Summary

Introduction:

Polycystic ovary syndrome (PCOS) is one of the most frequent endocrinological and metabolic diseases with varied manifestations. Nearly 13% of women in reproductive age are affected by this syndrome. Main manifestation of PCOS are menstrual disturbances, androgen excess and polycystic ovaries in ultrasound picture. This disease is often followed with frequent metabolic issues including but not limited to insulin resistance or obesity. Despite the numerous studies concerned on pathomechanism of PCOS its root cause is not yet unveiled. This is why precise diagnostics and quick treatment introduction is very difficult in this condition.

Recently, many publications indicate a possible interaction of different miRNAs in pathomechanism of some diseases, including PCOS. MiRNA are small non-coding molecules regulating gene expression on posttranscriptional level. One of their biggest advantages is their high stability in biofluids even after long term storage in active ribonuclease environment. These properties makes them a good potential biomarker for monitoring the progress of the disease. So far, there is no marker for PCOS that would be helpful in unambiguous diagnosis and thus accelerating diagnostics and giving contribution to a better understanding of the pathophysiology of the disease.

Aims:

The main aim of this study was to determine the characteristic profile of miRNA isoforms in the serum of women with PCOS that would differentiate them from the group of healthy women. Furthermore, an assessment of a relationship of chosen miRNAs with anthropometric and biochemical parameters was performed. Finally, a bioinformatical analysis for search of any relevant signalling pathway in which selected miRNAs may take regulatory function.

Material:

Genetical material obtained from 32 women aged 19 to 38 yrs was selected for the study. The study group consisted of 15 women diagnosed in the Department and Clinic of Endocrinology, Diabetes and Isotope Treatment at the Medical University in Wroclaw based on the Rotterdam criteria. The control group consisted of 17 healthy women with regular menstruations. Both groups were matched in terms of age and ethnicity.

Methods:

All women underwent medical interview with anthropometric parameters and biochemical tests (hormonal and metabolic parameters, lipid profile). Free androgen index (FAI), insulin resistance indexes (HOMA-IR and QUICKI) and atherogenic index of plasma (AIP) were calculated. Total RNA from serum was isolated for all patients, followed by cDNA synthesis. Serum-specific 179 miRNAs were expressed using real-time PCR. Results obtained from experiment were subjected to statistical analysis. miRNAs with differentiating expression (probability of less than 0.05) were considered statistically significant. Bioinformatical analysis was carried out for selected miRNAs to link them with the impact on individual processes in the body.

Results:

The group of patients with PCOS had significantly higher BMI, WHR and waist and hip circumferences. There were no differences between the groups in terms of blood pressure. The study group was characterized by significantly higher concentrations of total testosterone, androstenedione, dehydroepiandrosterone sulfate, lutropin and FAI value. No differences in the levels of sex hormone binding protein, follicle stimulating hormone, estradiol or thyrotropin were observed. Women with PCOS showed significantly higher insulin concentration and HOMA-IR values, while QUICKI value was significantly lower. There were no statistically significant differences in glucose concentration between the groups. No significant differences in lipid metabolism parameters were observed between the study and control groups. Statistical significance was demonstrated for C-reactive protein, which concentrations were higher in the study group.

Within the group of women with PCOS a significantly ($p = 0.023$) higher expression of the miRNA let-7e-5p isoform was demonstrated comparing to the control group. What is more, a positive correlation between let-7e-5p miRNA and fasting insulin has been presented ($R = 0.531$; $p = 0.00177$). After division of the study group based on biochemical studies, a subgroup was separated from the study group based on IR factor, which showed differences in expression that were statistically significant for four miRNAs: let-7e-5p, miR-99a-5p, miR-122-5p and miR-361-5p in comparison to the control group. Analysis of the correlation for overexpressed miR-99a-5p and miR-122-5p molecules with biochemical parameters showed that they are associated with insulin level and HOMA index. In contrast, correlation analysis of the miR-361-5p molecule, showing reduced expression relatively to the control group, showed close relationship with the FAI value.

After bioinformatic analysis, gene targets for let-7e-5p were identified, which included only one (PI3K-Akt) signalling pathway. In contrast, gene targets for let-7e-5p, miR-99a-5p

and miR-122-5p showed four signal pathways: the Wnt signal pathway, MAPK, type 2 diabetes and the PI3K-Akt signal pathway.

Conclusions:

Women with PCOS differ in miRNA profile compared to women in the control group. Serum expression of let-7e-5p was significantly increased in women with PCOS having higher insulin level and HOMA-IR index. Additional analysis showed that co-occurrence of IR and PCOS induces changes in four serum miRNAs: let-7e-5p, miR-122-5p, miR-99a-5p, miR-361-5p. MiRNAs with altered expression are involved in many important signalling pathways associated with metabolic dysfunctions in PCOS such as the PI3K-Akt signalling pathway, the Wnt signalling pathway, the MAPK signalling pathway, and the type 2 diabetes signalling pathway. Results obtained in this work might help in search of miRNA markers for PCOS and indicate their correlation with metabolic disturbances. However, further experiments on a larger group of patients is needed to confirm this outcome.