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Clinical short communication

Relevance of endoglin, IL-1α, IL-1β and anti-ovarian antibodies in females with multiple sclerosis

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ABSTRACT

Few studies support the concept of reduced fertility in females with multiple sclerosis (MS). Recently we reported on reduced serum levels of Anti-Müllerian Hormone (AMH) in reproductive-age females with MS, suggestive of reduced ovarian reserve. The cause for this observation is not evident and remains speculative. The aim of the study is to examine possible immunological mechanisms interfering with fertility, as well as ovarian reserve that might affect the reproductive potential in women with MS.

ELISA experiments were done to detect anti-ovarian antibodies (AOA), endoglin and interleukin (IL)-1α/-1β in sera of 85 MS females, including 15 women with known low AMH level as a marker of ovarian reserve, compared to 63 healthy controls.

Groups did not differ with respect to age, smoking habits, BMI, and use of oral contraceptives. MS females showed significantly increased endoglin values compared to healthy controls. Remarkable, the highest endoglin values were found in subjects with low AMH. AOA were neither detectable in MS patients nor control subjects. IL-1α and IL-1β levels did not differ between groups.

Our data established no relevance of IL-1α/-1β or AOA in ovarian insufficiency/dysfunction but suggests the involvement of endoglin in RRMS.

1. Introduction

For many females in whom the reason of infertility is not known, autoimmunity may be the pathogenic mechanisms. In women with multiple sclerosis (MS) the concept of reduced fertility is not clear cut and in fact having fewer children could be due to different biological or behavioral factors. Recently we demonstrated that reproductive aged females with relapsing remitting MS (RRMS) are characterized by lower serum levels of Anti Müllerian Hormone (AMH), suggestive of reduced ovarian reserve [1]. Yet, the reason of decreased AMH is not understood and in MS inflammatory processes are limited to the central nervous system. Nevertheless, similar observations in other immune mediated diseases, e.g., systemic lupus erythematosus support the assumption that uncontrolled inflammation adversely influences ovarian reserve, reproductive aging and fertility [2]. In line, Sepúlveda et al. (2015) reported on reduced AMH values in females with higher MS disease activity [3].

A recent paper highlighted the relevance of the proinflammatory cytokine interleukin (IL)-1 on the age-related exhaustion of ovarian reserve in rodents [4]. IL-1 seems also to be involved in endometriosis associated pelvic inflammation and related infertility [5]. Furthermore, immune mediated diseases along with the presence of anti-ovarian antibodies (AOA) may be involved in ovarian insufficiency/dysfunction [6]. AOA are a heterogeneous group of antibodies, critically involved in endocrine and reproductive function of human ovary and can adversely influence it, which in turn led to failed implantation and fertilization [7,8]. A probable involvement of AOA in females was discussed in numerous diseases including neurological disorders such as MS and Myasthenia gravis [6].

Endoglin (ENG) is a transmembrane glycoprotein, belongs like AMH to the TGF-β superfamily, and functions as a co-receptor for TGF-β. While endothelial endoglin expression was shown to be increased in human MS tissue, there are no studies reporting on soluble endoglin (sENG) in MS [9]. sENG showed an inhibitory activity on leukocyte adhesion and endothelial transmigration in inflammation and was suggested to skew the CD4 T cell population toward a proinflammatory homeostasis. These aspects may be of relevance in MS [10,11]. Additionally, sENG is increased in pre-eclampsia of pregnancy [11].

To the best of our knowledge, no studies have examined possible immunological mechanisms interfering with fertility that might affect the reproductive potential in women with MS. We analyzed serum levels of ENG, IL-1α/-1β, as well as AOA in females with RRMS characterized by

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normal and low AMH values compared to aged matched healthy controls (HC). We hypothesized that serum levels of these biomarkers would be different in women with known low AMH compared to MS females with normal AMH and HC, respectively.

2. Materials and methods

2.1. Participants

We included a total of 148 reproductive-aged women of white race/ethnicity. Females with RRMS (n = 85; fulfilling revised McDonald criteria) were characterized by either normal (>0.8 ng/ml; n = 70), low (<0.8 ng/ml; n = 4) or very low (<0.4 ng/ml; n = 11) AMH. The characteristics of this cohort were published recently [1]. Age matched healthy females (n = 63) served as controls. Exclusion criteria were: age < 18 years and >35 years, pregnancy, chronic liver, kidney or autoimmune (other than MS) disease, abnormal thyroid function, and history or treatment with drugs toxic to reproduction. Females seeking advice on hormonal stimulation were excluded. All participants gave written informed consent.

2.2. Sample collection and ELISA

Blood samples were collected and then centrifuged for 20 min at 4 °C at 2000 g. Corresponding serum samples were stored at −80 °C and brought to room temperature immediately before experiments. An enzymatically amplified-two-site immunoassay was used according to manufacturers' protocols in order to determine ENG, IL-1α/1β (R&D Systems) and AOA (Anti-ovary antibody ELISA, BIOSERV DIAGNOSTICS, Rostock, Germany). Positive controls were used to validate each assay. We defined subjects *naïve* to treatment if they had not received any immunomodulatory/-suppressive medication during the last 16 weeks.

2.3. Statistical analyses

Statistical analyses were performed using Prism software (GraphPad, San Diego, CA, USA). ELISA results shown are average of triplicates ± standard error of the mean (SEM). Statistical analyses were performed using Dunn's multiple comparison test.

3. Results

3.1. Clinical data

We included 148 women, 85 with RRMS and 63 HCs. The mean expanded disability status scale (EDSS) score in the RRMS group was 2.6 ± 0.89. 25 women with RRMS were admitted to the hospital due to appearance of new symptoms (active disease). Symptom onset was within 7 days prior to admission. All patients with active disease received intravenous methylprednisolone treatment after collection of blood samples. MS patients and HC did not differ significantly with respect to age, BMI, use of hormonal contraception and smoking habits. Characteristics of participants are summarized in Table 1.

3.2. ELISA results

AOA were neither detectable in RRMS subjects nor HC. Serum IL-1α/-1β levels did neither differ significantly between female MS patients with low and normal AMH nor MS patients and HC.

MS females showed significantly increased sENG values compared to HC (Fig. 1A, Table 2). The mean sENG level in HC was 1213 ± 24.48 pg/ml compared to 1403 ± 32.12 pg/ml in RRMS. Subjects with low and normal AMH showed also significantly increased sENG levels compared to HC. *ELISA* demonstrated increased sENG values in subjects with low AMH compared to normal AMH, although this effect failed to reach statistical significance (Fig. 1A, Table 1). Patients treated with natalizumab and treatment *naive* subjects showed significantly increased sENG values compared to HC while patients treated with other MS agents showed no significant sENG elevation (Fig. 1B, Supplementary Table 1). sENG levels did not differ in subjects with active MS compared to patients with stable disease (Fig. 1C, Table 2).

4. Discussion

In this observational, cross-sectional study, we have shown for the first time that sENG levels are significantly increased in women with RRMS compared to HC. In contrast to other immune mediated diseases we could not detect AOA, making a causative relationship between AOA and ovarian insufficiency in women with MS unlikely.

One relevant cause that adversely influences ovarian reserve and reproductive prognosis is autoimmunity [6]. Usually autoimmune diseases occur in reproductive-age women. Hence, if we can better understand the mechanisms involved in fertility issues in females with MS, patients with unrealized wishing for a baby or recurrent miscarriage might be managed more efficiently.

Our data established no relevance of sENG, IL-1α/-1β or AOA in ovarian insufficiency/dysfunction neither in females with normal serum AMH nor low serum AMH value but suggests the involvement of sENG in RRMS. Comparison of sENG levels showed significantly increased values in MS patients, even though levels did not reach levels found in pre-eclampsia. sENG levels were independent from AMH levels or disease activity. Interestingly, solely natalizumab treated and subjects naïve to treatment had increased sENG values while this was not found in any other MS therapy. Although the numbers were small, the consistent decrease of endoglin in subjects treated with IFN-beta, GLAT, fingolimod or rituximab suggests a treatment related effect. Increased serum levels of cytokines important in MS were reported in natalizumb treated patients 6–12 months after treatment initiation. Additionally, natalizumab treatment increased the percentage of activated leukocytes producing proinflammatory cytokines in the peripheral circulation [12, 13].

Yet, the meaning of increased sENG values in females with RRMS remains speculative. sENG is an inhibitor of TGF receptor signaling. In inflammation, TGF-β has a dual role on T-cells, as it contributes both to the induction of proinflammatory Th17 cells and immunosuppressive regulatory T-cells (Treg) [14]. Thus, sENG may influence Treg/Th17 homeostasis. Numerous studies have supported a particular need for a balanced regulation of both subsets in MS [15]. In pre-eclampsia,

Table 1
Patient characteristics.

	Control	RRMS	Low AMH	Normal AMH	Stable	Active
Number of patients, n	63	85	15	70	60	25
Mean age, years	28.6 ± 3.4	28.8 ± 4.8	27.2 ± 4.3	29.1 ± 4.1	29.6 ± 4.4	28.1 ± 4.5
Age range, years	19–35	18–35	20–35	18–35	18–35	19–34
Nicotine consumption	37.3%	32.9%	20.0%	35.7%	25%	40%
Use of birth control pill	54.2%	52.9%	53.3%	52.8%	43.3%	60%
ENG, pg/ml (±SEM)	1213 ± 24.5	1403 ± 32.1	1518 ± 94.8	1393 ± 33.5	1397 ± 34.4	1423 ± 76.5
EDSS		2.8 ± 1.6	2.66 ± 0.8	2.8 ± 0.7	2.6 ± 1.6	2.9 ± 0.5