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ORIGINAL ARTICLE

Study of Plasma Cholesterol in Adults with Phenylketonuria

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ABSTRACT

Objective: This study was designed to employ the NMR lipoprotein profiling technique to evaluate the spectrum of total cholesterol, LDL cholesterol, and HDL cholesterol. The major aim of this research was to investigate the subclasses of lipoprotein to investigate the pathophysiology of brain damage in PKU cases. However, we were also concerned with the possible cardiovascular risks caused by abnormal lipoprotein profiles.

Place and Duration: Pak International Medical College, Hayatabad, Peshawar(PIMC) From Jan 2021 to December 2021

Study type: Case control study

Methodology: Patients were asked for an overnight fast to get their plasma samples in the morning. Samples were frozen at − 20 °C to perform analysis by using a 600 MHz Bruker AVANCE IVDr spectrometer. For the statistical analysis, SPSS version 24.0 was used. A student t-test was applied to measure the statistical difference. The significant statistical difference was set as 0.05.

Results: In case group total cholesterol was reported as 179.4 while the control group had 200.9 mg/dL with a p value < 0.02. On the other hand, the LDL cholesterol was reported as 104.1 mg/dL in control while 79.5 mg/dL in the case group with p<0.003. Meanwhile, no statistical difference was observed between both groups when comparing triglycerides. No statistical difference was observed between subclasses of lipoprotein. Furthermore in current research correlation was observed between blood PHE levels andPHE control diet.

Conclusion: In conclusion, NMR spectroscopy provides unique patterns of lipoprotein profile which were previously seen in patients consuming statins as their treatment

Keywords: Plasma cholesterol, LDL cholesterol, phenylketonuria, PKU diet

INTRODUCTION

Phenylketonuria (PKU) is a product of high phenylalanine (PHE) in blood and brain which arises due to mutations occurring in the phenylalanine hydroxylase (PAH) gene. This rare disorder leads to intellectual disabilities, autism, and behavioral and psychiatric problems if not treated properly.[1] Many cases of PKU reported developmental problems, seizures, microcephaly, motor deficits, and eczematous rash. Although the role of the PAH system in converting PHE to tyrosine is well defined still there is no evidence that defines the pathophysiology of PKU and the influence of high PHE on the central nervous system.[2] Previous theories of PKU were concerned with neurotransmitter depletion, impairment in brain protein synthesis[3,4], and oxidative stress which causes early cell death and damages the mitochondrial function.[5,6] Along with these theories, lipid metabolism was the major focus of researchers as they tried to explore the concentration of essential fatty acid and their deficiency caused by a special PKU diet.[7,8] A systematic review observed that many studies investigate the direct relationship between cholesterol deficiency and hypomyelination resulting in intellectual disability but the results are quite controversial.[9] However, none of these studies used NMR metabolomic lipoprotein profiling. Lipoprotein profiling is now widely used to demonstrate the effect of statins in cardiovascular diseases.[10,11] So, this study was designed to employ the NMR lipoprotein profiling technique to evaluate the spectrum of total cholesterol, LDL cholesterol, and HDL cholesterol. The major aim of this research was to investigate the subclasses of lipoprotein to investigate the pathophysiology of brain damage in PKU cases.[12] However, we were also concerned with the possible cardiovascular risks[13] caused by abnormal lipoprotein profiles.

METHODOLOGY

This case-control study was conducted in Pak International Medical College Hayatabad Peshawer (PIMC) from January 2021 to December 2021. During the study framework total of 20 phenylketonuria patients were recruited. In this work patients aged above, 30 years were included. The maximum age limit for this study was set as 54. Out of these 22 patients, two were excluded due to triglycerides of 475 and 625 mg/dL respectively after lipoprotein evaluation. A total of 14 healthy controls were included for making a comparison. Participants who required additional cardiovascular investigations or had clinical symptoms or medical history were excluded from both case and control groups. Patients were strictly directed to take a PHE-restricted diet (eliminating those food which contain high protein amount including meat, fish, milk, nuts etc). This diet varied from patient to patient in adherence to plasma levels and treatment recommendations. Patients were asked for an overnight fast to get their plasma samples in the morning. Samples were frozen at − 20 °C to perform analysis by using a 600 MHz Bruker AVANCE IVDr spectrometer. The procedure mentioned in the study of Monsonis[14] was used to carry out the analysis.[15] For the statistical analysis, SPSS version 24.0 was used. A student t-test was applied to measure the statistical difference. The significant statistical difference was set as 0.05.

RESULTS

A total of 22 PKU patients were recruited for this study with a mean age of 38.7 (range 30–54) years. Out of these 22 cases, 16 were females while 8 were males. The overall average BMI of the case group was observed as 27.2 kg/m2 ranging from 20.7–to 51.3 kg/m2. In the control group, 14 patients were recruited with a mean age of 35.2 and the average BMI was reported as 23.9 kg/m2 in the control group. In the case of the group, total cholesterol levels and LDL cholesterol were significantly lower than the control group. In case group total cholesterol was reported as 179.4 while the control group had 200.9 mg/dL with a p value < 0.02. On the other hand, the LDL cholesterol was reported as 104.1 mg/dL in control while 79.5 mg/dL in the case group with p<0.003. Meanwhile, no statistical difference was observed between both groups when comparing triglycerides. There was no statistical difference observed between subclasses of lipoprotein. Furthermore in current research correlation was observed between blood PHE levels and diet. VLD5-cholesterol, VLDL5-free cholesterol, VLDL5-phospholipid, and VLDL5-triglyceride show a

negative correlation with blood PHE levels and confirmed the negative effect of PHE on subclasses (R = -0.49.1, p = 0.024; R = -0.55, p = 0.009, R = -0.54, p = 0.012 and R = -0.58, p = 0.006 respectively). Total cholesterol and LDL concentration were also negatively correlated with blood PHE levels. Glutamic acid and citric acid were significantly higher in case group than control while creatinine, glutamine, and tyrosine show statistical differences between both groups (Table 3).

Table 1: Biological characteristics of Case group[15]

Patient Id	Gender	Age	BMI	Allele 1	Allele 2	Actual PHE µmol/L	Diet adherence	genetic predicted value
1	Female	31	23.6	n/a	n/a	416	yes	n/a
2	Female	37	26.8	p.F39L	p.R252W	1207	No	1.4
3	Female	35	21.2	p.R261Q	IVS7 + 3 g > c	1183	No	1.3
4	Female	36	31.2	p.R158Q	p.R158Q	1106	No	0
5	Female	43	27	p.G239V	IVS10–11 g > a	957	Yes	n/a
6	Female	45	22.4	p.R408W	p.R408W	349	Yes	0
7	Female	44	25	p.L48S	p.Y387H	796	Yes	2.4
8	Female	31	24.9	p.R408W	IVS10-3C > T	929	Yes	0.9
9	Female	31	20.7	p.R158Q	p.R158Q	60	Yes	0
10	Female	41	21.2	p.R158W	p.R252Gfs*30	142	Yes	0
11	Female	30	21.8	n/a	n/a	402	Yes	n/a
12	Female	38	41.8	IVS10–11 g > a	p.R408W	1905	No	0
13		42	21.3	p.P281L	IVS12 + 1G > A	51	Yes	0
14	Female	33	32	IVS12 + 1G > A	IVS12 + 1G > A	1013	No	0
15	Female	39	31.2	p.L48S	p.R408W	648	Yes	2.4
16	Female	54	29.6	p.L48S	IVS10–11 G > A	238	Yes	2.4
17	Male	46	51.3	p.R408W	IVS12 + 1G > A	1318	No	0
18	Male	45	26.8	p.R158Q	p.R158Q	1532	No	0
19	Male	45	23.3	p.R261Q	p.G272X	730	Yes	1.3
20	Male	32	23.1	p.S349P	p.L348V	1017	Yes	n/a
21	Male	44	23.8	p.P281L	p.R408W	1409	Yes	0
22	Male	30	27.5	p.R261Q	p.R408W	866	Yes	1.3

Table 2: Comparison of plasma lipoprotein in case and control group[15]

Variables	Case	Control	P-value
TPCH [mg/dL]	179.4 ± 28.1	201 ± 33.2	0.02204
L2AB [mg/dL]	5.2 ± 2.9	9.6 ± 2.8	0.00005
L3FC [mg/dL]	3.3 ± 1.8	4.6 ± 1.7	0.0219
L2PN [nmol/L]	93.9 ± 53.5	173.9 ± 51.6	0.00005
L3TG [mg/dL]	2 ± 0.7	2.5 ± 0.7	0.01909
L2PL [mg/dL]	5.4 ± 3.1	9.7 ± 2.6	0.00007
LDAB [mg/dL]	54.7 ± 12.3	67.6 ± 18.5	0.00815
L2CH [mg/dL]	8.3 ± 6.4	17 ± 5.5	0.00009
LDPN [nmol/L]	994.6 ± 224	1229.8 ± 335.9	0.00815
L2FC [mg/dL]	3.1 ± 2	5.4 ± 1.6	0.00053
L3PL [mg/dL]	5.5 ± 3	8.5 ± 3.5	0.00579
L1CH [mg/dL]	18.2 ± 6.4	24.8 ± 3.8	0.00078
L3PN [nmol/L]	100.6 ± 56.1	158 ± 71.6	0.00555
L1AB [mg/dL]	10.2 ± 2.9	13.2 ± 2	0.00105
L3AB [mg/dL]	5.5 ± 3.1	8.7 ± 3.9	0.00554
L1PN [nmol/L]	185.1 ± 53.2	239.1 ± 36.6	0.00106
L3CH [mg/dL]	8.6 ± 5.6	14.5 ± 7	0.0045
L1PL [mg/dL]	11.2 ± 3	14.2 ± 2	0.00115
LDFC [mg/dL]	24.1 ± 6.9	31 ± 7.8	0.00414
L1FC [mg/dL]	5.6 ± 2	7.4 ± 1.3	0.00239
LDCH [mg/dL]	79.5 ± 21.8	104.1 ± 30	0.00382
LDPL [mg/dL]	48.9 ± 10.9	60.9 ± 14.2	0.00368

Table 3: Comparison of low molecular plasma parameters of both groups.[15]

Variables	Case	Control	P-value
Lactic acid	3033 ± 1646.8	2409.7 ± 405.2	0.08787
Phenylalanine	830.7 ± 503	49.2 ± 10.1	0.00000
D-Glucose	4830.8 ± 1188	4904.1 ± 481	0.41409
DL-Tyrosine	42.6 ± 18.7	56.9 ± 7.8	0.00514
Acetic acid	17.9 ± 17.6	19.1 ± 16	0.41959
Glutamine	611.4 ± 99.1	690.6 ± 88.4	0.01013
Glycine	318.7 ± 99.8	311.5 ± 101.2	0.41959
Glutamic acid	87.7 ± 57.3	50.5 ± 29.2	0.01596
Valine	222.4 ± 60.4	218.4 ± 43.9	0.41813
Creatinine	74.8 ± 16	86.1 ± 12.7	0.01647
Acetone	21.5 ± 18.7	23.3 ± 10.5	0.41777
Citric acid	186.9 ± 45.4	157.2 ± 30.9	0.01952
Threonine	69.5 ± 64.2	59.8 ± 99.2	0.37320
3-Hydroxybutyric acid	83.9 ± 102.3	47.6 ± 34	0.10514
Formic acid	16.7 ± 4.5	17.5 ± 6	0.36175
Acetoacetic acid	19.8 ± 30	10.1 ± 14.3	0.13468
Leucine	89.1 ± 24.4	93.1 ± 23.5	0.32376
L-Isoleucine	45.3 ± 11.5	50.1 ± 15.6	0.14610
Trimethylamine-N-oxide	18.3 ± 17.7	21.3 ± 16.9	0.31443
Creatine	11.8 ± 11.2	15.6 ± 10.2	0.15723
Alanine	432.8 ± 89.7	415.5 ± 116.5	0.31144
Histidine	90.7 ± 72	76.9 ± 25.5	0.24784
Pyruvic acid	96.7 ± 38.9	89 ± 38.5	0.31004
Ethanol	117.1 ± 45.1	107.2 ± 55.5	0.28115

DISCUSSION

The results of the study showed the impact of phenylketonuria on lipoprotein concentration in plasma. These results indicated that phenylketonuria affected the LDL regulations or synthesis of cholesterol. One of the animal models studies high PH which caused a reduction in 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and mevalonate-5-pyrophosphate decarboxylase in the brain and liver.[16] This reduction results in impairment of cholesterol synthesis. One of the theoretical perspectives claims that impairment in untreated PKU cases leads to mental retardation and hypomyelination. Later on, one study reported that HMGR activity is not completely impaired in the liver however the function of HMGR may reduce up to 40% in forebrain oligodendrocytes.[17] Therefore inconsistent findings were revealed in the past related to the impact of the cholesterol concentration. One of the recent systematic reviews of Montoya Parra et al[9] based on 20 studies observed low cholesterol concentration in 12 studies while the remaining studies did not show a reduction.

In this current work, unique patterns of lipoprotein profiles were observed in the case group. A significant difference was observed in cholesterol, LDL-cholesterol, and LDL subclasses. However, only a small negative correlation of PHE was found between total cholesterol level and LDL concentration which causes obstacles in the interpretation of intergroup differences hence not shown in the result section. Regardless of these insignificant correlations plasma PHE and higher VLDL subfractions showed a significant negative correlation with BMI. A study by Couc et al[18] had similar results to the current work. They also observed a low reduction in cholesterol concentration and LDL cholesterol in patients with classical PKU. A vegan diet supplemented with artificial PHE-free amino acid mixture plays a massive role in the treatment of PKU.[9] These diets show a positive effect on lipid profiles and cannot be excluded. A vegan diet has

the ability to reduce HDL cholesterol however study by Huang[19] reported no influence on LDL cholesterol after consuming a vegan diet. However, the results of the meta-analysis[20] were in contradiction to this observation. They observed the positive influence of a vegan diet by reducing LDL cholesterol and HDL cholesterol in patients. However, in current work analysis of extensive lipoprotein subclass showed a reduction in total cholesterol level, and LDL concentration but failed to lower HDL cholesterol. Therefore, these results open a door for discussion that how cholesterol and LDL may be influenced or decreased in PKU cases. In this study, higher PHE levels were observed in patients with a less well-controlled diet. Due to these higher levels, there should be a higher intake of natural protein which enhanced the risk of elevated protein more.

A comprehensive review by Goldstein and Brown[21] claims that regulation of LDL cholesterol is a complex mechanism in which endogenous synthesis provide cholesterol to cells via HMG CoA, receptor-mediated uptake, and lysosomal hydrolysis of LDL cholesterol. Statins reduced cholesterol synthesis by restraining HMG-reductase activity. Increased PHE may mimic this statin effect. Sterol regulatory element-binding protein-1 (SREBP) transcription factors can regulate the LDL receptor gene by activating endogenous cholesterol biosynthesis. A low cholesterol diet can cause activation of SREBPs which leads to the activity of LDL receptors and HMGR resulting in increasing cholesterol synthesis and decreasing LDL.[21] Proprotein convertase subtilisin/Kexin type 9 (PCSK9) is another player in LDL regulation however mutation in this protein gene encouraged the destruction of LDL receptors thus reducing the plasma LDL levels.[22,23] No data is available to demonstrate whether the high PHE interferes with protein function. Hence, the reduction mechanism of LDL cholesterol in PKU patients is unclear and demands further investigation. Compared to controls high citrate and glutamic acid concentration were observed in the case of the group due to statin-like the effect of high PHE.

There was no catabolism observed due to lower molecular mass metabolites however, higher acetoacetic and 3-OH-butyric acid was observed without any significant difference. In both case and control groups, plasma analysis reported elevated amounts of lactic acid in capillary blood. The lower amount of glutamine found in the case group echoes a previous study by Perry et al.[24] Increased excretion of N-acetyl glutamine in urine was the major cause of low glutamine in patients however the clinical significance of the difference in both groups is unclear. One of the previous study claims that a decrease in glutamine levels is the major reason for mental retardation in PKU infants because glutamine plays an important role in developing an infant's brain.[25] In the current study, patients were recommended to use amino acid supplements enriched with tyrosine still the tyrosine levels were significantly lower in the case group which may lead to impaired brain protein synthesis.[1,2, 26,27] Meanwhile, no significant difference was found between branched-chain amino acids due to PHE levels < 900 µmol/L in eleven patients while six patients reported PHE levels < 600 µmol/L as described in European studies.[28-30] Studies reported that patients with classical PKU may have a possibility of chronic kidney disease as comorbidity.[31,32] The current study reported a significant difference in creatinine due to lower muscle mass.[33] However, in current work, no patient with CDK was reported.

CONCLUSION

In conclusion, NMR spectroscopy provides unique patterns of lipoprotein profile which were previously seen in patients consuming statins as their treatment

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