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Microparticles: Inflammatory and haemostatic biomarkers in Polycystic Ovary Syndrome

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is associated with a chronic low-grade inflammation and predisposition to hemostatic and atherosclerotic complications. This case-control study evaluated the microparticles (MPs) profile in patients with the PCOS and related these MPs to clinical and biochemical parameters. MPs derived from platelets (PMPs), leuckocytes (LMPs) and endothelial cells (EMPs) were evaluated, as well as MPs expressing tissue factor (TFMPs), by flow cytometry, comparing women with PCOS ($n = 50$) and a healthy control group ($n = 50$). PCOS women presented increased total MPs, PMPs, LMPs and EMPs levels when compared to control group (all $p < 0.05$). TFMPs was similar between the groups ($p = 0.379$). In conclusion, these MPs populations could be useful biomarkers for association with thrombosis and cardiovascular disease in PCOS women.

1. Introduction

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder among women in reproductive age (Palomba et al., 2015; Silva et al., 2015). The etiology of PCOS is not completely understood (Dunaif, 2016; Sóter et al., 2015), but it results from environmental and genetic factors (Aytan et al., 2016; Insenser and Escobar-Morreale, 2013). The patients usually present hyperandrogenism, irregular menstruation and polycystic ovaries (Palomba et al., 2015; Aytan et al., 2016).

PCOS is an important cause of infertility due to chronic anovulation (Palomba et al., 2015). Furthermore, PCOS women present several metabolic complications, including obesity, insulin resistance (IR), type 2 diabetes mellitus (T2DM), dyslipidemia, metabolic syndrome and cardiovascular disease (Palomba et al., 2015; Silva et al., 2015; Repaci et al., 2011).

PCOS patients present unbalance between pro- and anticoagulant factors with higher atherothrombotic risk, besides increased levels of pro-inflammatory cytokines, which contribute to systemic and chronic low-grade inflammation (Palomba et al., 2015; Sóter et al., 2015; Aytan et al., 2016; Repaci et al., 2011). This subclinical inflammation also has been assumed to be the link between numerous metabolic complications, which are frequently associated with PCOS (Repaci et al., 2011). Furthermore, the inflammation probably contributes to an increased risk of cardiovascular disease in PCOS, because it is considered a hallmark of endothelial and hemostatic dysfunction (Repaci et al., 2011).

Microparticles (MPs) are extracellular vesicles with 0.1–1 μm released from the cell membrane during cell activation and apoptosis (Marques et al., 2013). MPs are important messengers in cell-cell communication and contribute to the induction of endothelial modifications, inflammation, differentiation, and angiogenesis (Marques et al., 2013). MPs membranes also carry signaling molecules, such as chemokines, cytokines, enzymes, growth factors, receptors, adhesion molecules, mRNAs and microRNA (Marques et al., 2013; Tan and Lip, 2005; VanWijk et al., 2002). The circulating MPs can affect the target cell properties by presenting

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these bioactive molecules, resulting in cell activation, phenotypic cellular modification, and the reprogramming function (Mause and Weber, 2010).

The initial step in the MPs formation is the membrane remodeling, with the development of blebs within it. This step requires an increase in intracellular calcium levels, resulting in the rearrangement and externalization of phosphatidylserine to the outer surface. Calcium-sensitive enzymes are activated and promote the cleavage of the filaments of the cytoskeleton leading to the formation of blebs on cell membrane and the release of MPs (Boulanger et al., 2006). Furthermore, MPs expose their membrane proteins provided from the specific cells that originated them, which can in turn be used to study their exact origin (Boulanger et al., 2006).

MPs levels are associated with a variety of pathological conditions, such as prothrombotic (Mooberry et al., 2006) and inflammatory disorders (Marques et al., 2012), cardiovascular (Alexandru et al., 2015; Burbano et al., 2015), autoimmune (Burbano et al., 2015) and infectious diseases (Campos et al., 2010), cancer (Goubran et al., 2015) and T2DM (Koga et al., 2005). Furthermore, MPs arising from adipose tissue can influence insulin signaling through protein kinase B pathway and expression of gluconeogenic genes (Kranendonk et al., 2014).

Few studies have evaluated the role of MPs from different cells origin in PCOS (Wills et al., 2014; Koiou et al., 2011, 2013). Because this syndrome is associated with pro-coagulant and pro-inflammatory states, we investigated MPs originated from different cell types (platelets, leukocytes, and endothelial cells) and MPs expressing TF in a case-control study. Moreover, we evaluated the association and the correlation of these MPs with clinical and laboratory parameters in PCOS women. Our data can help to clarify a possible role of MPs in PCOS and their investigation as biomarkers for this syndrome and its complications.

2. Material and methods

2.1. Subjects

We evaluated 50 women with PCOS (aged from 14 to 41 years) and 50 women without the syndrome (20–43 years). The PCOS group was recruited at Hospital Borges da Costa, UFMG (Belo Horizonte, Minas Gerais, Brazil), in the period 2011–2013. The control group was recruited among employees and students from UFMG in the same period, and consisted of healthy women with regular menstrual cycles and no signs of hyperandrogenism or micropolycystic ovary.

PCOS diagnosis was performed according to the European Society of Human Reproduction/Embryology and the American Society for Reproductive Medicine criteria (ESHRE/ASRM) (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), considering the presence of at least two of three criteria: (1) oligo/amenorrhea and anovulation; (2) clinical or laboratory hyperandrogenism and (3) ultrasonography showing micropolycystic ovaries, as defined by the presence of 12 or more follicles in the ovary each measuring 2–9 mm in diameter and/or increased ovarian volume (>10 mL).

Exclusion criteria for both groups were the presence of diabetes mellitus, autoimmune, adrenal, kidney and liver disease, thyroid disorders, cancer, acute inflammatory disease, orthopedic implant, hyperprolactinemia, hypogonadism, and pregnancy. Current or recent (past 3 months) users of the following medications were also excluded: steroidal and non-steroidal anti-inflammatory medications, anabolic steroids, isotretinoin, cyclosporine, antiretroviral, insulin and oral contraceptives. Individuals with C-reactive protein (CRP) > 10 mg/dL were also excluded.

2.2. Clinical and laboratorial evaluation

Venous blood samples were obtained after 12 h fasting using tubes with sodium citrate or anticoagulant-free (Vacuette®). The samples were centrifuged at 1500 xg for 20 min at 4 °C to obtain the plasma or serum. Aliquots were immediately processed or stored at −80 °C until the use.

The serum samples were used for measure fasting glucose, CRP and lipid profile using Vitros kits (Johnson and Johnson®). Insulin and testosterone levels were also measured in serum samples using Abbott Architect®. All procedures were conducted according to the manufacturer's instructions.

Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg at the time of interview, or regular use of antihypertensive medication (Chobanian et al., 2003). Hirsutism in PCOS group was assessed according to the modified Ferriman–Gallwey scale (Api et al., 2009) for a single observer to avoid subjectivity. The visual scoring of hair density on the upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, arms, and thighs were classified visually on a scale of zero to four. A total score was calculated.

We considered dyslipidemic women as those currently using lipid lowering medication or with altered lipid profile, according to the III Brazilian Guidelines on Dyslipidemia and Atherosclerosis Prevention (total cholesterol > 240 mg/dL, LDL-cholesterol > 160 mg/dL, HDL-cholesterol < 40 mg/dL, or triglycerides > 201 mg/dL) (Santos and De Cardiologia., 2001). Body Mass Index (BMI) was measured by weight in kilograms divided by the square of the height in meters (kg/m2) (Obesity, 2000). The waist circumference (WC) was measured between the lowest ribs and the iliac crest, as recommended by World Health Organization and International Diabetes Federation (Wen-Ya et al., 2013).

The Lipid Accumulation Product (LAP) index was calculated by using the formula: [(waist circumference cm − 58) × (triglycerides mg/dL)] (Lwow et al., 2016). The Homeostatic Model Assessment (HOMA) for IR was calculated using the following the formula: [insulin (mU/L) x glucose (mM/L)]/22.5 (Tang et al., 2015).

2.3. MPs flow cytometry assay

The purification of the MPs was performed according to Campos et al. (2010) (Campos et al., 2010). In order to obtain platelet-free plasma, the plasma samples were centrifuged at 15,300 xg for five minutes diluted 1:3 in phosphate buffered saline (PBS) containing heparin, and again centrifuged at 15,300 xg for 90 min at 15 °C. The subsequent MPs pellet was resuspended in 1X annexin V binding buffer (BD Pharmingen®).

MPs were isolated in a LSR Fortessa cytometer (BD Biosciences®) and gated on basis of their forward (FSC) and side (SSC) scatter distribution of synthetic 0.7–0.9 μm SPHEROTM Amino Fluorescent Particles (Spherotech®). The presence of phosphatidylserine residues on the MP surfaces was assessed for their positive staining with monoclonal antibodies against annexin V (BD Pharmingen®) labeled with fluorescein isothiocyanate (FITC).

Cell-specific monoclonal antibodies were used to identify the source of the MPs. CD41-PECY7 (eBioscience®), CD45-APC (eBioscience®), CD51/61-PE (BD Pharmingen™) and CD142-PE (BD Pharmingen™) were used to label platelet-derived MPs (PMPs), leukocyte-derived MPs (LMPs), endothelium cell-derived MPs (EMPs) and MPs that express TF (TFMPs), respectively. The antibodies were used in concentrations according to manufacturer's instructions. The specific monoclonal antibody was corrected for isotype-matched control antibodies. FACSDIVA 6.2 software (BD®) was used for data acquisition and the analysis was performed using the FlowJo® software (Tree Star).