ORIGINAL ARTICLE: FERTILITY PRESERVATION

High cytokine expression and reduced ovarian reserve in patients with Hodgkin lymphoma or non-Hodgkin lymphoma

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Objective: To investigate the ovarian reserve in female lymphoma patients and the potential relationships with the cytokine network.
Design: Age-matched control study.
Setting: Women's university hospital.
Patient(s): Seventy-three lymphoma patients (57 with classic Hodgkin lymphoma [HL] and 16 with non-Hodgkin lymphoma [NHL]), approaching our center for ovarian tissue cryopreservation (study group) were compared with 25 age-matched healthy volunteers (control group).
Intervention(s): Measurements of antimüllerian hormone (AMH), soluble interleukin-2 receptor (SIL-2R), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor α (TNF-α) levels.
Main Outcome Measure(s): The AMH and cytokine levels of the lymphoma patients and the healthy volunteers were compared. Correlations between AMH with SIL-2R, IL-6, and IL-8 levels were performed.
Result(s): The AMH showed significant lower concentrations in lymphoma patients than in the control group. Higher significant concentrations in lymphoma patients than in control group were found for SIL-2R and IL-6. No differences were observed comparing HL and NHL groups and within the stages of HL group for AMH and all the cytokines analyzed. Finally, significant inverse correlations were observed in lymphoma patients between AMH and SIL-2R, IL-6, and IL-8 levels, but not with TNF-α levels. Positive correlations between SIL-2R with IL-6, and IL-6 with IL-8 were also shown.
Conclusion(s): In patients with HL or NHL at baseline the cytokine network is particularly active and the ovarian reserve is reduced. A strong negative correlation between AMH and SIL-2R, IL-6, and IL-8 has been also evidenced. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Antimüllerian hormone, cytokines, Hodgkin lymphoma. non-Hodgkin lymphoma, fertility preservation

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Survival rates of patients suffering from Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) have increased significantly during the past decades as a result of a use of intensive chemotherapy regimens (1). However, HL and NHL survivors have to face the long-term side effects of the treatments, primarily premature menopause, reduced fertility and even infertility.

It is well established that the clinical and pathologic features in lymphoma disease reflect a defect in the cell-mediated immune response and that this defect may be attributable to the aberrant elaboration of a variety of cytokines produced by the malignant Reed-Sternberg cells and surrounding reactive inflammatory cells (2). Cytokines play important roles in the pathogenesis of lymphoma disease (3), and several of them are definitely elevated in patients with HL or NHL (4, 5). Furthermore cytokines may also affect ovarian function. Soluble interleukin-2 receptor (SIL-2R) into biological fluids is considered as the most active indicator of lymphocyte activation and proliferation and has a key role in tolerance and immunity of the immune system

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(6). The SIL-2R level demonstrated an activation of T cells that arrive in the ovary at the end of the follicular phase (7). Interleukin-6 (IL-6) is an important regulator of the acute phase inflammatory response influencing the growth and differentiation of B and T cells, induces plasma cell differentiation, is involved in angiogenesis, and may represent a physiologic link between the endocrine and immune systems modulating ovarian function (8). Interleukin-6 showed higher concentrations in follicular fluid (FF) than in serum (9) and was able to inhibit aromatase activity suppressing FSH-binding capacity in porcine granulosa cell (GC) cultures (10). Interleukin-8 (IL-8), the most representative α chemokine, is a potent activator of neutrophils and angiogenic agent and is indicative of inflammatory and growth regulating properties (11). Interleukin-8 was involved in follicular development and atresia, ovulation, steroidogenesis, and corpus luteum (CL) function (12), and inhibited estrogen (E) production and enhanced P production in bovine GCs (13). Tumor necrosis factor α (TNF-α) is a mediator of the immediate-early immune response (14) and, in particular, was demonstrated a powerful inhibitor of GC proliferation and gonadotropin-stimulated steroidogenesis in rats (15). The IL-6, IL-8, and TNF-α have also proven to have an important role in the oogenesis regulation (16).

On the other hand, a reduced ovarian reserve characterized by low antimüllerian hormone (AMH) levels in patients with HL and NHL has recently been observed even before the start of chemotherapy (17). In addition, adolescent and young women with HL have a significant damage to gametes in pretreatment conditions, therefore we have advanced the hypothesis of a relationship between the disease itself and the gonadal dysfunction (18).

In the present report we analyzed for the first time simultaneously the behavior of AMH, as indicator of ovarian reserve, and a panel of representative cytokines, including SIL-2R, IL-6, IL-8, and TNF-α studied in pretreatment conditions in a group of patients with HL or NHL and in a healthy control group. These patients are applicants for ovarian tissue cryopreservation. There is a need to evaluate the ovarian reserve in these patients and to establish possible relationships between the aberrant cytokine production and the potential ovarian dysfunction in lymphoma disease.

MATERIALS AND METHODS

Subjects

Between March 2011 and May 2015, 73 postpubertal white patients suffering from lymphoma disease (57 patients with classic HL and 16 patients with NHL) (mean ± SD age, 24.3 ± 6.2 years; range, 14–38 years) approached our Centre for ovarian tissue cryopreservation counseling. These patients were selected for the study. The HL patients were staged according to the Ann Arbor system (stages I through IV) (19). All 73 patients agreed to take part in the study (study group).

Twenty-five age-matched healthy volunteers of reproductive age (mean ± SD age, 26.1 ± 5.7 years; range, 18–39 years; $P=.204$ vs. lymphoma patients) were also enrolled in this study as the control group. These volunteers were from the medical and nursing staff of our medical school.

This study and the ovarian tissue cryopreservation procedure were approved by the Ethical Committee of S. Orsola-Malpighi Hospital (clinical trial N. 74/2001/O; date of application February 13, 2002). Inclusion criteria for all subjects studied (study and control groups) were no previous cytotoxic treatment, the risk of premature ovarian failure due to the forthcoming anticancer treatment >30%–40%, presence of regular menstrual cycles (26–32 days), no premenopausal condition identified by FSH and LH levels, no history of ovarian cancer or surgery, no evidence of endocrine/metabolic or systemic diseases that are accompanied by a reduction in the ovarian reserve, no medication or hormonal therapy in the 3 months before study entry. For early stage HL, particularly, additional inclusion criteria who met The International Prognostic Markers for relapse were used (20). The study group was subjected to AMH and cytokine investigations within a period of 24–36 hours before ovarian tissue cryopreservation in a random day of the menstrual cycle. The control group was evaluated the same way as the study group. All subjects (study and control groups) gave their informed consent for the analysis, and in the case of minors the consent was signed by parents.

Study Design: Hormone and Cytokine Assay

The study protocol was in accordance with the Helsinki Declaration of 1975. A blood sample was taken to measure serum AMH levels as a marker of ovarian reserve and serum levels of SIL-2R, IL-6, IL-8, and TNF-α as representative of the cytokine network. All serum measurements were performed at the Central Laboratory of S.Orsola-Malpighi Hospital, Bologna.

Serum AMH was assayed in duplicate by ELISA, using AMH Gen II ELISA kit (Beckman Coulter Inc.). Serum concentrations of IL-6, IL-8, and TNF-α were assayed in duplicate by BD Cytometric Bead Array (CBA) Human Inflammatory Cytokines kit (Becton Dickinson & Company, Biosciences). Serum concentrations of SIL-2R was assayed in duplicate by Immulite (Diagnostic Products) chemiluminescent immunometric assay. The sensitivity of the methods and respective intra-assay and inter-assay coefficient of variation (CV) were in agreement with those reported by manufacturer.

Statistical Analysis

Statistical analyses were performed by using the Statistical Program for Social Sciences (IBM SPSS, version 21.0, IBM Co.). Mean, SD, median, range, and 95% confidence interval are reported as descriptive statistics to describe continuous data. Absence of normality distribution of quantitative variables was assessed using Kolmogorov-Smirnov test. Nonparametric statistics for quantitative variables were then applied and the Mann-Whitney U test was used to determine differences between the two groups of subjects and Spearman rank correlation was used to test relationship between the AMH and cytokine variables. Two-tailed P values $<.05$ were considered statistically significant.