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ORIGINAL ARTICLE

Chromosomal aberrations level in peripheral blood lymphocytes of women with polycystic ovary syndrome

A. Nersesyan[1*], A. Martirosyan[2], G. Parsadanyan[3], G. Zalinyan[4]

[1]National Center of Oncology; [2]Research Center of Maternal and Child Health Protection; [3]Research Institute of Environmental Hygiene and Preventive Toxicology; [4]State University, Yerevan, Republic of Armenia

Summary

Purpose: *Polycystic ovary syndrome (PCOS), characterized by polycystic ovaries, hyperandrogenism, chronic anovulation and hirsutism, is a common endocrine disease in females worldwide. Many investigations have shown oxidative stress in such patients and the relationship between genetic instability and oxidative stress is well known. The aim of the present study was to investigate the background chromosomal aberrations (CAs) level in lymphocytes of females with PCOS.*

Patients and methods: *Fifteen females, diagnosed with PCOS (hirsutism score >6; significantly increased level of testosterone in blood; increased ovarian volume) and 15 healthy women of similar physical parameters (controls) were included in this investigation. The frequency of CAs in cultured lymphocytes was used as a biomarker of cytogenetic damage.*

Results: *The frequencies of all types of CAs were significantly higher in patients with PCOS, and the mitotic index was significantly lower.*

Conclusion: *Females with PCOS have increased CAs level in lymphocytes which is a sign of genetic instability.*

Key words: chromosomal aberrations, genetic instability, ovarian cancer, polycystic ovary syndrome

Introduction

PCOS, characterized by polycystic ovaries, hyperandrogenism, chronic anovulation and hirsutism, is a common endocrine disease in females worldwide. PCOS is a polygenic/multifactorial disorder causing female infertility [1]. Estimates of the prevalence of this syndrome in the general female population in the USA and Europe have ranged from 2-20% [1,2]. In Armenia the prevalence of PCOS is about 12.5% [3].

We had studied 25 Armenian females with hirsutism and found significantly increased level of micronuclei (MN) in exfoliated buccal mucosa cells (EBMC) compared with healthy females (1.52-fold) [4]. About half of them were diagnosed with PCOS while the remaining had various reasons for hirsutism.

Recently Esilada et al. [5] reported about 3-fold increase in MN level in lymphocytes of Turkish females with PCOS.

In the literature we could not find data concerning the spontaneous (background) level of CAs in lymphocytes of females with PCOS. The aim of this work was to investigate this parameter.

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Author and address for correspondence:

Armen K. Nersesyan, PhD
Environmental Toxicology Group
Institute of Cancer Research
Borschkegasse 8A
1090, Vienna
Austria
Tel: +431 4277 65146
Fax: +431 4277 9651
Email: armen.nersesyan@meduniwien.ac.at

Patients and methods

Subjects

The study included 15 females of Armenian nationality newly diagnosed with PCOS at the Institute

*Present address: Environmental Toxicology Group, Institute of Cancer Research, Borschkegasse 8A, 1090, Vienna, Austria

of Obstetrics and Gynecology, Ministry of Health, Yerevan, Armenia. Fifteen healthy women with similar age and physical parameters served as controls. All women gave written informed consent before entering the study.

All females with PCOS had the following symptoms: 1) hirsutism score > 6; 2) significantly increased level of testosterone in blood; 3) increased ovarian volume compared with healthy female controls. All biochemical analyses were carried out at the Laboratory of Biochemistry, Institute of Obstetrics and Gynecology. Hirsutism score was evaluated by a dermatologist using the Ferriman-Gallway scoring system.

Chromosomal analysis

The CA assay was carried out using conventional techniques [6]. Human blood samples were obtained from 15 females with PCOS and 15 healthy, non-smoking volunteers, 19-31 years old.

All reagents and chemicals used in this study, unless otherwise noted, were produced in Russia. Heparinized total blood (0.5 ml) was added to 4.5 ml medium, containing 78% RPMI 1640, 20% inactivated fetal bovine serum, antibiotics (penicillin and streptomycin) and stimulated with 2% of phytohaemagglutinin (Difco, USA), and incubated for 72 h at 37° C. Colchicine was added to a final concentration of 0.5 μg/ml culture medium, 2 h prior to harvesting. Lymphocytes were stained with azure-eosin (Sigma Chemical Co., USA). The end points analysed were mitotic index, total CAs, and percentage of aberrant metaphases. The mitotic index was determined by scoring the number of metaphases in 500 cells per sample. Nearly 100 well-spread metaphases containing 46±1 chromosomes were studied from each donor. The classification of aberrations was as described in ISCN [7]. Gaps were registered but not considered as CAs.

The slides were evaluated under a light microscope (Nikon Eclipse E200) with × 1000-fold magnification using oil immersion.

Table 1. Subject characteristics

Parameter	*Healthy controls (n=15)*	*Females with PCOS (n=15)*
Age (years)	25.5± 1.60	26.3± 1.75
Body mass index (kg/m^2)	21.2± 0.5	22.6± 0.5
Hirsutism score	3.7± 0.4	12.9± 0.8**
Total testosterone (μUmol/l)	1.5±0.1	2.4±0.4*
Insulin (U/ml)	6.1±0.7	8.9±1.1*

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

Statistical analysis

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

Results

Physical and biochemical data of healthy females and patients with PCOS are presented in Table 1. It can be seen that hirsutim score, total testosterone and insulin levels were significantly higher in patients with PCOS.

In both experiments all cytogenetic parameters in patients were significantly higher than those of healthy women (Tables 2 and 3). In the first experiment the number of cells with CAs and the number of CAs per 100 cells were 2.25- and 2.37-fold higher, respectively (Table 2). In patients, except chromatid and chromosomal breaks, exchanges were also registered. It is interesting that the mitotic index in patients with PCOS was significantly lower than in healthy women (1.63-fold) (Table 3).

Table 2. Total number of chromosomal aberrations in lymphocytes of females with PCOS and healthy subjects

Groups of females	*Number of studied cells*	*Number of aberrant cells*	*Aberrant cells (%)*	*Number of aberrations per 100 cells*	*Total number of aberrations*			
					Gaps	*Chromatid breaks*	*Chromosomal breaks*	*Exchanges*
PCOS (n=15)	1359	44	3.24±0.13*	3.75±0.2*	14*	21	26*	4**
Control (n=15)	1454	21	1.44±0.07	1.58±0.07	4	18	5	0

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

Table 3. Mitotic index and mean number of chromosomal aberrations per 100 cells in lymphocytes of females with PCOS and healthy subjects

Groups of females	*Mitotic index (‰)*	*CA per aberrant cell*	*Aberrations per 100 cells*			
			Gaps	*Chromatid breaks*	*Chromosomal breaks*	*Exchanges*
PCOS	3.2±0.29	1.16±0.05	1.03±0.13*	1.54±0.2	1.91±0.27*	0.27±0.27
Control	5.2±0.36*	1.10±0.05	0.28±0.13	1.24±0.2	0.34±0.13	0

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

Discussion

This study shows that in the somatic cells of females with PCOS an increased level of CAs was registered, suggesting genetic instability. Our data are in good agreement with our previous results obtained in exfoliated buccal mucosa cells of females with PCOS [4]. We had studied 25 females with PCOS and found that the level of cells with MN was 1.54-fold higher in patients ($p < 0.02$). These numbers are a little lower than those obtained in the present study, but a lot of literature data show that MN assay in exfoliated cells is less sensitive than MN and CAs assays in lymphocytes [8]. Our results on genetic instability in somatic cells (buccal mucosa and lymphocytes evaluated by means of MN and CAs assay) of women with PCOS are supported by Esilada et al., reporting about 3-fold increase in MN level in lymphocytes [5]. Hence, in somatic cells of females with PCOS genetic instability was documented by means of 3 cytogenetic end points - MN in lymphocytes and exfoliated buccal cells, and CAs in lymphocytes.

Genetic instability in somatic cells of females with PCOS is due to oxidative stress, a well documented fact by many investigators [9,10]. In such patients malondialdehyde and reduced glutathione (GSH) concentration in erythrocytes were increased and decreased, respectively. Possibly the main reason of oxidative stress is excess of testosterone in blood of females with PCOS because this hormone has such a property [11]. Increased chromosomal damage was also observed in patients with Parkinson's and Adamantiades-Behçet's diseases which are also associated with oxidative stress [12,13].

We also observed significantly decreased mitotic index in the lymphocytes of the patients. We suppose that this is due to DNA (chromosomal) damage because some hormones producing oxidative stress can damage chromosomes and decrease the mitotic index [14]. Increased background MN and CA level in somatic cells is confirmed by a study showing a significant increase in DNA strand breakage and H_2O_2-induced DNA damage in women with PCOS [15].

In another study all types of CAs (gaps, breaks and exchanges) were significantly higher in patients compared with healthy females which is an evidence of genetic instability [16].

As it was shown by some groups of investigators, the CA frequency predicts the overall cancer risk in healthy subjects [17,18]. Recent studies have addressed the possibility of an association between polycystic ovaries and ovarian cancer [15]. Data on genetic instability support the hypothesis of a relation between PCOS and ovarian cancer.

References

1. Franks S, McCarthy MI, Hardy K. Development of polycystic ovary syndrome: involvement of genetic and environmental factors. Int J Androl 2006; 29: 278-290.
2. Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. J Clin Endocrinol Metab 1998; 83: 3078-3082.
3. Dolyan GG. Evaluation of hirsutism in sterile Armenian women. In: Okoev GG. (ed): Actual Problems of Endocrinology. Luys Publ House, Yerevan, 1999, pp 51-78.
4. Nersesyan AK, Zalinyan GG, Vartazaryan NS, Arutyunyan RM. Micronucleus level in exfoliated buccal mucosa cells of women with hirsutism. Central Eur J Occup Envir Health 2001; 7: 39-44.
5. Yesilada E, Sahin I, Ozcan H, Yildirim III, Yologlu S, Taskapan C. Increased micronucleus frequencies in peripheral blood lymphocytes in women with polycystic ovary syndrome. Eur J Endocrinol 2006; 154: 563-568.
6. Moorhead PS, Nowell PC, Mellman WJ, Batipps DP, D.A. Hungeford. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res 1960; 20: 613-616.
7. ISCN, International System for Human Cytogenetics Nomenclature: Report of the Standing Committee on Human Cytogenetics Nomenclature. S. Karger, Basel, München, Paris, London, New York New Delhi, Singapore, Tokyo, Sydney, 1985, pp 66-69.
8. Nersesyan AK. Risk assessment in the first degree female relatives of breast cancer patients using the alkaline comet assay. Carcinogenesis 2001; 22: 679 (letter).

9. Sabuncu T, Vural H, Harma M, Harma M. Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. Clin Biochem 2001; 34: 407-413.
10. Fenkci V, Fenkci S, Yilmazer M, Serteser M. Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. Fertil Steril 2003; 80: 123-127.
11. Pansarasa O, D'Antona G, Gualea MR, Marzani B, Pellegrino MA, Marzatico F. "Oxidative stress": effects of mild endurance training and testosterone treatment on rat gastrocnemius muscle. Eur J Appl Physiol 2002; 87: 550-555.
12. Migliore L, Scarpato R, Coppede F, Petrozzi L, Bonuccelli U, Rodilla V. Chromosome and oxidative damage biomarkers in lymphocytes of Parkinson's disease patients. Int J Hyg Environ Health 2001; 204: 61-66.
13. Freitas JP, Filipe P, Yousefi A, Emerit I, Guerra Rodrigo F. Oxidative stress in Adamantiades-Behçet's disease. Dermatology 1998; 197: 343-348.
14. Djelic N, Spremo-Potparevic B, Bajic V, Djelic D. Sister chromatid exchange and micronuclei in human peripheral blood lymphocytes treated with thyroxine in vitro. Mutat Res 2006; 604: 1-7.
15. Dinger Y, Akcay T, Erdem T, Ilker Saygili E, Gundogdu S. DNA damage, DNA susceptibility to oxidation and glutathione level in women with polycystic ovary syndrome. Scand J Clin Lab Invest 2005; 65: 21-28.
16. Paz-y-Mino C, Davalos MV, Sanchez ME, Arevalo M, Leone PE. Should gaps be included in chromosomal aberration analysis? Evidence based on the comet assay. Mutat Res 2002; 516: 57-61.
17. Rossner P, Boffetta P, Ceppi M et al. Chromosomal aberrations in lymphocytes of healthy subjects and risk of cancer. Environ Health Perspect 2005; 113: 517-520.
18. Hagmar L, Stromberg U, Tinnerberg H, Mikoczy Z. The usefulness of cytogenetic biomarkers as intermediate endpoints in carcinogenesis. Int J Hyg Environ Health 2001; 204: 43-47.