disorders of sorbitol and myoinositol metabolism and the activity of sodium, potassium ATPase in the pathogenesis of peripheral neuropathy in patients with diabetes mellitus, *Polski Tygodnik Lekarski (Warsaw, Poland: 1960),* 47 (1-2) (1992) 56-58.