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Buccal micronucleus cytome biomarkers in Algerian couples with idiopathic infertility

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ABSTRACT

The buccal micronucleus cytome (BMCyt) assay is a useful and a minimally invasive cytogenetic method for measuring genomic damage. The aim of the present study is to evaluate the extent of chromosomal damage in couples with idiopathic infertility using a BMCyt.

This study included 54 patients (27 couples) with idiopathic infertility and 30 fertile subjects (15 couples). When evaluated by individual (each subject from the couple is considered separately), the frequencies of micronucleated cells (MNC), total micronuclei (TMN), nuclear buds (NBUD), and binucleated cells (BN) were significantly higher in the infertile individuals than in the fertile ones ($p = 0.009$, $p = 0.009$, $p = 0.003$ and $p < 0.0001$, respectively). Among the cells reflecting cell death events, condensed chromatin (CC), karyorrhectic (KHC) and pyknotic (PYK) cells were significantly higher in the infertile individuals ($p = 0.0001$, $p = 0.003$, $p = 0.001$, respectively). Identical results were obtained when data were analysed by couple (female + male). The frequencies of MNC, TMN, NBUD, and BN cells were significantly higher in the infertile couples ($p = 0.019$, $p = 0.021$, $p = 0.013$, and $p < 0.0001$, respectively). Likewise, CC, KHC and PYK cells were significantly higher in the infertile couples ($p = 0.002$, $p = 0.034$, $p = 0.008$, respectively). BN cells showed the most pronounced difference between the fertile and infertile groups. The basal (BAS) and karyolitic (KYL) cells did not show a significant difference. In conclusion, this study showed that, in comparison to controls, couples with idiopathic infertility had significantly higher frequencies of DNA damage biomarkers (MN and NBUD), biomarkers of cytokinesis-failure or arrest (BN cells) and cell death biomarkers (CC, KHC and PYK cells). These results suggest a possible role of chromosomal damage in idiopathic infertility that may be due to an imbalance between DNA damage rates and DNA repair mechanisms.

1. Introduction

Infertility can be defined as the failure of a couple to achieve a clinical pregnancy after at least one year of regular unprotected sexual intercourse [1]. Idiopathic infertility is diagnosed when a couple does not conceive and no underlying mechanism(s) of infertility can be identified following a complete evaluation of both partners. Despite the advances in diagnostic techniques, the estimation of average prevalence of idiopathic infertility is approximately 15%–30% of infertile couples [2–4]. A certain number of genetic mechanisms can lead to infertility and it is likely that most currently idiopathic infertility cases are due to genetic causes (Fenech [14]).

It is well known that genome damage and chromosome instability are associated with an increased risk of neurodegerative diseases, accelerated aging syndromes, cardiovascular diseases and cancers [5–8]. The micronucleus (MN) assay is one of the best cytogenetic techniques to evaluate the chromosome instability in humans [9,10]. The MN are formed by chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division [11]. They can be evaluated easily in erythrocytes, lymphocytes, and exfoliated epithelial cells (e.g. oral, nasal, urothelial) [12]. The BMCyt is a useful and a minimally invasive approach for measuring genetic damage in human exfoliated buccal cells [9,13]. It has been used to measure biomarkers of DNA damage, cytokinetic defects, cell proliferation, and cell death [9,10,13].

The use of MN assays to study the relationship between DNA damage and infertility in humans has greatly increased [14]. A number of studies have found an association between the high frequency of chromosome instability measured by the MN assays and the increased risk of infertility [15–21]. However, all these studies applied MN assays on peripheral blood lymphocytes. The present study was undertaken to

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Table 1
Demographic and medical characteristics of the infertile couples.

Characteristics			
Age (year)	Female	33.56 ± 5.26	24-41
	Male	39.48 ± 5.86	28-52
Occupational status	Female	Employed	5 (18.51%)
		Unemployed	22 (81.48%)
	Male	Employed	25 (92.59%)
		Unemployed	2 (7.40%)
Family history of infertility	Female	Yes	6 (22.22%)
		No	21 (77.77%)
	Male	Yes	5 (18.51%)
		No	22 (81.48%)
Infertility duration (year)	Couple	≤5	9 (33.33%)
		> 5	18 (66.66%)
Attempt of IVF and ICSI*	Couple	Yes	12 (44.44%)
		No	15 (55.55%)
Residence	Couple	Coastal	2 (7.40%)
		Rural	3 (11.11%)
		Urban	22 (81.48%)

Abbreviations: * IVF: *in vitro* fertilization, ICSI: Intracytoplasmic sperm injection.

investigate idiopathic infertility in couples using the BMCyt assay. To our knowledge, this study is the first to use the noninvasive BMCyt assay to investigate genetic damage biomarkers in the buccal cells of couples with idiopathic infertility. This is also the first evaluation of chromosomal instability by the BMCyt assay in an Algerian population.

2. Subjects, materials and methods

2.1. Subjects

This study included 27 couples with idiopathic infertility (54 patients) referred to the service of medically assisted reproduction in Sidi Mabrouk maternity, Constantine, Eastern Algeria. All participants underwent a standard basic infertility evaluation (semen analysis, assessment of ovulation, hysterosalpingogram and assessment of sexual dysfunction of the couple) and no definitive causes of infertility were found. Their ages ranged from 24 to 52 years with a mean (± SD) of 36.52 ± 6.27 (Table 1).

The control group is composed 15 fertile couples (they had at least one child) with matched age. Their mean age (± SD) was 34.50 ± 6.11years (range 23–51).

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of the Research Center in Biotechnology (CRBt, Constantine, Algeria).

A written informed consent was obtained from patients and controls. Before collecting the samples, a detailed questionnaire was answered by all participants regarding their medical history, residence and occupational status. Exclusion criteria for both groups included work-related exposure to mutagenic agents, smoking history, anticancer therapy, alcohol consumption and chronic illnesses.

2.2. Buccal micronucleus cytome assay (BMCyt)

Buccal cell samples were collected from all subjects after rinsing the mouth with tap water. Cells were obtained by scraping the cheek mucosa with a cytobrush. Then, they were transferred to a tube containing Saccomanno's fixative and transported to the histology laboratory where they were centrifuged for 10 min at 580 g at room temperature. The supernatant was removed and replaced with 10 ml of buccal cell buffer (1.6 g Tris − HCl, 37.2 g EDTA disodium salt, 1.2 g sodium chloride) at pH 7.0 and centrifuged for 10 min at 581 g. This process was repeated twice more, as the buffer helps to inactivate endogenous DNAases and aid in removing cell debris and bacteria that may cause difficulties in scoring. The cells were homogenized 2–3 minutes by a homogenizer, and then passed through a 100 µm nylon filter held in a Swinex holder to remove large cell aggregates. Cells were further centrifuged at 580 g for 10 min. After removing the supernatant they were resuspended in 1 ml of buccal cell buffer. One hundred twenty microliters of the cell suspension were dropped onto clean slides that were air-dried for 10 min and then fixed with a fresh mixture of ethanol: acetic acid (3:1) for 10 min. Later, the slides were stained using the Feulgen/Fast Green method.

The slides were scored by two scorers without knowledge from which group the samples were obtained. Each slide was scored twice and the result was the average of both readings. The observation was done under light microscope Axioskop 20; Carl Zeiss, (Göttingen, Germany), first at ×400 then at x1000 magnification in order to confirm the results. Initially, 1000 cells were scored per subject to find the frequency of various cell types observed in BMCyt assay. The observed cells included BAS, BN, PYK, KHC, CC and KYL cells. Then, DNA damage biomarkers including MN and NBUD were scored in 1000 differentiated and basal cells. The scoring was done according to the criteria defined by Thomas et al [9].

2.3. Statistical analysis

The data were analyzed using GraphPad Prism ver. 5.01 for Windows (GraphPad Software, USA). The non-parametric Mann-Whitney *U* test was used for comparison of data in both studied groups. The effect of age on MN and other BMCyt assay biomarkers was determined by the Spearman's correlation coefficient. The comparison of ages in groups was performed using the Student's *t*-test The data were presented as mean ± standard deviation (S.D.). A p value of less than 0.05 was considered significant for all statistical tests.

3. Results

The BMCyt assay biomarkers were analyzed by couple (data of the male and the female were analysed together) and by individual (data from each member of the couple were analysed separately). The results for the BMCyt assay the two studied groups are represented in Tables 2 and 3.

We studied frequencies of BMCyt assay biomarkers following age and sex. The results showed that, in the control group, the frequencies of the KHC and KYL cells were significantly higher in males compared to females ($p = 0.021$, $p = 0.032$, respectively). However, the other biomarkers (MNC, TMN, NBUD, BAS, BN, CC and PYK) did not show significant differences between females and males ($p > 0.05$).

The Spearman correlation coefficients for the BMCyt assay

Table 2
Age and buccal micronucleus cytome assay biomarkers evaluated by individual.

	Infertile	Controls	*p* value
Number of subjects[a]	54	30	
Age (years)	36.52 ± 6.27	34.50 ± 6.11	0.157
MNC (‰)	2.45 ± 2.64	0.96 ± 1.01	**0.009**
TMN (‰)	2.96 ± 3.71	1.01 ± 1.14	**0.009**
NBUD (‰)	2.08 ± 2.25	1.00 ± 0.96	**0.003**
BAS (‰)	2.99 ± 2.66	3.46 ± 2.28	0.183
BN (‰)	7.53 ± 3.22	3.95 ± 2.11	**< 0.0001**
CC (‰)	1.59 ± 1.79	0.48 ± 0.72	**0.0001**
KHC (‰)	3.68 ± 3.85	1.50 ± 1.87	**0.003**
PYK (‰)	4.45 ± 2.84	2.58 ± 1.54	**0.001**
KYL (‰)	186 ± 70.48	158.2 ± 77.65	0.100

Abbreviations: MNC: micronucleated cells, TMN: total number of micronuclei, NBUD: nuclear buds, BAS: basal cells, BN: binucleated, CC: condensed chromatin, KHC: karyorrhectic, PYK: pyknotic, KYL: karyolitic. Data are presented as means ± SD. Data in bold is statistically significant ($p < 0.05$).

[a] Subjects were members of couples; so, half of each group were females and half were males.