RESEARCH ARTICLE

Multi-Parameter Approach for Evaluation of Genomic Instability in the Polycystic Ovary Syndrome

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Abstract

**Background:** The polycystic ovary syndrome (PCOS), characterized by hyperandrogenism and chronic anovulation, is a common endocrine disorder in women. PCOS, which is associated with polycystic ovaries, hirsutism, obesity and insulin resistance, is a leading cause of female infertility. In this condition there is an imbalance in female sex hormones. All the sequelae symptoms of PCOS gradually lead to cancer in the course of time. It is heterogeneous disorder of unknown etiology so it is essential to find the exact cause. **Materials and Methods:** In this study both invasive and non-invasive techniques were employed to establish the etiology. Diagnosis was based on Rotterdam criteria (hyperandrogenism, ovulatory dysfunction, PCOM) and multiparameters using buccal samples and dermatoglypic analysis and cytogenetic study for 10 cases and four age and sex matched controls. **Results:** In clinical analysis we have observed the mean value of total testosterone level was 23.6nmol/L, total hirsutism score was from 12-24, facial acne was found in in 70% patients with 7-12 subcapsular follicular cysts, each measuring 2-8 mm in diameter. In dermatoglypic analysis we observed increases in mean value (45.9°) of ATD angle when compared with control group and also found increased frequency (38%) of Ulnar loops on both fingers (UU), (18%) whorls on the right finger and Ulnar loop on left finger (WU) and (16%) arches on right and left fingers (AA) were observed in PCOS patients when compared with control subjects. Features which could be applied as markers for PCOS patients are the presence of Ulnar loops in middle and little fingers of right and left hand. The buccal micronucleus cytome assay in exfoliated buccal cells, we found decrease in frequency of micronuclei and significant increases in frequency of karyolysed nuclei in polycystic ovarian syndrome patients. Chromosome aberration analysis revealed a significant increase in frequency of chromosome aberrations (CAs) in PCOS patients when compared with controls. **Conclusions:** From this present work it can be concluded that non-invasive technique like dermatoglypics analysis and buccal micronucleus cytome assays with exfoliated buccal cell can also be effective biomarkers for PCOS, along with increased CAs in lymphocytes as a sign of genetic instability. There is a hypothesis that micronuclei and chromosomal aberrations could have a predictive value for cancer. From this present work it can be concluded to some extent that non-invasive technique like dermatoglypics and buccal cell analysis can also be effective for diagnosis.

**Keywords:** Polycystic ovary syndrome - cytogenetic - dermatoglypics - buccal micronuclei analysis

Asian Pac J Cancer Prev, 16 (16), 7129-7138

Introduction

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous disorder, affecting approximately 7% of women in reproductive age (Diamant Kandarakis, 2008). It is distinguished by chronic anovulation, hyperandrogenemia, altered LH: FSH ratio (>2/3:1) and polycystic ovaries. The syndrome is a major cause of anovulatory infertility (Dunaif et al., 1997).

The etiology and pathogenesis of this syndrome still remains mysterious but likely to be multifactorial consisting of genetic and environmental components. Women with PCOS are frequently obese which contributes an extrinsic component of Insulin Resistance (IR). It is known that IR progresses towards the development of compensatory hyperinsulinemia, which drives Hyperandrogenemia in these women (Poretsky et al., 1999). Increased production of androgen levels lead to menstrual disturbances, development of ovarian cysts, facial acne, Hirsutism and other related disorders. The diverse nature of its clinical and biochemical features, it has been suggested that PCOS represents a range of disorders rather than a single entity (Simpson et al., 1992). Although it seems likely that there is more than one cause of this disorder, there are certain biochemical features which are common to all groups of subjects with ultrasonographic evidence of polycystic ovaries irrespective of the clinical appearance. Clustering of cases in families strongly support the role of
Materials and Methods

Collection of samples

A total number of 10 cases of buccal samples, dermatoglyphics and blood samples from PCOS patients aged between 18 to 28 years have been collected from Sandhya Hospital, Vellore. Detailed information about the family history of each affected individual was recorded in a standard proforma. Epidemiological studies for these cases were done and their clinical records including Hirsutism, Acne, Irregular period, obesity, diabetics, cardio problem, Acanthosis Nigricans and Ovarian morphology were recorded. Hirsutism scoring was recorded according to Ferriman Galloway scoring system (Ferriman and Galloway, 1961). This tool is used to evaluate hair growth at seven sites: upper lip, chin/face, chest, back, abdomen, arms, and thighs. A score of 0 is given in the absence of terminal hair growth and a score of 4 is given for extensive growth. A total score of 8 or more is indicative of Hirsutism (Unluhizarci K., 2012). Blood collection was done by venipuncture and the blood was collected in sterile vacutainers (Heparin & EDTA). Buccal samples, Dermatoglyphics and blood samples were collected from (n=04) from control normal individuals simultaneously. Informed consent was taken from all individuals included in this study. The study was approved by the University Human Ethical Committee of the VIT University

Dermatoglyphics analysis

Dermatoglyphics samples were taken for 10 cases of PCOS and 4 control samples by touch the ink with finger on its side and roll smoothly across the ink 180 degree to the opposite side of the finger and lift. Repeat the motion on paper, transferring the print. Palm prints require a similar procedure. the palm needs to be slowly rolled to transfer the edge (Cummins Midlo, 1943). Remove ink from hands with soap and water. The Dermatoglyphic patterns were recorded and parameters like fingertip patterns (ulnar loops, radial loops, whorls, arches) and “a-t-d” angle were studied.

Buccal micronucleus cytome (BMCyt) assay

Buccal Cell Sampling and Preparation: Buccal cells (BCs) were collected from 10 clinically confirmed cases of PCOS patients from Sandhya Hospital, Vellore and 4 control subjects were carried out. Prior to Buccal cell collection the mouth was rinsed thoroughly with water to remove any unwanted debris. Buccal cell samples were obtained by rubbing the inside of both cheeks using a cyto brush.

Buccal micronucleus cytome (BMCyt) assay

The BMCyt assay test was carried out on Buccal epithelial cells of 10 clinically confirmed cases of PCOS and 4 controls subjects. Oral Buccal cells obtained were smeared on a pre-cleaned slide, fixed in methanol and stained with 4% Giemsa. A total of 500 cells per individual were scored for analysis of micronuclei.

Scoring criteria for micro nuclei assay: For the
Micronuclei analysis, 500 Buccal cells were evaluated per subject according to the criteria established by (Nina et al., 2008) under a microscope at 100X magnifications to identify Binucleated cells, Micronuclei, Nuclear Buds and Fragmented Nucleus.

Statistical Analysis: The samples were coded at the time of preparation and scoring. They were decoded before statistical analysis for comparison. Mean and standard deviation (SD) were calculated for biomarkers. Mean values and standard deviations were computed for the scores and the statistical significance (P < 0.001).

Cytogenetic analysis

Chromosome Preparation: Chromosome preparations were obtained from Phytohaemagglutinin-stimulated peripheral blood lymphocytes (Hungerford, 1965). About 2 ml of venous blood sample was collected from each PCOS patients and control subjects in sterile Heparinized syringes. 0.5ml of the blood was inoculated into the vials containing 5ml of Ready mix RPMI 1640 medium containing 1 ml of Fetal Bovine Serum (FBS) and 0.2ml of Phytohaemagglutinin under aseptic condition. The culture vials were then placed in an incubator at 37°C. The cultures were shaken every 24th hour and carbon dioxide was released once in 24 hours. At the 72nd hour harvested the culture. At the end of the incubation period (72nd hour), the dividing cells were arrested at metaphase by adding 2 drops of 0.1% Colchicine solution to each culture vial. The cultures were incubated further for 20 minutes at 37°C. (Hungerford et al.1965). Lymphocytes were harvested after 20 min by centrifuging cell suspension to remove culture medium (2000 rpm), addition of Hypotonic solution (KCl 0.075 M) at 37°C for 6-7 min to swell the cells, and treated twice with Carnoy’s fixative (3:1 ratio of methanol: acetic acid). Slides were carefully dried on a hot plate (40°C). Later, the slides were stained and banded with Giemsa stain.

Slide preparation: The cell button was suspended in a small quantity of freshly prepared fixative. A test slide was prepared by gently placing a drop of the cell suspension on a cleaned glass slide and dried immediately on a hot plate (400C). The test slide was examined under the microscope for cell density and metaphase spreads. Other slides were prepared after suitable modifications.

Staining procedure: The slides were stained in 4% Giemsa solution for 4 minutes and destained in double distilled water for 2minutes.

Scoring: A minimum of 50 technically good metaphase plates of each sample was analyzed. Scoring of chromosomal aberrations including chromatid and chromosomal breaks and deletions were carried out in well spread and stained cells under oil immersion objective lens (100X) of the light microscope (Magnus MPX) to identify numerical and structural Chromosomal aberrations.

Giemsa Banding: 2-3 days old slide with good metaphase spreads were taken. The slides were treated with 0.0125% freshly prepared trypsin solution for 4-5 seconds and rinsed with double distilled water. The above treated slides were stained using Giemsa stain for 4 minutes and were thoroughly air dried.

Microphotography: Well spread metaphases of Giemsa stained and Giemsa banded spreads were selected and photographed under oil immersion objective lens (100X) of Leica DM2000 microscope with Metasystems camera and the photomicrographs of banded spreads were karyotyped using automatic IKaros software (Metasystems).

Statistical analysis: The samples were coded at the time of preparation and scoring. They were decoded before statistical analysis for comparison. Mean and standard deviation (SD) were calculated. The significance of the differences was found between control and PCOS. Mean values and standard deviations were computed for the scores and the statistical significance (P < 0.001).

Results

Clinical analysis

This study is carried out with 10 PCOS patients with 4 Control samples with age between 18 to 28 years. Among the entire patient group we have observed that they have irregular periods and 70% patients having facial acne but we did not observe any insulin resistance, Cardio or Acanthosis Nigricans symptoms. In this study we have observed the range of total Hirsutism score from 12-24 and mean value of total testosterone level was 23.6nmol/L. Among the patient group some females have been suffering from PCOS from long term and we found that one of the female has age of onset of irregular periods from of 13 years (Table 1A). In our study we have observed 7-12 Subcapsular follicular cysts; each measure 2-8mm in diameter. Ovarian morphology was recorded and presented in Table1B.

Dermatoglyphics analysis

Dermatoglyphics analyses were carried out for all the 10 cases of PCOS and 4 control subjects. The Dermatoglyphics patterns observed in right and left hands of controls and PCOS patients were presented in Table 2A. The frequency of inheritance patterns observed in controls and PCOS patients were presented in Table 2B. The Dermatoglyphics illustrations were presented in Figure1. In this study we have observed that mean value of ATD angle of right and left hand of control is 41.87° and for PCOS patient is 45.95° and it conclude that PCOS patients have the elevated ATD angle when compare with control group (Figure 1). The inheritance pattern type observed in PCOS patients are 3 (30%) dominant type inheritance and 7 (70%) semi dominant type of inheritance and for control are 1 (25%) of them are dominant inheritance and 3 (75%) semi dominat type inheritance. Increased frequency of inheritance pattern observed in PCOS patients are 19 (38%) Ulnar loops on both fingers (UU)=Semidominant type of inheritance, 9 (18%) Whorls on right finger and Ulnar loop on left finger (WU)=Dominant type of inheritance and 8 (16%) Arches on right and left finger (AA)=Dominant type of inheritance and for controls are 6 (30%) Whorls on right and left finger (WW)=Semidominant type of inheritance, 4 (20%) Ulnar loops on both fingers (UU)=Semidominant type of inheritance, 8 (15%) Arches on left finger and Ulnar loops on right finger (AU)=Dominant type of
inheritance. Percentage of predominant dermatoglyphic pattern observed in control and PCOS patients were presented in Figure 2.

**Buccal micronucleus cytome (BMCyt) assay**

Buccal Cell Analysis: Exfoliated Buccal cell micronuclei analysis was carried out in all the 10 PCOS patients and 4 age and sex matched control samples. The Buccal cell aberrations observed in PCOS patients and control samples in following frequencies. The percentage of Binucleated cells was found to be in 7.4% for PCOS patients and for control 9.25%. The frequency of Binucleated cells per 500 cells was found to be significantly lower in PCOS patients than controls. [PCOS patient’s (mean±SD): 37±23.24 versus controls 46±31.45]. The percentage of micronuclei in cells was 0.10% in PCOS patient’s and 0.25% in controls, the frequency of total number of micronuclei per 500 cells (mean±SD) is 0.5±1.58 in PCOS patient’s and for controls it is 1.25±2.5.

The percentage of Trinucleated cells was 1.10% in PCOS patient’s and 2.7% in controls. The frequency of trinucleated cells per 500 cells (mean±SD) is 0.6±0.9 in PCOS patient’s and for controls it is 1.4±0.8.

## Table 1. Clinical and Medical History of PCOS Patients

| Patient Code | Age | Height | Weight | BMI | Marital Status | Age of Onset | Acne | Cardio problem | Obesity | Diabetics | Total Testosterone (nmol/L) | Irregular periods | Hirsutism total score | Total Testosterone (nmol/L) | Acne | Cardio problem | Obesity | Diabetics | Total Testosterone (nmol/L) | Irregular periods | Hirsutism total score | Total Testosterone (nmol/L) |
|--------------|-----|--------|--------|-----|----------------|-------------|------|----------------|---------|------------|------------------------|-----------------|------------------------|------------------------|------|-----------------|---------|------------|------------------------|-----------------|------------------------|------------------------|
| PCOS 01      | 24  | 143    | 18.97  | N   | N              | 24          | N    | N             | N       | N          | 22.87                  | N               | N                      | 22.87                  | N    | N              | N       | N          | 22.87                  | N               | N                      | 22.87                  |
| PCOS 02      | 24  | 150    | 26.66  | N   | N              | 18          | N    | N             | N       | N          | 40                     | N               | N                      | 40                     | N    | N              | N       | N          | 40                     | N               | N                      | 40                     |
| PCOS 03      | 18  | 160    | 26.88  | N   | N              | 19          | Y    | Y             | Y       | Y          | 26                     | Y               | Y                      | 26                     | Y    | Y              | Y       | Y          | 26                     | Y               | Y                      | 26                     |
| PCOS 04      | 19  | 156    | 32.87  | N   | N              | 19          | Y    | Y             | Y       | Y          | 21                     | Y               | Y                      | 21                     | Y    | Y              | Y       | Y          | 21                     | Y               | Y                      | 21                     |
| PCOS 05      | 18  | 150    | 32.87  | N   | N              | 18          | Y    | Y             | Y       | Y          | 23                     | Y               | Y                      | 23                     | Y    | Y              | Y       | Y          | 23                     | Y               | Y                      | 23                     |
| PCOS 06      | 18  | 150    | 32.87  | N   | N              | 18          | Y    | Y             | Y       | Y          | 24                     | Y               | Y                      | 24                     | Y    | Y              | Y       | Y          | 24                     | Y               | Y                      | 24                     |
| PCOS 07      | 18  | 150    | 32.87  | N   | N              | 18          | Y    | Y             | Y       | Y          | 24                     | Y               | Y                      | 24                     | Y    | Y              | Y       | Y          | 24                     | Y               | Y                      | 24                     |
| PCOS 08      | 18  | 150    | 32.87  | N   | N              | 18          | Y    | Y             | Y       | Y          | 24                     | Y               | Y                      | 24                     | Y    | Y              | Y       | Y          | 24                     | Y               | Y                      | 24                     |
| PCOS 09      | 18  | 150    | 32.87  | N   | N              | 18          | Y    | Y             | Y       | Y          | 24                     | Y               | Y                      | 24                     | Y    | Y              | Y       | Y          | 24                     | Y               | Y                      | 24                     |
| PCOS 10      | 18  | 150    | 32.87  | N   | N              | 18          | Y    | Y             | Y       | Y          | 24                     | Y               | Y                      | 24                     | Y    | Y              | Y       | Y          | 24                     | Y               | Y                      | 24                     |

*Y-Yes, N-No, Me-Medium, L-Little, M-Married, UM-Unmarried*
### Table 1B. Ovarian Morphology of PCOS Patients

| Patient Code | Ovary Measurement | No of Follicles | Size of Follicles |
|--------------|-------------------|----------------|------------------|
|              | Right(cm)         | Left(cm)       | Right Ovary      | Left Ovary      | Right | Left |
| PCOS 01      | 3.6 X 2.0         | 3.5 X 2.0      | 10               | 11              | 3mm   | 5mm  |
| PCOS 02      | 2.1 X 1.6         | 2.5 X 1.5      | 10               | 10              | 4mm   | 4mm  |
| PCOS 03      | 4.3 X 3.2         | 3.7 X 3.4      | 9                | 12              | 5mm   | 4mm  |
| PCOS 04      | 2.8 X 3.1         | 3.5 X 2.7      | 12               | 11              | 4mm   | 6mm  |
| PCOS 05      | 3.7 X 2.8         | 4X 2.5         | 8                | 11              | 5mm   | 5mm  |
| PCOS 06      | 4.0 X 26.1        | 4.41 X 2.35    | 9                | 10              | 3mm   | 3mm  |
| PCOS 07      | 4.7 X 2.5         | 4.2 X 1.7      | 8                | 9               | 6mm   | 5mm  |
| PCOS 08      | 3.5 X 2.4         | 3.1 X 2.2      | 10               | 12              | 5mm   | 5mm  |
| PCOS 09      | 3.3 X 2.8         | 4 X 2.7X 2.5   | 7                | 9               | 3mm   | 5mm  |
| PCOS 10      | 4.5 X 3.5         | 3.7 X 3.1      | 11               | 13              | 4mm   | 4mm  |

cm-Centimeter. mm-Millimeter

### Table 2A. Dermatoglypics Pattern Observed in Right and Left Hand of Controls and PCOS Patients

| Lab Code | Thumb finger | Index finger | Middle finger | Ring finger | Little finger | Inheritance pattern | Right hand palm angle | Left hand palm angle | Mean value of right and left hand angle |
|----------|--------------|--------------|---------------|-------------|---------------|---------------------|-----------------------|----------------------|----------------------------------------|
| CON01    | WW(SD)       | UU(SD)       | AU(D)         | WW(SD)      | UU(SD)        | SD                  | 40                    | 40                   | 40                                      |
| CON 02   | AA(D)        | AA(D)        | RU(RE)        | UU(SD)      | UU(SD)        | SD                  | 43                    | 42                   | 42.5                                   |
| CON 03   | AU(D)        | UA(D)        | AU(D)         | WA(D)       | WU(D)         | D                   | 43                    | 41                   | 42                                      |
| CON 04   | WW(SD)       | WW(SD)       | WW(SD)        | WW(SD)      | WU(D)         | SD                  | 42                    | 44                   | 43                                      |
| Mean Value |              |              |               |             |               |                     | 42                    | 41.75                 | 41.87                                  |
| PCOS 1   | WW(SD)       | WU(SD)       | WU(D)         | WW(SD)      | WU(D)         | SD                  | 46                    | 42                   | 44                                      |
| PCOS 2   | AA(D)        | AA(D)        | AA(D)         | AA(D)       | D             |                     | 46                    | 47                   | 46.5                                   |
| PCOS 3   | AA(D)        | AU(D)        | UU(SD)        | WU(D)       | UU(SD)        | D                   | 50                    | 47                   | 48.5                                   |
| PCOS 4   | UU(SD)       | WW(SD)       | WU(D)         | UU(SD)      | UU(SD)        | SD                  | 46                    | 44                   | 45                                      |
| PCOS 5   | AU(D)        | RW(RE)       | UU(SD)        | UU(SD)      | UU(SD)        | SD                  | 54                    | 48                   | 51                                      |
| PCOS 6   | UW(SD)       | UU(SD)       | UU(SD)        | UW(SD)      | UU(SD)        | SD                  | 47                    | 46                   | 46.5                                   |
| PCOS 7   | WW(SD)       | WU(D)        | UU(SD)        | WU(D)       | WU(D)         | D                   | 47                    | 47                   | 47                                      |
| PCOS 8   | UU(SD)       | UW(SD)       | UU(SD)        | AU(D)       | AW(D)         | SD                  | 39                    | 46                   | 42.5                                   |
| PCOS 9   | AA(D)        | UA(D)        | UU(SD)        | UU(SD)      | UU(SD)        | SD                  | 42                    | 47                   | 44.5                                   |
| PCOS 10  | AU(D)        | AA(D)        | UU(SD)        | UU(SD)      | UU(SD)        | SD                  | 42                    | 46                   | 44                                      |
| Mean Value |              |              |               |             |               |                     | 45.9                  | 46                   | 45.95                                  |

PCOS patient’s and for controls 0.25%, the frequency of total number of Trinucleated cells per 500 cells (mean±SD) is 5.5±6.85 is in PCOS patient’s and for controls it is 1.25±2.5. The percentage of fragmented nucleus was 0.10% in PCOS patient’s and for controls 0%, the frequency of total number of fragmented nucleus per 500 cells was (mean±SD) 0.5±1.58 in PCOS patient’s and for controls 0.00±0.00. The percentage of karyolysed nucleus was 30.50% in PCOS patient’s and for controls 7.25%, the frequency of total number of karyolysed nucleus per 500 cells was (mean±SD) 0.10% in PCOS patient’s and for controls 0%, the frequency of total chromosome aberrations is 1.5±1.78. In PCOS patients with 4 cases of age and sex matched controls, the results were presented in Table 4A and 4B. G banded Karyotyping analysis revealed 46. XX karyotype in all the 10 PCOS patients and 4 controls. In chromosome aberration analysis of PCOS patients, we have observed different types of chromosome aberrations like chromosome breaks as well chromatid breaks. The total chromosome aberrations in controls were found 4 out of 200 metaphases, in which percentage of aberrations were 2.0% and the frequency of total aberration is 1±0 (mean and SD) (Figure 4A). In PCOS patients the total number of chromosome aberration is 15 out of 500 metaphases, in which percentage of aberrations were 3% and the frequency of total chromosome aberrations is 1.5±1.78. The percentage and frequency of chromosome aberrations were significantly higher in PCOS patients than in controls (Figure 4B). Mean values and standard deviations were presented in Table 3B.

#### Cytogenetic analysis

Cytogenetic analysis was performed in 10 PCOS patients with 4 cases of age and sex matched controls, the results were presented in Table 4A and 4B. G banded Karyotyping analysis revealed 46. XX karyotype in all the 10 PCOS patients and 4 controls. In chromosome aberration analysis of PCOS patients, we have observed different types of chromosome aberrations like chromosome breaks as well chromatid breaks. The total chromosome aberrations in controls were found 4 out of 200 metaphases, in which percentage of aberrations were 2.0% and the frequency of total aberration is 1±0 (mean and SD) (Figure 4A). In PCOS patients the total number of chromosome aberration is 15 out of 500 metaphases, in which percentage of aberrations were 3% and the frequency of total chromosome aberrations is 1.5±1.78. The percentage and frequency of chromosome aberrations were significantly higher in PCOS patients than in controls (Figure 4B). Mean values and standard deviations were presented in Table 3B.
Table 2B. Frequency of Patterns Observed in Controls and PCOS Patients

| Patterns | Thumbs | Index | Middle | Ring | Little | Total | % | Patterns | Thumbs | Index | Middle | Ring | Little | Total | % |
|----------|--------|-------|--------|------|--------|-------|----|----------|--------|-------|--------|------|--------|-------|----|
| AA       | 1      | 1     | 0      | 0    | 0      | 2     | 10 | AA       | 3      | 2     | 1      | 1    | 1      | 8     | 16 |
| AR       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | AR       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| AU       | 1      | 0     | 0      | 0    | 0      | 1     | 5  | AU       | 2      | 1     | 0      | 1    | 0      | 4     | 8  |
| AW       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | AW       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| RA       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | RA       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| RR       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | RR       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| RU       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | RU       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| RW       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | RW       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| WA       | 0      | 0     | 0      | 1    | 0      | 1    | 5  | WA       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| UA       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | UA       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| UR       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | UR       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| UW       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | UW       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| WA       | 0      | 0     | 0      | 1    | 0      | 1    | 5  | WA       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| WR       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | WR       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| RU       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | RU       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| RW       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | RW       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| WA       | 0      | 0     | 0      | 1    | 0      | 1    | 5  | WA       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| WR       | 0      | 0     | 0      | 0    | 0      | 0     | 0  | WR       | 0      | 0     | 0      | 0    | 0      | 0     | 0  |
| WW       | 2      | 1     | 1      | 2    | 0      | 6     | 30 | WW       | 2      | 1     | 0      | 1    | 0      | 4     | 8  |

Table 3A. Statistical Analysis of the Buccal Cell abnormalities observed in the Patient and Control Samples

| Patients Code | No of cell scored | Frequency of MN Cells | Frequency of BN Cells | Frequency of Mni Cells | Frequency of cells with Tri nucleated | Frequency of fragmented nucleus | Frequency of karyolysis nucleus | Total no. of aberrations | % of aberration |
|---------------|-------------------|-----------------------|-----------------------|------------------------|--------------------------------------|---------------------------------|-------------------------------|------------------------|----------------|
| PCOS 01       | 500               | 420                   | 15                    | 5                      | 0                                    | 0                              | 60                            | 80                     | 16             |
| PCOS 02       | 500               | 301                   | 55                    | 0                      | 0                                    | 0                              | 0                             | 144                    | 199            |
| PCOS 03       | 500               | 305                   | 50                    | 0                      | 10                                   | 0                              | 135                           | 195                    | 39             |
| PCOS 04       | 500               | 250                   | 10                    | 0                      | 0                                    | 0                              | 0                             | 240                    | 250            |
| PCOS 05       | 500               | 335                   | 50                    | 0                      | 20                                   | 0                              | 95                            | 165                    | 33             |
| PCOS 06       | 500               | 220                   | 30                    | 0                      | 5                                     | 0                              | 245                           | 280                    | 56             |
| PCOS 07       | 500               | 310                   | 25                    | 0                      | 10                                   | 5                              | 150                           | 190                    | 38             |
| PCOS 08       | 500               | 345                   | 50                    | 0                      | 0                                    | 0                              | 0                             | 150                    | 155            |
| PCOS 09       | 500               | 275                   | 55                    | 0                      | 10                                   | 0                              | 160                           | 225                    | 45             |
| PCOS 10       | 500               | 280                   | 75                    | 0                      | 0                                    | 0                              | 145                           | 220                    | 44             |
| Total         | 5000              | 3041                  | 370                   | 5                      | 55                                   | 5                              | 1524                          | 1959                   | 391.8          |

Mean ±SD 304.1±55.3, 37 ±23.24, 0.5±1.58, 5.5±6.85, 0.5±1.58, 152.4±56.35, 195.9±55.31, 3.9±11.06

Statistical Analysis of the Buccal Cell Observed in the Control

| Patients Code | No of cell scored | Frequency of MN Cells | Frequency of BN Cells | Frequency of Mni Cells | Frequency of cells with Tri nucleated | Frequency of fragmented nucleus | Frequency of karyolysis nucleus | Total no. of aberrations | % of aberration |
|---------------|-------------------|-----------------------|-----------------------|------------------------|--------------------------------------|---------------------------------|-------------------------------|------------------------|----------------|
| Con01         | 500               | 440                   | 30                    | 0                      | 0                                    | 0                              | 30                            | 60                     | 12             |
| Con02         | 500               | 425                   | 70                    | 0                      | 5                                    | 0                              | 10                            | 85                     | 17             |
| Con03         | 500               | 335                   | 75                    | 0                      | 0                                    | 0                              | 90                            | 165                    | 33             |
| Con04         | 500               | 470                   | 10                    | 5                      | 0                                    | 0                              | 15                            | 30                     | 6              |
| Total         | 2000              | 1670                  | 185                   | 5                      | 5                                    | 0                              | 145                           | 340                    | 13.6           |

Mean ±SD 417.5±58.09, 46±31.45, 1.25±2.5, 1.25±2.5, 0.00±0.00, 36.25±36.82, 85±57.8, 1.78±11.58

*MN=Mon nucleated, BN=Bi nucleated, Mni=Micro Nuclei
Multi-Parameter Approach for Evaluation of Genomic Instability in the Polycystic Ovary Syndrome

Discussion

In the present study, we have used multi-parameter analysis techniques including clinical, Dermatoglypics, exfoliated Buccal cell micronuclei and chromosome analysis were carried out to study the genomic instability computed for the scores and it is proved the statistically significance (P < 0.001).

Table 3B. Statistical Analysis of the Buccal Cell Abnormalities Observed in the Patient and Control Samples

| BMCyt Assay variables | Controls | Patient |
|-----------------------|----------|---------|
| percentage            | Mean ± SD| percentage | Mean ± SD |
| Bi-nucleated          | 9.25%    | 46±31.45 | 7.41%    | 37±23.24 |
| Micronuclei           | 0.25%    | 1.25±2.5 | 0.10%    | 0.5±1.58 |
| Tri nucleated         | 0.25%    | 1.25±2.5 | 1.10%    | 5.5±6.85 |
| Fragmented nucleus    | 0%       | 00±00    | 0.10%    | 0.5±1.58 |
| Karyolysed nucleus    | 7.25%    | 36.25±36.82 | 30.50% | 152.4±56.35 |
| Total no of aberrations | 17%    | 85±57.88 | 39.21 | 195.9±55.31 |

Table 4. A) Cytogenetic Analysis of PCOS Patients; B) Cytogenetic Analysis of Control Samples

| Patients Code | Karyotype | Ch. Bks. | Chd. Bks. | Dic. ch | Rg. Ch. | Tt. No. of Ch Abs | % of Ch. Abs | No. of Abs Per Cell |
|---------------|-----------|----------|-----------|---------|---------|------------------|-------------|-------------------|
| A. Cytogenetic analysis of PCOS patients |
| PCOS 01       | 46,XX     | 50       | 0         | 0       | 0       | 0                | 0           | 0                 |
| PCOS 02       | 46,XX     | 50       | 2         | 2       | 0       | 0                | 4           | 8                 |
| PCOS 03       | 46,XX     | 50       | 2         | 1       | 0       | 1                | 4           | 8                 |
| PCOS 04       | 44XX      | 50       | 0         | 0       | 0       | 0                | 0           | 0                 |
| PCOS 05       | 46,XX     | 50       | 0         | 0       | 0       | 0                | 0           | 0                 |
| PCOS 06       | 46,XX     | 50       | 0         | 0       | 0       | 0                | 0           | 0                 |
| PCOS 07       | 46XX      | 50       | 0         | 0       | 0       | 0                | 0           | 0                 |
| PCOS 08       | 46,XX     | 50       | 0         | 0       | 0       | 0                | 0           | 0                 |
| PCOS 09       | 46,XX     | 50       | 0         | 3       | 0       | 0                | 3           | 6                 |
| PCOS 10       | 46,XX     | 50       | 0         | 3       | 0       | 0                | 3           | 6                 |
| TOTAL         | 500       | 5        | 9         | 0       | 1       | 15               | 3           | 0.03±0.03         |
| Mean ±SD      | 0.5±0.85  | 0.9±1.29 | 0         | 0.1±0.32| 1.5±1.78 | 3±0.55          | 0.03±0.03   |
| B. Cytogenetic analysis of control samples |
| Con01         | 46,XX     | 50       | 0         | 1       | 0       | 0                | 1           | 2                 |
| Con02         | 46,XX     | 50       | 1         | 0       | 0       | 0                | 1           | 2                 |
| Con03         | 46,XX     | 50       | 1         | 0       | 0       | 0                | 1           | 2                 |
| Con04         | 46,XX     | 50       | 0         | 1       | 0       | 0                | 1           | 2                 |
| Total         | 200       | 2        | 2         | 0       | 4       | 2                | 4           | 0.02±0.02         |
| Mean ±SD      | 0.5±0.57  | 0.5±0.57 | 0±0       | 0±0     | 1±0     | 2±0              | 2±0         | 0.02±0            |

Tt. No.=Total number; Ch=Chromosome; Chd: Chromatid; Bks: Breaks; Dic;Dicentric ; RG: Ring; Abs: Aberrations

Figure 3. Buccal Cell Micronuclei Assay in PCOS Patients. A-represent mono-nucleated cell ; B- represents bi-nucleated cell; C-represents karyolysed cell; D-represents tri-nucleated cell; E-represents micro-nucleated cell

Figure 4. A-Giemsa Stained Chromosome Picture of Control Sample; B-Giemsa Stained Chromosome Picture of PCOS Patient and Arrow Represents the Chromatid Breaks

computed for the scores and it is proved the statistically significance (P < 0.001).
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women with polycystic ovarian syndrome in the region of Vellore, Tamilnadu. The study group included 10 PCOS women and a control group of 4 women. The age in our study was 18 - 28 years. Menstrual disturbances commonly observed in PCOS include Oligomenorrhea, Amenorrhea, and prolonged erratic menstrual bleeding (Farquhar, 2007). Among the entire patient group we have observed that they have irregular periods with little or Medium Acne, but we did not observe any Diabetic, Cardio or Acanthosis Nigricans symptoms. Approximately 85%-90% of women with Oligomenorrhea have PCOS while 30%-40% of women with amenorrhea will have PCOS (Hart, 2007). More than 80% of women presenting with symptoms of androgen excess have PCOS (Aziz et al., 2004). In our study we have observed the mean value of total testosterone level was 23.6 nmol/L and total Hirsutism score from 12-24 in PCOS patients. (Elif Yesilada et al., 2006) also reported that they have observed the average value of total testosterone level was 24 nmol/L and a total Hirsutism score of 4 in healthy control subjects and a total score of 19.5 in PCOS patients. Hirsutism is a common clinical presentation of Hyperandrogenism occurring in up to 70% of women with PCOS (Fauser et al., 2012). We could find acneal in 70% patients.

Study has reported that approximately 15%-30% of adult women with PCOS present with acne (Wijeyaratne et al., 2002; Aziz et al., 2004). Some experts recommend that women presenting with acne be asked about their menstrual history and be evaluated for other signs of Hyperandrogenism (Lowenstein, 2006). A polycystic ovary defined as the presence of at least 10 subcapsular follicular cysts, each measures 2-8mm in diameter and arranged around or within thickened ovarian stroma and is diagnosed by pelvic ultrasonography (Adams et al., 1985). This study we have observed the 7-12 subcapsular follicular cysts, each measure 2-8mm in diameter. Obesity is the often characteristics of PCOS. The prevalence of insulin resistance in PCOS ranges from 50%-70% and occurs independently of obesity (Dunaif et al., 1989).

In this study we did not observed obesity and insulin resistance in PCOS patients, may be because of small sample size.

Dermatoglyphics has been considered as a growing science with an immense practical value and it has been emphasized as one of the diagnostic tool which aids the Cytogeneticist to look for specific abnormalities and identification of abnormal chromosomes. In the present study, we found increase in mean value (45.9°) of ATD angle of right and left hand of PCOS patients. In one of study (Nikha et al., 2013) reported that increase in the ATD angle value (44.3°) in obese females. In one of the study they have observed increased frequency of total radial loops and total whorls and decreased frequency of total ulnar loops and total arches (Shweta et al., 2014). This study we have observed increased frequency (38%) of Ulnar loops on both fingers (UU), (18%) Whorls on right finger and Ulnar loop on left finger (WU) and (16%) Arches on right and left finger (AA) were observed. Among the frequency of finger print patterns, loops seemed to be of higher frequency in controls as well as in amenorrheic subjects (Meenakshi et al., 2006). This increase in the loop pattern has also been reported by Forbes 4 and (Mutalik et al., 2003) in their study on gonadal dysgenesis and amenorrhea respectively. But in this study we observed (30%) Whorls on right and left finger (WW), (20%) Ulnar loops on both fingers (UU) and (15%) Arches on left finger and Ulnar loops on right finger (AU).

Features which could be applied as markers for PCOS patients are the presence of Ulnar loops in Middle and Little fingers of right and left hand. (Meenakshi S, 2006) reported that the features which could be applied as markers for amenorrhea are the presence of arch pattern in the 2nd left finger, loop pattern on the 5th right finger. The present study has emphasized the application of Dermatoglyphics as one of the diagnosis tools for referral of PCOS patients for Ultrasound scan. In the present study we found reduction in frequency of Whorls on right and left finger when compare with control subjects. Reduction in frequency Total Loops (TL) and total arches (TA) were observed in primary amenorrhea cases (Meenakshi S, 2006). Some authors found increased ulnar loops (6 of 10 cases) when Dermatoglyphic study was carried out in ten female patients with sex chromatin abnormalities and genital tract anomalies complaining of primary amenorrhea (Mutalik et al., 1968). We also found increased ulnar loops when Dermatoglyphic analysis on 10 PCOS cases. Some authors found increased frequency of ulnar loops on first finger of right hand when study was carried out in 52 women with the Ullrich-Turner syndrome (Otto PA and Otto PG, 1980). Another author found higher frequency of loops in both controls and primary amenorrhea cases frequency was especially higher in subjects with abnormal karyotype (Meenakshi et al., 2006). Dermatoglyphics may be a useful screening method to identify the patients at risk and referral of such individuals for ultrasound scan so that a watch may be kept for the early onset of symptoms of polycystic ovarian syndrome.

The Buccal Micronucleus Cytome assay in exfoliated Buccal cells is utilized as biomarkers for DNA damage, cell death and basal cell frequency. It offers great opportunity to evaluate Genotoxicity by the way of quantifying mean frequencies of Micronuclei, Binucleated cell, broken egg, karyolysis, karyorrhexis, pyknosis and condensed chromatin. This assay is sensitive, minimally invasive, simple, cheap, easy and fast (Yadav et al., 2015; Jaggi et al., 2015).

According to (Holland et al., 2008) about 90% of all cancers are derived from epithelial cells. Since more than 90% of all human cancers are of epithelial origin, MN assay with Buccal epithelial cells is the most suitable biomonitoring approach for the detection of increased cancer risk in humans. Buccal cells have limited DNA repair capacity relative to peripheral blood lymphocytes, and therefore, may more accurately reflect age-related genomic instability event in epithelial tissue (Dhillon et al., 2004). A significant increase in the frequencies of MN was observed in exfoliated Buccal cells of polycystic ovarian syndrome patients (Nerseyan and Chobanyan, 2010). In this study we found a insignificant frequencies of MN was observed in exfoliated buccal cells of polycystic ovarian
syndrome patients, may be because of very small sample size. Karyolysis represent an advanced stage of necrosis and apoptosis (Majno and Joris, 1995). In our study we found significant increases in frequencies of Karyolyised nucleus in exfoliated Buccal cells PCOS patients. Micronuclei in exfoliated buccal cells reflect Genotoxic events that occurred in the dividing basal layer 1-3 weeks earlier (Stich et al., 1983, 1984). The frequency of occurrence of MNII is a measure of chromosome breakage in early cell divisions, and the number of micronuclei is known to increase with carcinogenic stimuli, long before the development of clinical symptoms (Stich et al., 1984).

Genetic instability can have very serious consequences for PCOS patients because of established correlations of increased levels of MN and chromosomal aberrations with cancer incidence (Bonassi et al., 2005).

(Andrea et al., 2001) stated that family studies have indicated a genetic susceptibility to PCOS. Another group studied chromosomal aberrations level in lymphocytes of 15 PCOS patients from Armenia and found 2.25-fold significant increase of this parameter compared with healthy females (Nersesyan et al., 2006). In one of the previous study on beedi workers, we observed 3.63-fold significant increase of chromosomal aberrations when compared with healthy control group (Rajiv et al., 2013). In this study we found significant increase in frequency of chromosome aberration (CA) in PCOS patients when compare with control group. (Nersesyan et al., 2006) concluded that females with PCOS have increased chromosomal aberrations (CAs) level in lymphocytes which is a sign of genetic instability. There is a hypothesis that micronuclei and chromosomal aberrations could have a predictive value for cancer and therefore substitute chromosomal aberrations as cancer risk biomarkers (Aardema et al., 1998). Women with PCOS have a nearly 3 times increased risk for developing endometrial cancer, the association between PCOS and endometrial cancer involves prolonged endometrial exposure to unopposed estrogen by cyclic progesterone due to anovulation (Tokmak et al., 2013).

Acknowledgements

The authors are indebted to patients for providing us with blood samples. The authors would also like to thank the management of VIT University for providing the facilities to carry out this work. The author Nishu is grateful to VIT University for providing the financial assistance during this tenure.

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