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ORIGINAL ARTICLE

Micronuclei and other nuclear anomalies levels in exfoliated buccal cells and DNA damage in leukocytes of patients with polycystic ovary syndrome

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Summary

Purpose: *To evaluate the genetic instability in somatic cells of patients with polycystic ovary syndrome (PCOS) by means of study of micronuclei (MN) level in exfoliated buccal cells and DNA damage in leukocytes.*

Methods: *The levels of MN in exfoliated buccal cells and DNA damage in leukocytes of 17 PCOS patients and 17 healthy women were studied. Except MN, other nuclear anomalies connected both with genotoxicity and cytotoxicity were evaluated. DNA damage was evaluated by means of the comet or single-cell gel electrophoresis assay in leukocytes.*

Results: *The results of our study showed significantly increased frequencies of MN but not of other nuclear anomalies in exfoliated buccal cells of PCOS patients. DNA in leukocytes was also found significantly damaged compared with healthy females.*

Conclusion: *Genetic instability can have very serious consequences for PCOS patients because of established correlations of increased levels of MN and chromosomal aberration with cancer incidence. Hence, more scrupulous investigations in this area are certainly warranted.*

Key words: comet assay, DNA damage, exfoliated cells, leukocytes, micronuclei, polycystic ovary syndrome

Introduction

PCOS is the commonest endocrinopathy among women of reproductive age with an estimated prevalence of about 10% (range 2-20%) [1]. PCOS is characterized by hyperandrogenism and chronic anovulation. This disease, associated with polycystic ovaries and hirsutism, and, to a lesser extent, with obesity and insulin resistance, is a leading cause of female infertility. The prevalence of this disease in Armenia is about 12% [2].

Data on the incidence of endometrial, breast and ovarian cancers in patients with PCOS are conflicting [1].

Recently Yesilada et al. [3] reported about 3-fold increase in MN level in lymphocytes of Turkish females with PCOS. Australian women with PCOS also displayed increased genomic instability (higher frequencies of MN/1.4-fold, and chromosome malsegregation in lymphocytes) compared to healthy women [4].

Our 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 [5].

Now, within the frame of $HUMN_{XL}$ Project, attempts are being carried out to standardize MN test in exfoliated cells [6]. The complete protocol to study MN in exfoliated cells includes also the so-called nuclear anomalies (NA), such as cells with 2 nuclei (binucleates), karyolysis (lysed nucleus), karyorrhexis (nucleus broken to pieces), nuclear buds (broken egg phenomenon), and condensed chromatin in the nucleus. The precise nature of these parameters is unknown, but there is evidence connecting both cytotoxicity and genotoxicity with these phenomena [6]. The frequencies of MN and NA in exfoliated cells of PCOS patients are unknown. Unknown also is the possible DNA damage in somatic cells (leukocytes) of PCOS patients.

The aim of the present investigation was to fill in the mentioned gaps in knowledge and to evaluate the levels of MN and NA in exfoliated buccal cells and DNA damage in leukocytes of PCOS patients.

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Methods

Subjects

The study comprised 17 females of Armenian nationality newly diagnosed with PCOS at the Diagnostic Center, Yerevan, Armenia. Seventeen healthy women with similar age and physical parameters were included in the investigation as controls. All the subjects gave written consent for study inclusion and the Ethics Committee of the Diagnostic Center approved the investigation. All females with PCOS had the following symptoms: 1) hirsutism score >6; 2) significantly increased level of testosterone and luteinizing hormone in blood; 3) increased ovarian volume compared with healthy female subjects. All biochemical analyses were carried out at the Diagnostic Center. Hirsutism score was evaluated by a dermatologist using the Ferriman-Gallwey score [7].

Methods

MN assay was applied to exfoliated buccal cells which were obtained by means of a wooden spatula. Cells were washed by centrifugation (500 g) with PBS 3 times to get rid of microbes. Then, they were dropped to slides, air-dried overnight, fixed in 80% cold methanol, and stained with Schiff's reagent [6,8]. Cells were studied under light microscope MBI-6 (USSR) with oil immersion (×1000).

DNA damage was evaluated by means of the comet assay (single-cell gel electrophoresis) as described earlier [9]. The only difference was the use of some drops of whole blood instead of cells, but all other details are identical. Thirty µl of blood were obtained with finger prick, mixed with agarose, lysed with detergent and high salt, and electrophoresed at pH > 13. Under these conditions the presence of DNA breaks allows DNA to extend to form a "comet tail" observed by fluorescence microscopy after staining with ethidium bromide. The relative intensity of tail DNA fluorescence assessed by visual scoring of 100 comets on a scale of 0-4 category of damage (giving 0-400 arbitrary units) reflects the breaks' frequencies [10]. The comets were studied under fluorescence microscope LM-4 (USSR).

Table 1. Subjects characteristics

Parameter	*Healthy controls (n=17)*	*PCOS patients (n=17)*
Age (years)	27.8±1.80	29.3±1.76
Body mass index (kg/m^2)	22.4±0.7	23.1±0.8
Hirsutism score	3.0±0.5	14.8±1.1**
Total testosterone (µUmol/l)	1.3±0.2	2.4±0.5*
Luteinizing hormone (mU/ml)	6.0±0.7	8.9±1.1*

Values are given as means ± S.E. *p <0.05, **p <0.01 (Mann-Whitney U-test)

All chemical and reagents used in this study were from Sigma-Aldrich (St. Louis, USA).

Statistical analysis

Non-parametric Mann-Whitney U-test was used to compare the data in the 2 studied groups (Microsoft Word GraphPad Prism, version 3.02).

Results

The data of the study participants are presented in Table 1. As can be seen, age and body mass indexes of patients and control subjects were similar. The patients were hirsute, and total testosterone and luteinising hormone levels were substantially increased in the PCOS patients (by 85% and 48%, respectively). The data concerning MN and NA levels as well as DNA damage parameters are presented in Table 2. As can be noted, only the numbers of cells with MN and total MN count were significantly higher in patients (2.4- and 2.5-fold, respectively) than in healthy women. Also, Table 2 shows that DNA in leukocytes of PCOS patients had significantly more damage (increased by 36% comet score expressed in arbitrary units) than in controls.

Discussion

In this paper we reported for the first time signifi-

Table 2. Mean frequencies of micronuclei and other nuclear anomalies levels in exfoliated buccal cells and DNA damage (comet score) in leukocytes of PCOS patients and healthy women

Subjects	*Cells with MN*	*Total number of MN*	*BE*	*BN*	*KR*	*CC*	*P*	*KL*	*Comet score (a.u.)*
Healthy women	0.91± 0.12	1.12±0.14	0.29±0.08	6.18±0.66	4.56±0.54	9.2±1.10	1.29±0.27	9.62±0.97	32.00±1.48
PCOS patients	2.18±0.18*	2.82±0.22*	0.59±0.16	6.32±0.69	4.12±0.47	6.50±0.66	1.47±0.32	8.4±0.67	43.59±2.24*

Values are given as means ± SE; *p<0.01 (Mann-Whitney U-test)
MN: micronuclei, BE: broken egg, BN: binucleated cells, KR: karyorrhexis, CC: condensed chromatin, P: pycnosis, KL: karyolysis, a.u.: arbitrary unit

cantly increased levels of MN in exfoliated buccal mucosa cells and also DNA damage in leukocytes of PCOS patients. MN are either whole chromosome or chromosomal fragments, and, hence, increased number of MN reflects clastogenic and/or aneugenic effects in cells. In our recent study we reported about both structural and numerical aberration in lymphocytes of PCOS patients [5]. Total testosterone and luteinizing hormone levels in the patients under study were close to the ones reported previously by our research group [5]. Also, they were close to the ones published by Yesilada et al. [3].

As for the so-called NA, at present there are no strict explanations about their nature, yet they should be included in the complex study of exfoliated cells, so-called "cytome assay" [6].

Both our findings are quite logical, and are in accordance with previous reports concerning an increased genetic instability in PCOS patients. It is well known that in many cases significant relationships between MN and DNA damage in lymphocytes and MN in exfoliated buccal mucosa cells were shown [6].

The parameters of cytogenetic damage and cytotoxicity in the control subjects were close to the data obtained previously in Armenian women [8].

Hence, genetic instability in somatic cells of PCOS patients has been shown by means of MN in exfoliated buccal cells and lymphocytes, chromosomal aberrations in lymphocytes and DNA damage in leukocytes.

One possible reason of genetic instability in cells of PCOS patients is oxidative stress [2,11,12]. In many investigations overproduction of malondialdehyde (MDA) in PCOS patients has been shown [2,12]. Recently Karadeniz et al. [12] reported about non-significant elevation of MDA, nitric oxide and disulfide levels in non-obese, relatively young PCOS patients. In our study non-obese, relatively young (mean age 29 years) women were included, but as it was shown in the study of Dolyan et al. [2], the levels of MDA were significantly increased in the aforementioned category of Armenian women. The levels of MDA in Armenian women were close to the ones reported by Karadeniz et al. [12].

It is well known that excess of MDA production leads to increased genetic instability, such as elevated levels of chromosomal aberrations, MN and DNA damage in somatic and germ cells of such a group of subjects [13].

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 [14]. Although only conflicting results were published concerning a relation of PCOS and cancer, more scrupulous investigation in this area is certainly warranted [1].

Our results confirm the data of some investigators and our own about increased cytogenetic damage of somatic cells in PCOS patients (MN and chromosomal aberrations in lymphocytes) [3-5].

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