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Original Research Article

A study on micronucleus frequency in cervical smear as biomarker for genetic damage in polycystic ovarian syndrome

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a multifactorial endocrine disorder with metabolic and reproductive consequences. Genomic damage and susceptibility to cancer are noteworthy concerns in PCOS. Relationship between PCOS and endometrial and ovarian cancer has already been established. The presence of high body mass index, excess triglycerides, oxidative stress and occurrence of metabolic syndrome is a frequent occurrence in PCOS. This may succeed into genetic damage and susceptibility to cervical cancer as they are also known risk factors of the same. The aim of the study was to estimate the frequency of micronuclei in cervical smear and to determine if it can be used as a biomarker of genomic instability and susceptibility to cervical changes in the future in PCOS.
Methods: This observational case control study included 38 subjects diagnosed with PCOS by Rotterdam's criteria and 38 controls and was conducted between September 2018 to March 2020 in VIMS and RC. Data regarding age and anthropometric details and cervical smear samples was collected from all the subjects. The frequency of micronuclei in cervical smears was expressed as mean±standard deviation (SD). Differences between the PCOS group and the control group were examined for statistical significance using two-sample independent t-test. A p value of ≤0.05 denoted statistically significant difference.
Results: The mean±SD of micronuclei frequencies in cervical smears was observed to be 1.69±0.69 and 0.33±0.18 (p value <0.0001) in the subjects with PCOS and control group, respectively.
Conclusions: Micronuclei frequency was found to be elevated in cervical smears of women with PCOS when compared to controls indicating genetic instability and probable susceptibility to cervical cancer in the future in women with PCOS.

Keywords: Micronucleus, PCOS, Biomarker

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a multifactorial complex endocrinopathy with an estimated global prevalence of 4% to 20% in women of reproductive age group.[1] It is a polygenic lifelong disorder where metabolic and reproductive consequences have been widely documented at all stages of life. Aside from being a leading cause of female infertility, the condition is a well-known risk factor of type 2 diabetes, impaired glucose tolerance, obesity and metabolic syndrome.[2] Although the clinical signs of PCOS arise in adolescence, it may begin in the very early phases of development, potentially even during intrauterine life.[3] This condition is either genetically inherited or caused by epigenetic changes in the intrauterine microenvironment.[4] Due to the interplay of postnatal environmental parameters and genetic predisposition, PCOS has a complex aetiology.[5] Women with PCOS have a higher number of chromosomal aberrations in their somatic cells, signifying genetic instability.[6] Genetic damage and chromosomal aberrations are commonly assessed by a biomarker called the micronucleus.[7] They are minute entities that form when an

acentric chromosome or whole chromosomes lag during anaphase and fail to integrate with the main nucleus during telophase.[8] Their appearance is an account of rise in carcinogen exposed tissues long before any clinical signs develop.[9,10] Women with PCOS have an elevated frequency of micronuclei in lymphocytes.[11-14] Even though PCOS is linked to several cancers like endometrial, ovarian and breast cancer, there is unsatisfactory data to evaluate an association between PCOS and cervical cancer.[7,15-17]

Studies show an elevation in frequency of micronuclei with progression of different stages of cervical cancer.[9] PCOS and cervical cancer have identical symptoms which includes high body mass index (BMI) and blood pressure, elevated triglycerides, oxidative stress due to DNA damage and occurrence of metabolic syndrome.[18] Since presence of micronuclei in cervical smear can produce evidence of genetic damage in cervical cells and there is only one study which has linked the same with statistical significance, this study was undertaken to assess genetic damage and susceptibility to cervical cancerous changes in women with PCOS.[14]

METHODS

Study subjects and design

This is an observational case–control study carried out in 76 women attending the obstetrics and gynaecology outpatient department (OPD) in Vydehi Institute of Medical Sciences and Research Centre (VIMS and RC), Bangalore, India. R software was used to calculate the sample size where two-sided two-sample t test and considering a significance level of 0.05 and 95% power, a total of 73 subjects (36 per arm) were required to be enrolled for the study.

The study was conducted between September 2018 to March 2020 and study included women aged between 18 to 45 years. 38 women diagnosed with PCOS based on the Rotterdam's 2003 criteria and 38 age matched controls with regular menstrual cycles and normal ovaries were recruited.[19] Women clinically diagnosed with congenital adrenal hyperplasia, Cushing's syndrome, androgen secreting tumours, functional hypothalamic amenorrhea and Smokers were excluded from the study. The study protocol was approved by institutional ethics committee (VIEC/2018/APP/048).

The study was conducted in accordance with the ethical principles mentioned in the Declaration of Helsinki. Written informed consent was obtained from all the women included in the study.

Anthropometric measurements like weight (in kg) and height (in cms) were assessed to calculate the BMI of the subjects. The height of the subjects was measured barefoot, head straight, arms hanging freely in the sides, line of vision straight to the body and heels in contact with the measuring board. The weight was measured using a calibrated electronic weighing machine. Subjects were wearing light clothes, standing straight with an evenly distributed body where both their feet were in the middle of the weighing machine without footwear. In accordance with World Health Organization (WHO) classification, BMI was calculated by first measuring weight in kilograms and height in centimetres and then using the following formula.

$$BMI = weight/height^2$$

BMI calculated as weight (kg)/ height $(m)^2$. BMI was later classified based on WHO criteria, where BMI <18.5 is considered underweight, 18.5 to 25 as normal, 25 to 30 as overweight, 30 to 35 as obese and BMI >35 as being severely obese.

Sample collection, processing and staining

Cervical smear samples were collected from all the subjects to assess the frequency of micronuclei. Smear samples was collected with cytobrush and placed into the vial containing the fixative and was thoroughly mixed. 3 ml of cell separator solution was added to the Tarson tube along with 7 ml of the smear obtained and was centrifuged for 5 minutes at 2000 rpm. The supernatant was discarded. A drop of saline was added to the Tarson tube containing the sample pellet and mixed thoroughly. The opticoat slide was fixed to the nanocyt slide holder and two drops of the sample was added to it. The slide holder with the slide was placed in the nanocyt centrifuge and centrifuged for 2 minutes at 2000 rpm. The slides were then hydrated in running tap water and dehydration in 70% alcohol 2 minutes. It was then stained in haematoxylin for 7 minutes and cyto stain for 2 minutes. Excess stain was removed under running tap water and after 2 to 3 washes in xylene, the slides were mounted in DPX. Collection and processing of samples has been depicted in Figure 1.

Scoring of micronuclei

The slides were observed by light microscopy under 100x. For each sample, 1000 epithelial cells were counted by zig-zag method. Micronuclei with well-defined nuclei and cell borders and those with similar texture, staining and diameter less than $1/3^{rd}$ of the main nucleus were counted.[20] Cells showing features of degeneration and apoptotic changes and those in clumps were not included.

Statistical analysis

The data was analysed using R software and expressed as mean±SD. Differences between the test group and the control group were examined for statistical significance using two-sample independent t-test.

A p value of ≤0.05 denoted the presence of a statistically significant difference.

RESULTS

A total of 76 patients, 38 subjects with PCOS who fulfilled the Rotterdam criteria and 38 controls were included in the study.

The age group of the subjects ranged between 20 years to 39 years with a mean of 28.24±4.33 years whereas, the mean age group in the control group was observed to be 26.53±4.40 years ranging from 20 to 34 years.

The mean weight and BMI of the subjects with PCOS were observed to be elevated when compared to the controls as depicted in Table 1.

Table 1: Anthropometric details of subjects with PCOS and controls.

Anthropom-etric details	PCOS group (mean±SD) N=38	Control group (mean±SD) N=38	P value
Weight (kg)	70.14±15.47	57.79±10.26	<0.0001
Height (cm)	156.31±5.32	157.64±6.61	0.3371
BMI (kg/m^2)	28.82±5.64	23.20±4.06	<0.0001

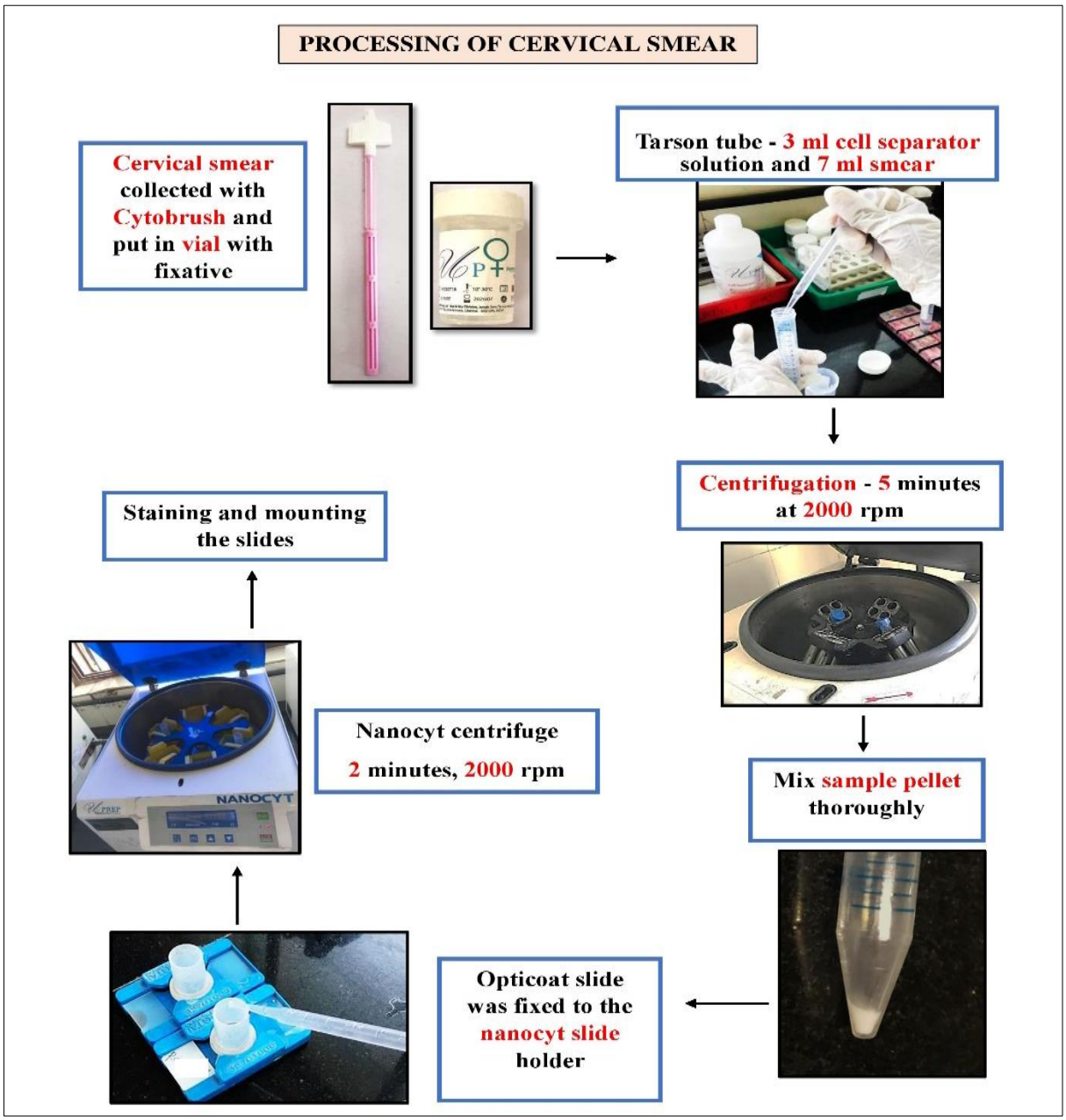

Figure 1: Steps followed in processing of cervical smears.

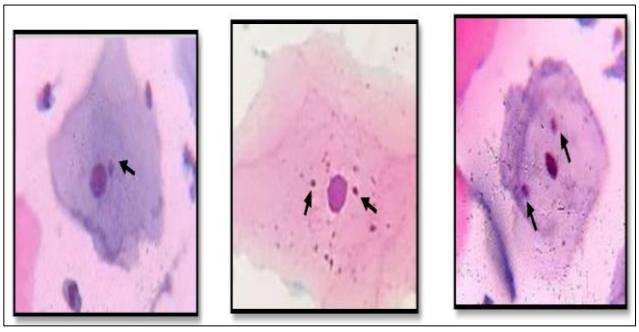

Figure 2: Micronuclei under 100x observed in cervical smears.

Based on two-sample independent t-test, mean micronuclei frequency in cervical smears was found to be elevated in subjects with PCOS when compared to the control group. The mean SD which was observed to be statistically significant has been depicted in Table 2. Micronuclei obtained in cervical smears has been depicted in Figure 2.

Table 2: Descriptive statistics of micronuclei in test and control group.

BMI (kg/m$^{2)}$	No. of subjects (%)	P value
Test group (N=38)	1.69±0.69	<0.0001
Control group (N=38)	0.33±0.18	

Table 3: Comparison of mean micronuclei frequency with normal and overweight BMI in subjects with PCOS.

BMI (kg/m$^{2)}$	No. of subjects (%)	Mean MN frequency
≤25	11 (28.9)	1.64±0.72
>25	27 (71.0)	1.74±0.95

The micronuclei frequency in subjects with PCOS whose BMI >25 kg/m^2 was observed to be higher when compared to subjects with a BMI ≤25 kg/m^2 as represented in Table 3.

DISCUSSION

PCOS is a multifaceted condition with a wide range of symptoms that affects not just women of reproductive age but also adolescents and postmenopausal women.[21] Due to the complexity and heterogeneity of this syndrome, a genetic basis has not yet been firmly established. However, a strong genetic component to the etiopathogenesis of PCOS is apparent.[22] It is evident that PCOS is genetically determined as the incidence of PCOS is twice as high in women with an affected twin as compared to general population.[23,24]

DNA damage is known to play a role in increasing the risk of PCOS especially in women with obesity and metabolic syndrome.[13,18] Susceptibility to cancer is initiated by genetic damage. Oxidative stress is a crucial component in PCOS pathogenesis and might be one of the key underlying causes of increased risk of gynaecological malignancies in PCOS.[7] It is possible that an excess of testosterone in PCOS maybe the major source of oxidative stress.[25] It is well documented that oxidative stress causes DNA damage, alters cell signalling pathways and has an impact on progression into cancers.[26] PCOS is known to coexist with endometriosis and these women have an increased probability of developing endometrial cancer due to absence of ovulation and long-term exposure to oestrogen.[20,27] PCOS women have a threefold increased risk of endometrial cancer and a twofold increased risk of ovarian cancer.[17] However, there are very few, but unsatisfactory data to assess a relationship between PCOS and cervical cancer. Presence of elevated BMI, blood pressure and triglycerides, oxidative stress and metabolic syndrome are common features of PCOS and cervical cancer.[18] Oral contraceptive pills (OCPs) have long been the first-line medication for women with PCOS as they continue to play an essential role in symptom management.[28] The usage of OCPs is also linked to an increased risk of cervical cancer.[29]

Micronuclei acts as a predictive biomarker of carcinogenesis much before the actual cancer appears.[10] Micronucleus assays are frequently assessed in stratified squamous non keratinized epithelial cells as they are actively dividing, undergoing wear and tear and as it can be exfoliated from the mucosa effortlessly.[30] In routine gynecological examinations, micronuclei in epithelial cells can be used as an additional criterion for the early detection of cytogenetic damage.[31] A positive linear correlation between the number of micronuclei and the risk of cervical cancer has been established and studies have concluded that mean micronuclei frequency in cervical smears gradually increases from subjects with atypical squamous cells of undetermined significance (ASC-US), cervical intraepithelial neoplasia (CIN), CIN II, CIN III and invasive cancer.[32] In the present study, the mean micronuclei frequency in cervical smears was found to be elevated (1.69±0.69) in PCOS group when compared to the control group (0.33±0.18) indicating that women with PCOS maybe susceptible to cervical changes in the future. A similar study which included 15 PCOS women and 11 controls, the mean MN frequency was found to be 1.19±0.57 and 0.74±0.34 in the test and control group, respectively.

Obese and overweight women have a higher risk of cervical cancer compared to normal and underweight women as determined by a retrospective cohort study of 9,44,227 women.[33] Obesity and body fat distribution were associated more strongly with adenocarcinoma of cervix in a case control study.[34] This shows that elevated BMI increases the risk of cervical cancer. High BMI is a hallmark symptom of PCOS. Women with PCOS have a

higher prevalence of android obesity where there is visceral fat accumulation which further exacerbates metabolic and reproductive features.[35-37] Increased mean BMI was observed in the PCOS group subjects in the present study. In the test group it was observed as 28.82±5.64 kg/m^2 and in the control group, it was found to be 23.20±4.06 kg/m^2.

These results were concomitant to many studies where the mean BMI in PCOS group was increased when compared to control group.[38-40] Increased genomic instability is seen in women with PCOS, especially those with a higher BMI.[38] In the present study, in women with PCOS with BMI >25 kg/m^2, micronuclei frequency in cervical smear were found to be increased when compared to women with BMI ≤25 kg/m^2. Similarly, in a cross-sectional study that included women with and without PCOS whose BMI was >25 kg/m^2, it was observed that micronucleus frequency was higher in women with PCOS than those without PCOS.[12]

CONCLUSION

PCOS is a multifaceted endocrine disorder. It is known to have a strong familial aggregation and multiple candidate genes has been investigated. The present study shows that DNA damage and susceptibility to cervical cancerous changes is increased in women with PCOS as assessed by presence of micronuclei in cervical smears. Micronuclei acts as a predictive biomarker of carcinogenesis, much before the actual cancer manifests and hence can be used in routine gynecological examinations as an additional criterion for the early detection of cytogenetic damage.

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