Chemical Sciences Journal
ISSN: 2150-3494
Kaur et al., Chem Sci J 2020, 11:1
Research Article
Open Access

Identification of Metabolites using ^{1}H NMR in Polycystic Ovary Syndrome Cases from North India

Ratneev Kaur[1], Tajinder Kaur[2], Palwinder Singh[3] and Anupam Kaur[1*]

[1]*Department of Human Genetics, Guru Nanak Dev University, Amritsar, Punjab, India*

[2]*Department of Obstetrics and Gynaecology, Hartej Hospital, Ranjit Avenue, Amritsar, Punjab, India*

[3]*Department of Chemistry, Guru Nanak Dev University, Amritsar, Punjab, India*

[*]**Corresponding author:** Kaur A, Department of Human Genetics, Guru Nanak Dev University, Amritsar, Punjab, India, Tel: +919872239393; E-mail: anupamkaur@yahoo.com

Received date: November 27, 2019; **Accepted date:** January 28, 2020; **Published date:** February 5, 2020

Abstract

Polycystic Ovary Syndrome (PCOS) is a common, heterogenous endocrine disorder in women of reproductive age. Alterations in numerous metabolic pathways have been implicated in its pathophysiology. The systemic changes in PCOS initate not only changes in the ovarian function but also changes in the whole-body metabolism. Indian women are at higher risk of developing type 2 diabetes and insulin resistance and hence give the drive to carry metabolomics study on Indian women. The present study focused on the metabolic profiling of PCOS women in North India. A total of 28 females of reproductive age were included. ^{1}HNMR technique was used to identify metabolites. Orthogonal projection to the latent structure with discriminant analysis (OPLS-DA) was applied for model identification. Important metabolites were identified using variance of importance (VIP) score and s-plot and the significance was verified by univariate analysis. The levels of leucine, glutamine, citric acid, proline and, methionine were decreased and that of valine, tyrosine and phenylalanine were elevated in PCOS women as compared to control females. The differences in the metabolic profiles between PCOS cases and controls may be attributable to the alterations in various pathways. The present study is first of its kind from North India.

Keywords: Nuclear Magnetic Resonance (NMR); Metabolites; Polycystic ovary syndrome; Metabolic pathways

Introduction

Polycystic Ovary Syndrome (PCOS) is a commonly occuring, heterogenous endocrine disorder in women of reproductive age with a prevalence of 5-10% worldwide. The characteristics of PCOS are hyperandrogenism, chronic anovulation and polycystic ovaries [1]. According to Rotterdam 2003 criteria, PCOS can be divided into four different phenotypes: hyperandrogenism, chronic anovulation and polycystic ovaries; chronic anovulation and polycystic ovaries but no clinical or biochemical hyperandrogenism; hyperandrogenism and chronic anovulation but normal ovaries; hyperandrogenism and polycystic ovaries but ovulatory cycles. PCOS is reproductive as well as metabolic disorder and is a foremost cause of anovulatory infertility among premenopausal women [2]. It is reported that oocytes of PCOS females have low quality and fertilization rate which might be due to abnormal levels of androgen or insulin. The obesity, genetic factors, environmental factors, play a synergistic role in PCOS females [3]. Alterations in numerous metabolic pathways have been implicated in the pathophysiology of PCOS [4]. However, PCOS women either having hyperandrogenism with normal menstrual cycle or anovulation with normal levels of androgens, are llikely to have lesser metabolic abnormalities [5]. It has been reported by different studies that PCOS women have increased tendency to obesity and abnormalities of lipids and lipoproteins [6,7].

Ovulatory dysfunction of PCOS patients is associated with the raised production of serine, threonine, phenylalanine, tyrosine and ornithine [8]. The systemic changes in PCOS initate changes not only in the ovarian function but also in the whole-body metabolism [9]. This emphasized the need to understand the metabolic dysfunction in PCOS for deterrence of long-term complications through apposite screening, diagnosis and intervention. Metabolomics has become an imperative tool in distinguishing changes in metabolic pathways and the verdict of human disease [10,11]. Comprehensive approaches to gain insights into metabolic variation and diseases, by more refined metabolic phenotyping, have become increasingly popular [12]. Metabolomics, aim at the recognition and quantification of all metabolites and impart a way to find functional biomarkers for the diagnosis of PCOS and scrutinize changes in biochemical pathways [13,14]. Due to its non-destructive nature, reproducibility and high resolution, nuclear magnetic resonance (NMR) technique is used in present study for metabolic profiling of PCOS [15].

Studies on metabolomics from different countries have been reported whereas there is only a single study from India. Indian women are at higher risk of developing type 2 diabetes and insulin resistance [16]. A study on Punjabi females suggested a prevalence of 14.8% overweight females and 13.8% obese females [17], which is characteristic of PCOS and gives the drive to carry metabolomics study on North Indian women. The present study focused on the metabolic profiling of PCOS women and could provide baseline data of metabolites in PCOS women for further studies in North India.

Materials and Methods

Participant selection and sample collection

In the present study, a total of 28 women participants were included. All the 15 PCOS participants were diagnosed by the doctor of Hartej

Hospital, Amritsar and fulfilled Rotterdam 2003 criteria [1], in which at least two features out of three were present i.e.,

- Polycystic ovaries
- Chronic anovulation and/or
- Clinical or biochemical signs of hyperandrogenism

We enrolled 13 age-matched healthy controls without any signs of PCOS and having a normal menstrual history. All the PCOS and normal participants included were aged between 14-30 years. The study was approved by the ethical committee of Guru Nanak Dev University, Amritsar, India. A predesigned questionnaire was filled which included: general information of the participant, medical and reproductive history, family history and detailed family pedigree. Informed consent was taken from participants before any investigation. Venous blood was taken in clot activator tubes and was kept at room temperature. Serum was separated by centrifugation from all samples and was stored at -80°C untill further use.

NMR spectroscopy

For the ^{1}H NMR measurement, serum samples were removed from -80°C and were thawed. Aliquotes of 500 µl of serum samples were prepared by adding methanol twice the volume of serum, to denature and precipitate the proteins. Samples were kept for 20 minutes at -20°C. Samples were thawed and then centrifugation was done at 14000 rpm for 10 minutes. The supernatant was freeze-dried for 3-4 hours in a speed vacuum concentrator (Eppendorf). The dried samples were resuspended in 140 µl of 99% D_2O and 460 µl 20 mM phosphate buffer, in NMR tubes [18]. The D_2O provided a field-frequency lock solvent for the NMR spectrometer. The internal standard for the chemical shift used was D_2O. ^{1}H NMR spectra with solvent suppression were recorded at Bruker 500 MHz NMR spectrophotometer with acquisition temp of 300K (water suppression using excitation sculpting with gradients) keeping spectral width -3.30 to 12.71 ppm. The sweep width was 16.0214 ppm. The numbers of scans were 16 and the receiver gain was set automatically. The phase correction and baseline correction of the collected spectra were done.

NMR data processing

The spectrum region of 0.8 to 8.0 ppm was binned to 166 spectral bins of equal width (0.04 ppm), the peaks containing water region from 4.2 to 4.9 ppm were excluded to avoid any noise created due to water. All the integral data were normalized to a total spectral area and was saved to text files in ASCII format, using Mestrenova (version 6.0.2-5475). The ASCII text files were further exported to Microsoft Excel 2007. The 166 bins were data scaled using autoscaling (mean-centered and divided by the standard deviation of each variable), to makes all the variances equal, using Metaboanalyst version 4.0 [19].

Statistical analysis

Multivariate pattern recognition was done to understand and classify metabolomic data, using Metaboanalyst version 4.0 and SIMCA-P version 15 (Umetrics, Umea, Sweden). After all the thorough pre-processing steps, Principal component analysis (PCA) was performed to look for the overview of the metabolomic dataset and relationships between groups [20] using metaboanalyst version 4.0. After the removal of the extreme outliers, 26 samples were selected (14 cases and 12 controls). The final step for the supervised method adopted for the present metabolomic study was orthogonal projection to the latent structure with discriminant analysis (OPLS-DA) with unit variance scaling (using SIMCA version 15). All the models were built according to OPLS-DA. The goodness of fit of the model was assessed by R2X and R2Y. The predictive ability of the model was quantified by Q2. The values ranged from 0-1, where 1 indicates the highest fit model and 0 signifies no fit model [21]. Very low or negative values of Q2 denoted no statistical difference between the groups. Then 100 permutations test was applied to the best components to cross-check the model significance. Score plot was generated to reveal group separation between PCOS and controls with predictive (Pp) and an orthogonal component (P_o) (Figure 1).

Metabolites detection

The major metabolites were identified in three steps. First, the difference of metabolites between PCOS and controls was given by a coefficient. The correlation values of particular metabolites, $r>0.6$ were considered important. Second, a variable of the importance projection score (VIP) ≥ 1.0 was taken for confirming the importance of metabolites. Third, the t-test was done to cross-check the significant difference of identified metabolites concentrations between PCOS and controls. The value $p<0.01$ was taken to be statistically significant.

The resulted metabolites were further cross-checked by volcano-plot with a fold-change threshold and t-test threshold using Metaboanalyst version 4 [19]. The important metabolites discovered were identified from the previous literature and using the Human Metabolome Database (HMBD 4.0) [22]. Moreover, the receiver operating characteristic (ROC) curve was generated to visualize the performance of metabolites and area under the curve (AUC) was measured for the identified metabolites in PCOS cases and controls.

Pathway analysis

Pathway analysis was carried out on important metabolites to see their role in specific pathways related and to verify their impact in respective pathways using Kyoto Encyclopedia of Genes and Genomes (KEGG) database in MetPA (http://metpa.metabolomics.ca) [23]. Two types of analysis were carried out, first the topology analysis, which takes the estimation of relative-betweenness centrality and the pathway impact factor and second is enrichment analysis (Tables 1 and 2).

Results and Discussion

The implication of different pathways participating in the etiology of PCOS leads to its complexity. Being a multifactorial disorder, it has various abnormalities related to genetics, metabolomics, endocrine, and environmental factors. Through NMR spectra analysis, the present study suggested an alteration in metabolites concentrations between PCOS cases and controls.

To improve the variation between the groups in the OPLS-DA model, strong outliers were removed. The goodness of fit was measured with R2=0.743 and Q2=0.632. In our study, the 100 permutations test was applied which confirmed the significance of the OPLS-DA model. The score plot showed a difference between PCOS cases and controls. The metabolites having $r>0.6$ and $p \leq 0.01$ were shortlisted and were checked for VIP score ≥ 1.0 in the OPLS-DA model from the VIP plot generated by SIMCA (version 15). The absolute correlation values were obtained from S-plot. The metabolites satisfying both the conditions were selected as important ones and were further studied for pathway analysis. Out of 166 bins, only 20 bins showed significance, corresponding to 16 important metabolites. To confirm the significance, univariate analysis was done using the t-test.

Previously identified metabolites showed a statistically significant difference between PCOS cases and controls ($p \leq 0.01$). Volcano-plot was generated with the t-test and fold-change threshold, which further confirmed the significance of discovered metabolites. The significant metabolites identified were fatty acids, succinate, adipic acid, methionine, tyrosine, pyruvate, citric acid, lipids (VLDL and LDL), lipoprotein, lysine, valine, glutamine, acetate, phenylalanine, histidine and N-acetyl glycoproteins (Tables 1 and 2). The metabolites were identified by their chemical shift. The metabolites and their percentage fold change between cases and controls are scheduled in Table 1. Fatty acids, lipids, lipoprotein, methionine, succinate, citric acid, tyrosine, VLDL and LDL, acetate, valine, glutamine, pyruvate and histidine showed the maximum predictive efficacy (>0.8) in terms of AUC 0.892, 0.892, 0.858, 0.850, 0.842, 0.842, 0.842, 0.825, 0.825, 0.817, 0.817, 0.808 and 0.800 respectively, on applying ROC. All the identified metabolites showed significance according to their AUC in PCOS cases and controls (Figure 2).

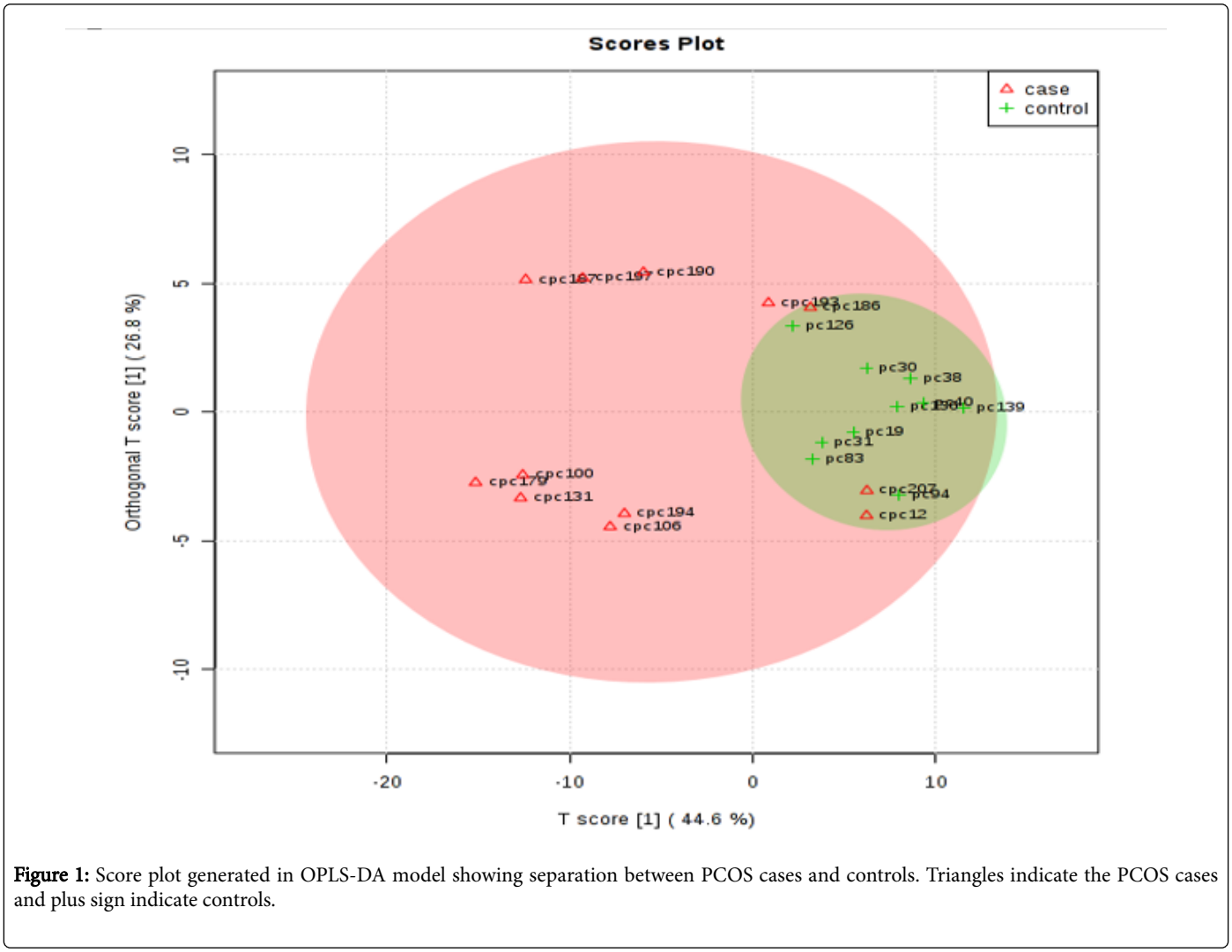

Figure 1: Score plot generated in OPLS-DA model showing separation between PCOS cases and controls. Triangles indicate the PCOS cases and plus sign indicate controls.

Dyslipidemia is related to insulin resistance, cardiovascular risk and is one of the most frequent metabolic abnormalities in women with PCOS. The present metabolomics study showed an increase in lipid metabolism, lipoproteins, very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) in PCOS cases as compared to controls. A study by Zhao et al., showed similar results of increased lipid metabolism in a Chinese population [5]. Sun et al., also showed similar results in Chinese women with PCOS [24]. Despite these, some studies have reported lower lipid levels in PCOS women [25].

The studies have reported that concentrations of branched-chain amino acids such as valine, leucine, and isoleucine, may lead to the development of obesity and related insulin resistance that may further contribute towards the progression of type 2 diabetes and PCOS phenotype [26,27]. Hyperglycemia may be caused by an alteration in the metabolism of these amino acids as well as aromatic-amino acids [28]. In the present study, leucine levels were decreased in PCOS cases as compared to controls. Leucine by the activation of the mTOR pathway might rescue insulin resistance and its increased levels can improve insulin resistance and follicle development [29,30]. Valine levels were increased in the present study that suggests it might affect gluconeogenesis because alterations from normal levels may lead to progression of complications such as obesity, insulin resistance and oxidative stress [31]. Our results were similar to Roy et al. [32], which showed increased valine levels in Indian women with PCOS as compared to controls. Acetate levels were found to be increased in our study and increased levels are known to lower fatty acid metabolism in

PCOS [18]. A study by Roy et al. also reported increased levels of acetate in PCOS women [32].

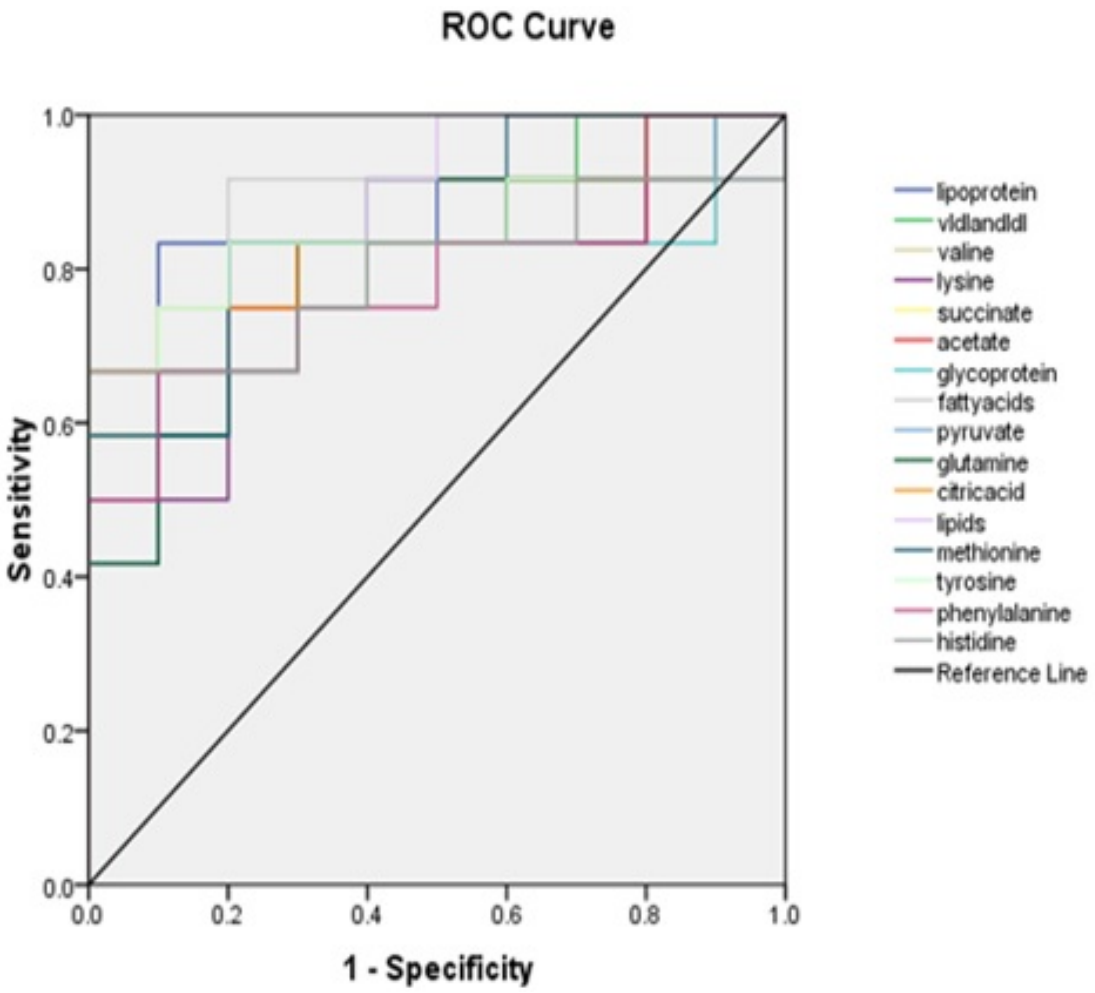

Figure 2: ROC curve showing area under the curve for different identified metabolites in PCOS cases and controls.

ppm	Metabolite present	VIP	% Fold Change	P-value
1.76	Succinate	1.16	37.4	0.006
1.68	Lysine	1.11	36.1	0.01
1.00	valine	1.11	28.1	0.008
0.88	lipids (VLDL and LDL)	1.09	24.9	0.009
0.84	Lipoprotein	1.07	27.2	0.004
2.36	Pyruvate	1.14	1	0.009
2.48,2.44	Glutamine	1.12	0.86	0.005
2.52	citric acid	1.12	28.3	0.002
2.24	Fatty acids	1.11	32.1	0.003
1.96	N-acetyl signal of glycoprotein	1.09	26.9	0.01

1.92	Acetate	1.03	3.35	0.01
2.64	Methionine	1.02	22.3	0.01
2.76, 5.36	Lipids	1.1	0.85	0.0007
7.2	Tyrosine	1.16	0.19	0.009
7.32, 7.44	Phenylalanine	1.13	0.29	0.01
7.76	Histidine	1.12	0.24	0.01

Table 1: Identified important metabolites showing significant changes between PCOS cases and controls.

Pathway analysis

Pathway analysis was done using KEGG, which identified 22 related pathways for 17 identified metabolites. Out of these 22 related pathways, 8 pathways had 0 impacts. The significant pathways ($p<0.05$) were Aminoacyl-tRNA biosynthesis, Valine, leucine and isoleucine biosynthesis, propanoate metabolism, phenylalanine metabolism, citrate cycle (TCA cycle), alanine, aspartate and glutamate metabolism, glyoxylate and dicarboxylate metabolism, taurine and hypotaurine metabolism, tyrosine metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, glycolysis or gluconeogenesis, pyruvate metabolism, butanoate metabolism, cysteine and methionine metabolism. The six pathways with highest impact (>0.1) by topology analysis were lysine degradation, phenylalanine metabolism, citrate cycle, Alanine, aspartate and glutamate metabolism, pyruvate metabolism and histidine metabolism (Figure 3).

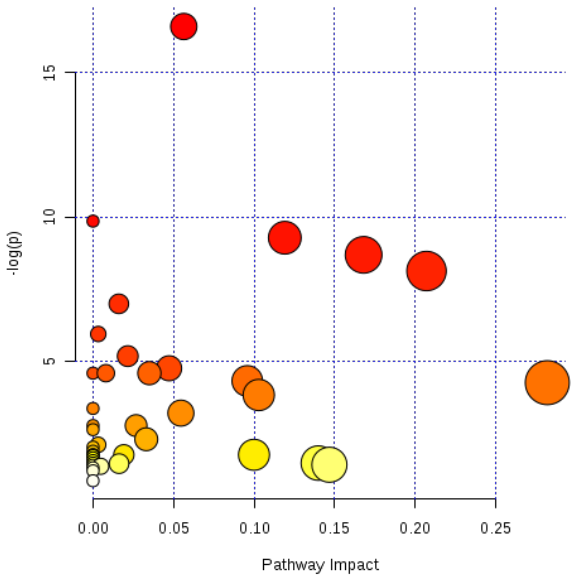

Figure 3: Topology analysis showing impact of different pathways conducted by MetPA.

Tricarboxylic acid cycle (TCA) is known to be impaired in PCOS women as suggested by previous studies [25,33,34]. An aerobic organisms use a sequence of reactions to generate energy by using acetyl-CoA also known as the TCA cycle. An increase or decrease in amino acid levels leads to alteration in the TCA cycle. The levels of citric acid, proline and methionine were decreased in PCOS women as compared to normal in the present study. TCA cycle has also been known to play a role in oocyte maturation [35]. Our results were similar to the study by Atiomo [25]. Sun et al., also showed increased levels of arginine, citrulline, glutamine, proline, and methionine, in a study conducted on Chinese PCOS women [24]. A study conducted by Zhao et al., also showed results in favor of our study [5]. These amino acids are a source of energy generation and any alteration in these may also alter glucose metabolism. The decrease in glutamine levels was seen in the present study which may alter glutamine/glutamate metabolism, these results were similar as shown by Sun et al., and Roy et al. [24,32]. High energy in the form of ATPs, RNA, and proteins is required by growing oocytes, which is fulfilled by an adequate amount of glutamine [36]. These alterations in amino acids may progress towards oxidative stress, insulin resistance, and obesity. Steroidogenesis needs an adequate level of glutamine for the synthesis of cholesterol. These amino acids are an abundant source of carbon intermediates providers for carrying out different pathways which help in meeting up energy demands of growing oocytes [32]. Therefore downregulation of such amino acids may scarce the demand for energy needed and may alter different pathways.

Histidine is known to play a role in oxidative stress and inflammation by negative association [37]. Lower levels of histidine may increase oxidative stress in PCOS cases [38]. Lowers levels of histidine were seen in the serum of PCOS cases as compared to controls in the present study. The results were similar as shown by Roy et al. and Whigham et al. [18,32]. Increased levels of pyruvate were found in PCOS women serum in our study. The increased levels of pyruvate produced during gluconeogenesis, indicate its decreased conversion to acetyl-CoA which in turn will not be oxidized to carbon dioxide and will further affect the TCA cycle and energy production.

In our study, the levels of endogenous amino acids such as tyrosine and phenylalanine were found to be elevated in the serum of PCOS cases as compared to controls. The elevated levels of these amino acids indicate protein degradation during ovarian dysfunction. Tyrosine and phenylalanine are indicated as important biomarkers for prediction of future development of diabetes. The study was conducted by Wang et al., which concluded the importance of these metabolites in predicting the development of diabetes [27]. Diabetes can further lead to

complications in PCOS. Our results were consistent with the study of Zhao et al., [5]. They showed that there was increased production of serine, threonine, phenylalanine, tyrosine, and ornithine which were significantly elevated PCOS, implied the relation of these amino acids to ovulatory dysfunction.

Division of groups

OPLS-DA models were generated by separating PCOS cases and controls into subgroups according to low and high body mass index (BMI). PCOS cases were compared with controls, both having low BMI levels and same was done with high BMI PCOS cases and controls. The score plot of the OPLS-DA model with low BMI cases and controls showed a difference (Figure 4).

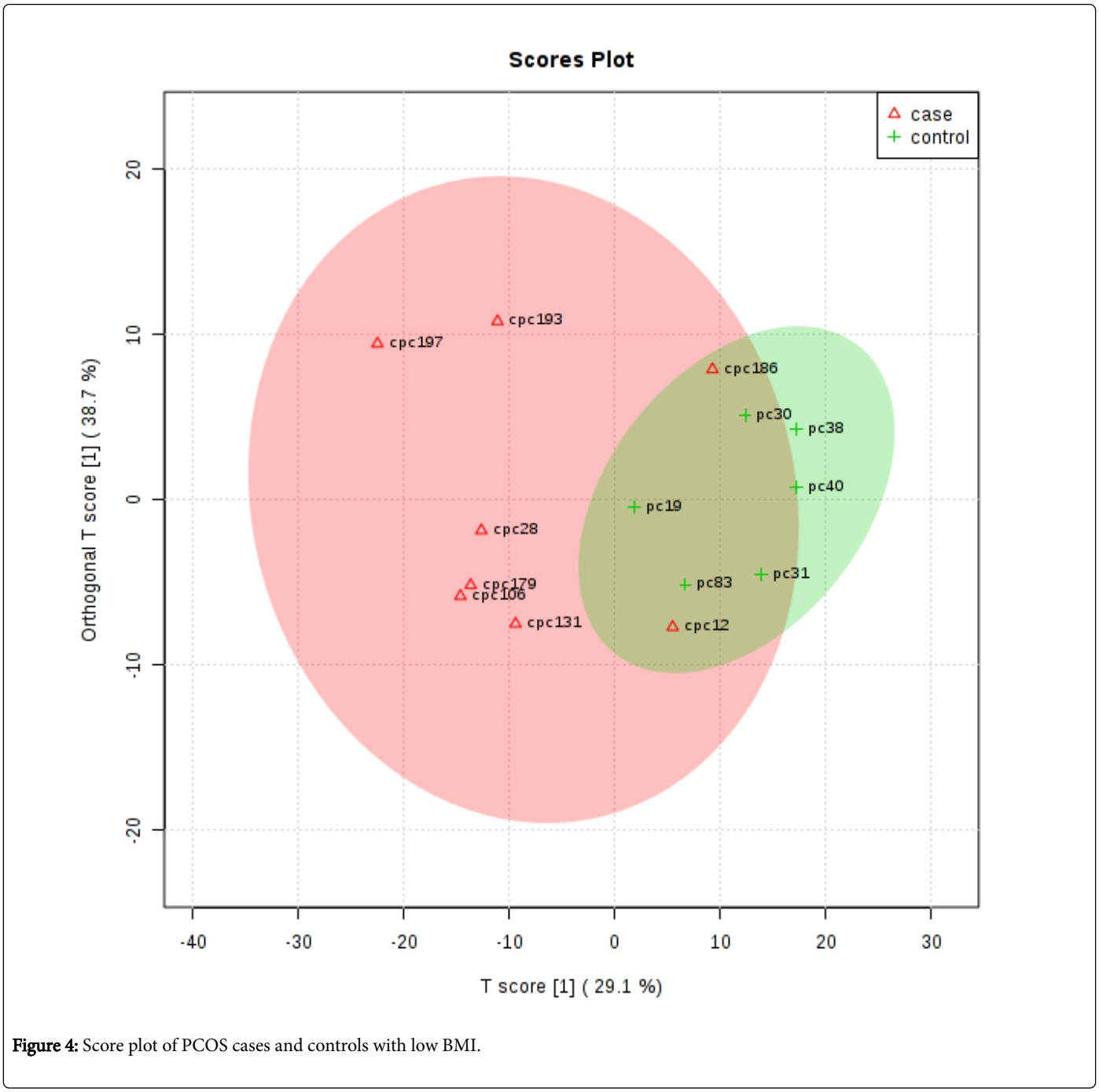

Figure 4: Score plot of PCOS cases and controls with low BMI.

The fitness of model values was R2=0.536 and Q2=0.161. There was a significant difference shown between PCOS cases and controls with high BMI by the score plot. The R2 and Q2 values obtained were 0.99 and 0.406 respectively (Figure 5). This predicted a good fit model.

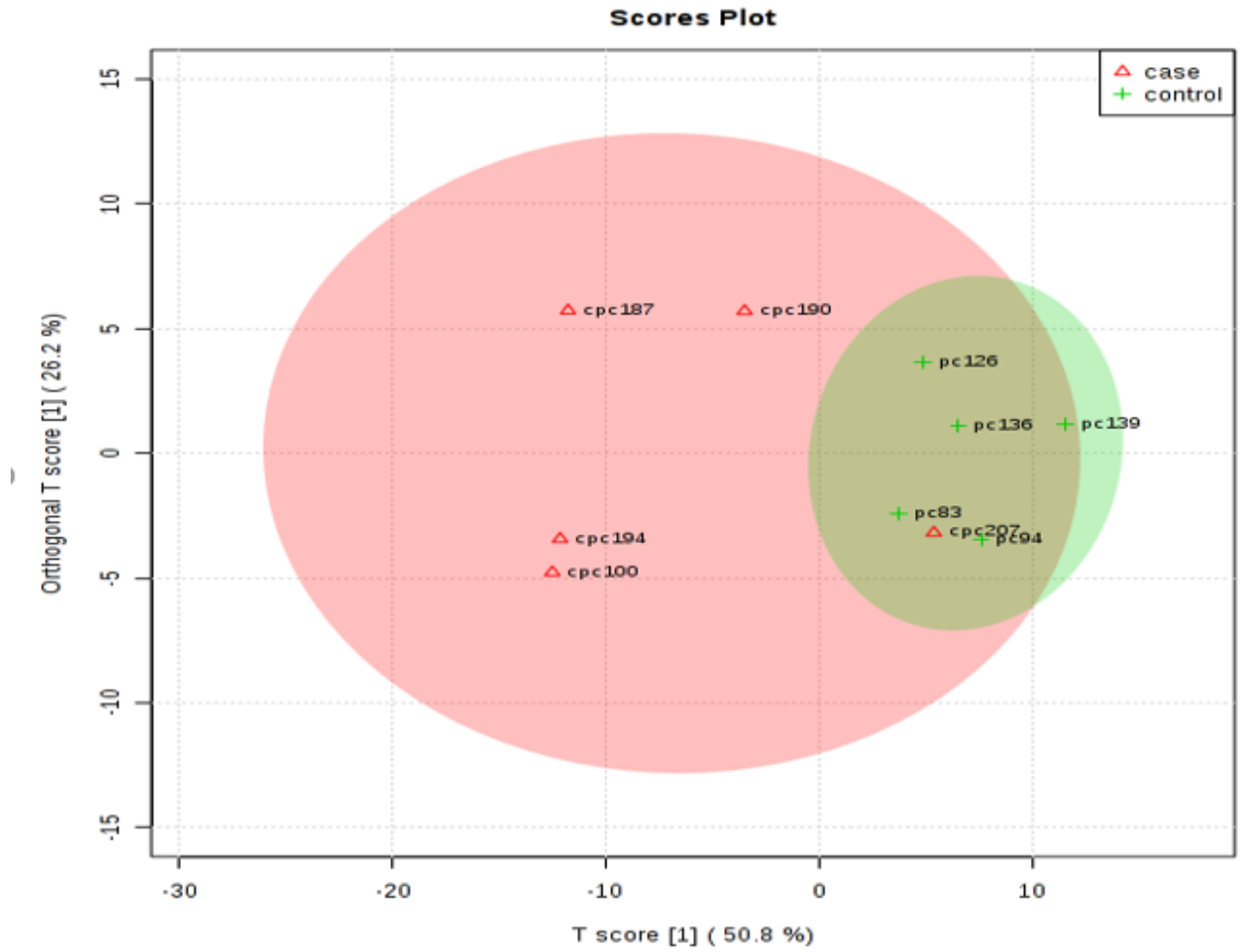

Figure 5: Score plot showing difference between PCOS cases and controls with high BMI.

Pathway name	Total metabolites	Detected metabolites	p-value	impact
Aminoacyl-tRNA biosynthesis	75	7	2.94E-06	0.05634
Phenylalanine metabolism	45	4	9.32E-05	0.119
Citrate cycle	20	3	0	0.167
Glyoxylate and dicarboxylate metabolism	50	3	0.002	0.003
Taurine and hypotaurine metabolism	20	2	0.005	0.021
Tyrosine metabolism	76	3	0.008	0.04
Phenylalanine, tyrosine and tryptophan biosynthesis	27	2	0.01	0.008
Glycolysis or gluconeogenesis	31	2	0.013	0.095
Pyruvate metabolism	32	2	0.014	0.282
Butanoate metabolism	40	2	0.02	0.102
Cysteine and methionine metabolism	56	2	0.04	0.05

Sulfur metabolism	18	1	0.1	0.033
Selenoamino acid metabolism	22	1	0.12	0.003
Vitamin B6 metabolism	32	1	0.171	0.019
Valine, leucine and isoleucine biosynthesis	27	2	0.015	0.034
Propanoate metabolism	35	3	0	0.016
Histidine metabolism	44	1	0.228	0.139
Ascorbate and aldarate metabolism	45	1	0.232	0.016
D-Glutamine and D-glutamate metabolism	11	1	0.06	0.026
Alanine, aspartate and glutamate metabolism	24	3	0	0.207
Lysine biosynthesis	32	1	0.11	0.09
Lysine degradation	47	1	0.17	0.099
Fatty acid metabolism	50	1	0.255	0.004

Table 2: Pathway enrichment analysis showing detected metabolites in corresponding pathways with their significance and impact.

Conclusion

Insulin resistance, hyperandrogenism, and obesity are the key features of PCOS and any alteration in the related metabolic pathway may lead to the progression of the disease. Thus, the present study analyzed different metabolic profiles in PCOS women and it is first of its kind from North India to the best of our knowledge. The differences in the metabolic profiles among PCOS cases and controls might be attributable to the alterations in various pathways, for instance, TCA cycle, gluconeogenesis, pyruvate metabolism, etc. The data presented may be insightful in identifying a molecular markers for diagnosis as well as management of PCOS. However, the limitations associated with the present study include measurement of insulin resistance and androgen levels to assess their relation with metabolic profiles in PCOS cases.

References

1. Eshretr P, ASRM-Sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertility and sterility 81: 19-25.
2. Balen AH, Rutherford AJ (2007) Managing anovulatory infertility and polycystic ovary syndrome. BMJ 335: 663-666.
3. Arya BK, Haq AU, Chaudhury K (2012) Oocyte quality reflected by follicular fluid analysis in poly cystic ovary syndrome (PCOS): a hypothesis based on intermediates of energy metabolism. Med Hypotheses 78: 475-478.
4. Jonard S, Robert Y, Cortet-Rudelli C, Decanter C, Dewailly D (2003) Ultrasound examination of polycystic ovaries: Is it worth counting the follicles?. Hum Reprod 18: 598-603.
5. Barber TM, McCarthy MI, Wass JAH, Franks S (2006) Obesity and polycystic ovary syndrome. Clin Endocrinol 65: 137-145.
6. Barber TM, Wass JAH, McCarthy MI, Franks S (2007) Metabolic characteristics of women with polycystic ovaries and oligo-amenorrhoea but normal androgen levels: implications for the management of polycystic ovary syndrome. Clin Endocrinol 66: 513-517.
7. Taponen S, Martikainen H, Järvelin MR, Sovio U, Laitinen J, et al. (2004) Metabolic cardiovascular disease risk factors in women with self-reported symptoms of oligomenorrhea and/or hirsutism: Northern Finland Birth Cohort 1966 Study. J Clin Endocrinol Metab 89: 2114-2118.
8. Diao FY, Xu M, Hu Y, Li J, Xu Z, et al. (2004) The molecular characteristics of polycystic ovary syndrome (PCOS) ovary defined by human ovary cDNA microarray. J Mol Endocrinol 33: 59-72.
9. Zhao Y, Fu L, Li R, Wang LN, Yang Y, et al. (2012) Metabolic profiles characterizing different phenotypes of polycystic ovary syndrome: plasma metabolomics analysis. BMC Med 10: 153.
10. Rosenfield RL, Ehrmann DA (2016) The pathogenesis of polycystic ovary syndrome (PCOS): The hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev 37: 467-520.
11. Dong F, Deng D, Chen H, Cheng W, Li Q, et al. (2015) Serum metabolomics study of polycystic ovary syndrome based on UPLC-QTOF-MS coupled with a pattern recognition approach. Anal Bioanal Chem 16: 4683-4695.
12. Murri M, Insenser M, Escobar-Morreale HF (2014) Metabolomics in polycystic ovary syndrome. Clin Chim Acta 429: 181-188.
13. Xiao JF, Zhou B, Ressom HW (2012) Metabolite identification and quantitation in LC-MS/MS-based metabolomics. Trends Analyt Chem 32: 1-14.
14. Shulaev V (2006) Metabolomics technology and bioinformatics. Brief Bioinform 7: 128-139.
15. Gowda GAN, Raftery D (2015) Can NMR solve some significant challenges in metabolomics? J Magn Reson 260: 144-160.
16. Wells JCK, Pomeroy E, Walimbe SR, Popkin BM, Yajnik CS (2016) The elevated susceptibility to diabetes in India: An evolutionary perspective. Front Public Health 4: 145.
17. Kaur A (2016) Survey of obesity among various age group girls of Punjab. Int J Phys Educ Sports Health 3: 296-299.
18. Whigham LD, Butz DE, Dashti H, Tonelli M, Johnson LK, et al. (2014) Metabolic Evidence of Diminished Lipid Oxidation in Women With Polycystic Ovary Syndrome. Curr Metabolomics 2: 269-278.
19. Xia J, Mandal R, Sinelnikov IV, Broadhurst D, Wishart DS (2012) MetaboAnalyst 2.0-a comprehensive server for metabolomic data analysis. Nucleic Acids Res 40: 127-133.
20. Psihogios NG, Kalaitzidis RG, Dimou S, Seferiadis KI, Siamopoulos KC, et al. (2007) Evaluation of Tubulointerstitial Lesions' Severity in Patients with Glomerulonephritides: An NMR-Based Metabonomic Study. J Proteome Res 6: 3760-3770.

21. Worley B, Powers R (2013) Multivariate Analysis in Metabolomics. Curr Metabolomics 1: 92-107.
22. Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, et al. (2013) HMDB 3.0-the human metabolome database in 2013. Nucleic Acids Res 41: 801-807.
23. Xia J, Wishart DS (2010) MetPA: A web-based metabolomics tool for pathway analysis and visualization. Bioinformatics 26: 2342-2344.
24. Sun L, Hu W, Liu Q, Hao Q, Sun B, et al. (2012) Metabonomics Reveals Plasma Metabolic Changes and Inflammatory Marker in Polycystic Ovary Syndrome Patients. J Proteome Res 11: 2937-2946.
25. Atiomo W, Daykin CA (2012) Metabolomic biomarkers in women with polycystic ovary syndrome: A pilot study. Mol Hum Reprod 18: 546-553.
26. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, et al. (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 9: 311-326.
27. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, et al. (2011) Metabolite profiles and the risk of developing diabetes. Nat Med 17: 448-453.
28. Wurtz P, Tiainen M, Makinen VP, Kangas AJ, Soininen P, et al. (2012) Circulatingmetabolite predictors of glycemia in middle-aged men and women. Diabetes Care 35: 1749-1756.
29. Li F, Yin Y, Tan B, Kong X, Wu G (2011) Leucine nutrition in animals and humans: mTOR signaling and beyond. Amino Acids 41: 1185-1193.
30. Macotela Y, Emanuelli B, Bang AM, Espinoza DO, Boucher J, et al. (2011) Dietary leucine-an environmentalmodifier of insulin resistance acting on multiple levels of metabolism. Plos One p: 6.
31. Wu G (2009) Amino acids: metabolism, functions, and nutrition. Amino Acids 37: 1-17.
32. RoyChoudhury S, Mishra BP, Khan T, Chattopadhayay R, Lodh I, et al. (2016) Serum metabolomics of Indian women with polycystic ovary syndrome using ^{1}H NMR coupled with a pattern recognition approach. Mol Bio Syst 12: 3407-3416.
33. Escobar-Morreale HF, Samino S, Insenser M, Vinaixa M, Luque-Ramírez M, et al. (2012) Metabolic heterogeneity in polycystic ovary syndrome is determined by obesity: plasma metabolomic approach using GC-MS. Clin Chem 58: 999-1009.
34. Gaster M, Nehlin JO, Minet AD (2012) Impaired TCA cycle flux in mitochondria in skeletal muscle from type 2 diabetic subjects: marker or maker of the diabetic phenotype? Arch Physiol Biochem 118: 156-89.
35. Jimenez PT, Frolova AI, Chi MM, Grindler NM, Willcockson AR, et al. (2013) DHEA-mediated inhibition of the pentose phosphate pathway alters oocyte lipid metabolism in mice. Endocrinol 154: 4835-4844.
36. Collado-Fernandez E, Picton HM, Dumollard R (2012) Metabolism throughout follicle and oocyte development in mammals. Int J Dev Biol 56: 799-808.
37. Niu YC, Feng RN, Hou Y, Li K, Kang Z, et al. (2012) Histidine and arginine are associated with inflammation and oxidative stress in obese women. J Nutr 108: 57-61.
38. Unni CS, Lakshman LR, Vaidyanathan K, Subhakumari KN, Menon NL (2015) Alterations in the levels of plasma amino acids in polycystic ovary syndrome- A pilot study. Indian J Med Res 142: 549-554.