ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
April 2021; 20(2):198-204.
Doi: 10.18502/ijaai.v20i2.6053

Distribution of Myeloid and Plasmacytoid Dendritic Cell Subpopulations in Peripheral Blood of Hyperprolactinemic Women

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Received: 20 August 2020; Received in revised form: 20 October 2020; Accepted: 5 November 2020

ABSTRACT

Dendritic cells (DCs) play key roles in regulating the immune response using the specialized function of processing and presenting antigens. Prolactin (PRL), a hormone produced by the pituitary gland, participates in DC maturation and function. The present study was aimed to determine the frequencies of peripheral blood DC subpopulations of myeloid DC (MDC) and plasmacytoid DC (PDC) in hyperprolactinemic (HPRL) women compared to normal healthy volunteers.

This study was conducted on 70 women, including 35 HPRL patients and 35 matched healthy controls, whose PRL serum levels were in the normal range (lower than 25 ng/mL). Serum thyroid-stimulating hormone (TSH) levels were measured in both groups as an indicator of normal thyroid function. The electrochemiluminescence immunoassay method was applied to measure the serum levels of TSH and PRL. The frequencies of MDC and PDC in the peripheral blood samples of both groups were determined by flow cytometry.

The mean serum PRL levels in the HPRL patients and healthy individuals were 46.41±21.96 and 13.75±11.19, respectively (p<0.0001); however TSH levels in both groups were similar and within the normal range (0.4–4.5 mIU/mL) (p=0.2). The frequencies of both MDC and PDC subpopulations in the peripheral blood of HPRL patients were significantly lower than they were in the healthy controls. However, the ratio of MDCs/PDCs in HPRL patients was not significantly different between the two groups (p=0.8).

Our study revealed that an increased level of serum PRL may lead to a reduction in the number of MDC and PDC subpopulations. These results could help clarify the complex relationship between the immune system and the neuroendocrine axis and may be of potential use in understanding the pathogenesis of endocrine and immune disorders.

Keywords: Dendritic cells; Hyperprolactinemia; Myeloid; Prolactin

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INTRODUCTION

The immune and the endocrine systems have a two-way relationship. A complex network of immune-endocrine interactions is implicated in the cellular and humoral immune responses; dysfunction in this interaction can lead to autoimmune diseases, malignancies, atherosclerosis, and infertility.[1-3] Prolactin (PRL) plays an important role in the human immune system. The different functions of this hormone in a variety of immune responses are the subject of many studies in neuroendocrine immunology.[4]

PRL is a peptide hormone mainly synthesized and secreted by the anterior pituitary lactotrophs cells, but extra-pituitary tissues and cells in the prostate, decidua, breast epithelium, endothelial cells, skin cells, and immune cells are also capable of producing PRL.[5,6] Various roles, including homeostasis, regulation of osmotic pressures and metabolism, and regulation of the immune and nervous system in various vertebrate species, have been identified for this hormone.[7] PRL can stimulate many cells of the immune system, including T cells, B cells, natural killer (NK) cells, CD34+ hematopoietic cells, neutrophils, macrophages, and dendritic cells (DCs).[8]

DCs are the primary and specific antigen-presenting cells, like other immune cells; originating from hematopoietic stem cells in the bone marrow. DCs can regulate different immunologic mechanisms and play a key role in immunogenic tolerance. These cells are essential for the communication between innate and adaptive immune responses.[9,10] In human, DCs comprise a small percentage of the mononuclear cells in peripheral blood and include two major distinct subsets, myeloid DC (MDC) and plasmacytoid DC (PDC), with mutually exclusive phenotypes and functions. Generally, these cell populations are positive for human leukocyte antigen DR (HLA-DR) and negative for other lineages (monocytoid, T, B, and NK cells) markers.[11] For Example, MDCs express CD11c, produce interleukin-12 (IL-12) and induce the T helper type 1 (Th1) of T-cell differentiation.[12] PDCs originate from lymphoid precursors, express CD123 (IL-3R α-chain), and produce type 1 interferon (IFN), and their important roles are inducing Th1/Th2 differentiation and controlling virus infection.[13]

Hyperprolactinemia (HPRL), defined as serum PRL above the normal range (higher than 20 ng/mL in males and higher than 25 ng/mL in females), is one of the most common endocrine disorders of the hypothalamus-pituitary axis. HPRL can have physiological, pharmacological, and pathological causes or may be idiopathic. Pituitary adenoma, chronic kidney disease, hypothyroidism, and some drugs are the main causes of HPRL. Besides, physiological HPRL has been observed during pregnancy and lactation.[14-16] However, 16–35% of HPRL cases remain idiopathic.[17]

Many studies have investigated the function of PRL as a regulating factor in the immune system. These studies suggest that PRL is capable of affecting the proliferation, survival, and function of cells in the cellular, and humoral immune systems.[2]As there has been no study on the effect of increased serum PRL on the distribution of peripheral blood dendritic cell subpopulations, the present study aimed to evaluate the percentages of MDCs and PDCs, and their ratios in peripheral blood samples obtained from HPRL patients and compare them with those of normal healthy volunteers.

MATERIALS AND METHODS

Patients

This case-control study was conducted on 70 women aged between 18 and 50 years, consisting of35 newly diagnosed idiopathic HPRL patients who had not received any previous treatment for this disease (with serum PRL levels higher than 30 ng/mL) as the case group and 35 matched healthy volunteers (serum PRL<25 ng/mL). Patients who had taken drugs such as dopamine receptor blockers, dopamine synthesis inhibitors, opiates, H2 antagonists, antidepressants, calcium channel blockers, hormones, and/or immunosuppressive drugs, which increase serum PRL levels, in the previous three months were excluded from this study. Also, pregnancy, lactation, thyroid dysfunction, and chronic kidney disease were considered as exclusion criteria.

Fifteen-milliliter venous blood samples were collected between 08.00 and 09.00 AM after an overnight fast by clean venipuncture. Ten mL of the sample was transferred to EDTA-containing tubes for flow cytometry analysis and the rest was kept in the tube without anticoagulant for hormonal tests.

The study participants were recruited from endocrinology outpatient clinics of a tertiary reference

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

medical center. All of them signed the written informed consent. The Ethics Committee of Kerman Medical University approved the study protocol (Ethic Code: IR.KMU.REC.1394.540).

Hormonal Tests

Serum TSH and PRL levels were measured using the electrochemiluminescence immunoassay (ECLIA) method with associated kits (Roche Diagnostics Mannheim, Germany) and equipment (Elecsys 2010) in both groups: case and control.

Flow Cytometry Analysis

Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples by density gradient centrifugation on Ficoll-Paque (Biosera, France). The isolated mononuclear cells were washed three times in phosphate-buffered saline and then suspended in this buffer for flow cytometry analysis.

The subpopulation of DCs in peripheral blood can be recognized by the surface expression of blood dendritic cell antigens (BDCAs). MDCs are defined as a population positive for BDCA1 (CD1c) and negative for CD19 and CD14. BDCA2/CD303 is specific for blood PDCs.

Due to the very low frequency of DCs in peripheral blood, the indirect immunofluorescence staining method was used for specific markers of MDC and PDC populations. This method is more sensitive than direct staining. Monoclonal anti-BDCA1 (mouse IgG2a) and anti-BDCA2 (mouse IgG1) conjugated to biotin-conjugated antibody were used in the first step. After washing, anti-biotin-PE (mouse, IgG1) was added for detection. Direct staining with anti-CD19-FITC (mouse, IgG1) and CD14-FITC (mouse, IgG2a) was performed to exclude CD1c-positive CD19+ B cells and CD1c-positive CD14+ monocytes. All monoclonal antibodies and appropriate isotype controls were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany).

Partec PAS-III cytometer and FloMax software was used for the enumeration of MDCs and PDCs in PBMCs of HPRL patients and healthy controls, (Figure 1).

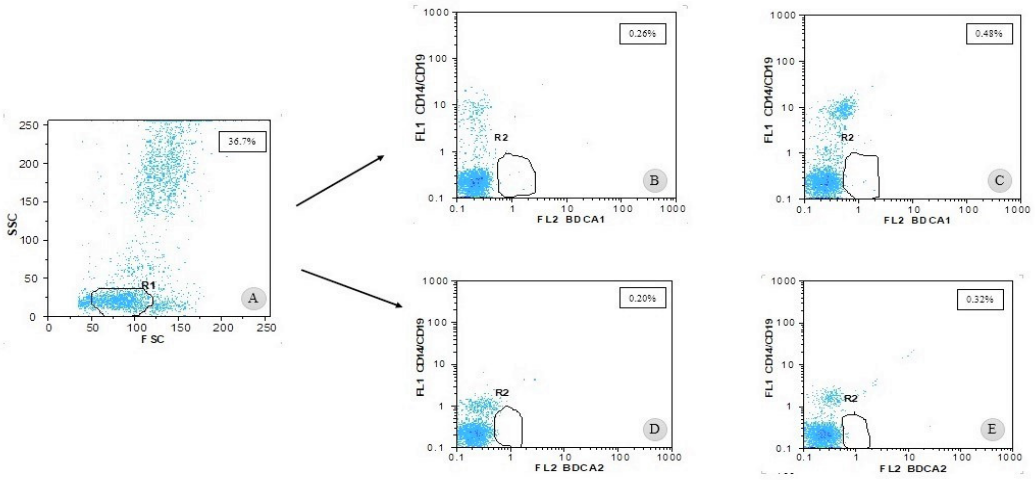

Figure 1. Flow cytometry analysis of myeloid dendritic cells (MDCs) and plasmacytoid dendritic cells (PDCs) in peripheral blood samples. The peripheral blood mononuclear cells (PBMCs) were stained according to the method described in Materials and Methods. For data acquisition, about 2.5×10^5events in the sample were collected and flowing dot plots were produced. A. Forward scatter (FSC) vs. side scatter (SSC) dot plot for excluding death cells, granulocytes, and debris. CD19-FITC/CD14-FITC vs. either (BDCA1)-PE or (BDCA2)-PE dot plots for excluding B cells and monocytes and enumeration of MDCs and PDCs. B. Flow cytometry results of MDCs in hyperprolactinemic (HPRL) patients. C. Flow cytometry results of MDCs in healthy volunteers. D. Flow cytometry results of PDCs in HPRL patients. E. Flow cytometry results of PDCs in healthy volunteers.

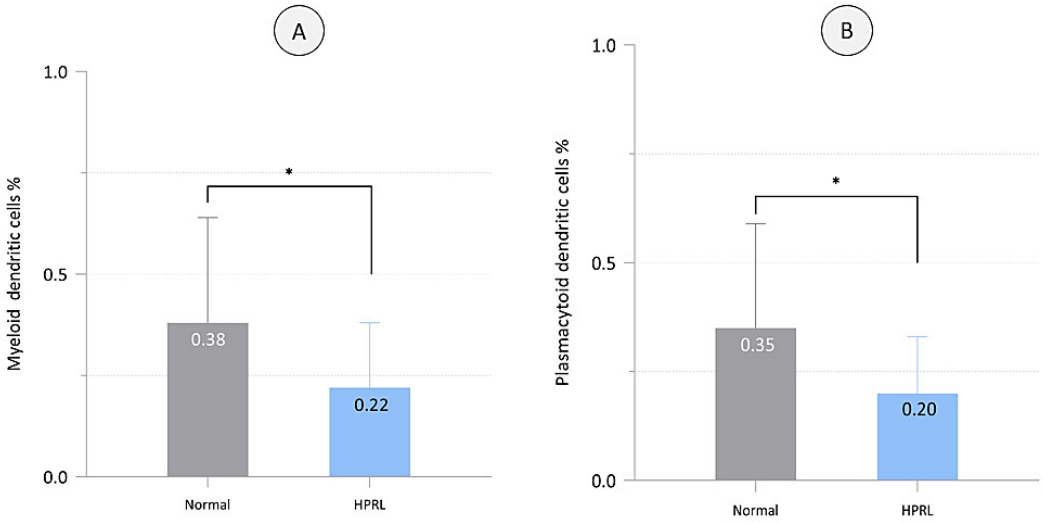

Figure 2. Comparison of the percentages of myeloid dendritic cells (MDCs) and plasmacytoid dendritic cells (PDCs) by standardized flow cytometry in peripheral blood of case and control groups: A. The percentages of MDCs in the peripheral blood of hyperprolactinemic (HPRL) patients and healthy volunteers. B. The percentages of PDCs in the peripheral blood of HPRL patients and healthy volunteers. Statistical significance was determined by the Mann-Whitney U test, $p<0.05$.

Statistical Analyses

The data were expressed as mean±standard deviation. A t-test was used to compare the mean values of serum TSH levels in HPRL patients and healthy volunteers. Mann-Whitney U test was applied to compare serum PRL levels and DC subpopulation frequencies between the case and control group. The normality of variables was assessed with the Kolmogorov-Smirnov test. Also, the covariance analysis (ANCOVA) was used to compare the DC subpopulation ratio in the two groups with the PRL as variable control. Spearman's *p* was also used to evaluate the correlation coefficients between PRL and MDC and PDC. A *p*-value lower than 0.05 was considered to be significant. SPSS software, version 18 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS

Patients' Characteristics

The groups were age-matched, with average ages of 28.45±6.19 and 29.17±6.42 in the case and control group, respectively (p=0.2).

HPRL was defined as serum PRL level above the normal range (higher than 30 ng/mL). The mean serum PRL levels in the HPRL and the healthy group were 46.41±21.96 and 13.75±11.19, respectively (p<0.0001).

The results of previous studies have shown that the thyrometabolic status influences the serum PRL level and the phenotype and function of human peripheral blood DC subtypes.[1,18,19] Accordingly, serum thyroid-stimulating hormone (TSH) levels were measured in both groups.

Data showed serum TSH concentrations were 2.86±0.73 mIU/mL and 2.31±1.01 mIU/mL in the case and control group, respectively (p=0.2) (normal range was 0.4–4.5 mIU/mL).

The Frequency and Ratio of Peripheral Blood DC Subtypes

The results showed that the MDC percentage in the peripheral blood of HPRL patients with a mean of 0.22±0.16 was significantly lower than that of healthy volunteers with a mean of 0.38±0.26 ($p\leq0.005$, Figure 2A). The data also showed that the PDC percentage in the peripheral blood of HPRL patients with a mean of 0.2±0.13 significantly decreased compared with normal individuals with a mean of 0.35±0.24 ($p\leq0.002$, Figure 2B).

Data analysis revealed that the ratios of MDCs/PDCs in HPRL patients were 1.21±0.63, which

did not have a significant difference compared to normal individuals with a mean of 1.6±1.74 (p=0.83). Covariance analysis (ANCOVA) showed that the MDC/PDC ratios in both cases and control groups show an insignificant difference after removing the effect of PRL level as a confounding variable (p=0.928). Spearman's correlation coefficient between PRL and MDCs was-0.325, which shows an inverse association between the two variables (p=0.008). Also, the association between PRL and PDCs was-0.30, which is also statistically significant (p=0.014).

DISCUSSION

The roles of some hormonal factors including androgen, estrogen, progesterone, and vitamin D in the regulation of functional and phenotypic characteristics of DCs and their subpopulations have been identified.[1] However, information about the effects of hypothalamic-pituitary axis hormones, especially PRL, on DC subtypes and their biology is limited. Most studies aiming to investigate the effect of PRL on DCs have been conducted on animal models and *in vitro* and focused more on the role of this hormone in puberty stimulating and the function of these cells.[20-22]

For the first time, in this study, we evaluated the percentages of MDC and PDC subpopulations and their ratios in peripheral blood of HPRL women compared to normal subjects. Our analysis showed that the MDC and PDC population in peripheral blood of patients with HPRL decreased in comparison with those of healthy volunteers. Also, we found the MDC/PDC ratio was significantly different in neither of the two groups. According to the results, it can be stated that increased serum PRL level reduces the number of DC subsets in peripheral blood but does not affect their proportion and balance.

In Ueda et al.'s study, the percentages of MDCs and PDCs and their ratios in peripheral blood samples obtained from women were evaluated during pregnancy as well as at the time of delivery and in the umbilical cord blood. Their results showed that the ratio of MDCs/PDCs decreased in pregnant women, partly because of hormonal changes, including changes in PRL. The reduction of MDCs during pregnancy can play an important role in maintaining immune tolerance against the embryo.[11]

A study was conducted to investigate the individual and combined effect of granulocyte-macrophage colony-stimulating factor and PRL on the maturation of DCs from blood monocytes under serum-free conditions. The results showed that the physiological concentration of PRL had a synergistic effect on GM-CSF on the maturity and function of DCs.[20]

The effect of PRL on the maturation, proliferation, and DC functions of the thymus in rats was investigated in another study. This study showed that PRL presence in thymocyte cultures did not increase the number of DCs, but stimulated their differentiation.[22]

Yang et al. demonstrated that PRL increased the expressions of CD40, MHC-II, IL-6, IL-10, IL-12, and TNF-α in murine spleen CD11c-positive dendritic cells (SDCs) while it decreased the level of CD54, NF-κBp65, and the endocytosis in these cells. It was assumed that PRL up-regulates the stimulatory capacity and viability of SDCs.[8]

The percentages and particular balance between MDCs and PDCs are essential for polarizing T-cell mediated immune responses and contributing to Th1 or Th2 differentiation. There are several disorders whose onset is influenced by changes in the distribution of DC subpopulations.[23] One of the most common and important of these disorders is autoimmunity. Given the crucial role of DCs in maintaining immune tolerance and initiating an immune response, they are the most important cells involved in the induction and development of autoimmune disorders. Therefore, in autoimmune diseases, alteration and activation of DC subsets is a necessary first step in activating self-reactive lymphocytes, producing pathogenic autoantibodies, and processing chronic inflammation reactions.[9,24] On the other hand, there is much controversy about the potential role of PRL in the pathogenesis of autoimmune disorders. The results of studies, suggesting an increase in serum levels of PRL in many autoimmune diseases as well as an increase in the level of autoantibodies in HPRL patients, support this claim.[25-29] Therefore, considering the possible effect of HPRL in autoimmune disorders and the key role of DC subpopulation balance in these diseases, practical investigations on the influence of elevated serum PRL levels on MDC/PDC ratios can shed light on the complex mechanism of autoimmunity and its treatment.

In conclusion, this study revealed that an increased level of serum PRL is concurrent with a decrease in the number of peripheral blood MDC and PDC

subpopulations. Conducting this research on a larger sample size will help better define this relationship. Regarding the effect of prolactin on the DC subset population, it is possible that this hormone affects the maturation and function of these cells. Further studies to investigate this possibility seem necessary.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

This study was supported by grants from the Endocrinology and Metabolism Research Center of Kerman University of Medical Sciences (No.94/686). The authors express great appreciation for the volunteers for their participation in this study.

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