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Significance of growth differentiation factor 15 in primary ovarian insufficiency: inflammatory, biochemical, and hormonal correlates

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Summary

Purpose: To investigate the levels of growth differentiation factor-15 (GDF-15) in primary ovarian insufficiency (POI) and to evaluate its correlation with hormonal, biochemical, and inflammatory indicators. *Materials and Methods:* This comparative, cross-sectional study was carried out in 60 cases consisting of 30 healthy controls (mean age: 29.2 ± 5.0 years) and 30 patients with POI (mean age: 28.9 ± 6.8 years). Two groups were compared in terms of serum levels of glucose, lipids, thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), GDF-15, and neutrophil-lymphocyte ratios (NLR). Correlation between GDF-15 and NLR with these variables was sought. *Results:* Serum levels of FSH ($p < 0.001$), LH ($p < 0.001$), NLR ($p < 0.001$) and TSH ($p = 0.020$) were increased significantly in POI group. In POI patients, a correlation was detected between levels of GDF-15 levels and PRL ($p = 0.049$). *Conclusion:* The authors suggest that NLR can serve as a promising marker for diagnosis and follow-up of POI, whereas GDF-15 seems not to have such a potential.

Key words: Primary ovarian insufficiency; Growth differentiation factor-15; Neutrophil lymphocyte ratio; Inflammation.

Introduction

Primary ovarian insufficiency (POI) is characterized with the absence, non-functionality or early depletion of the ovarian reserve that may in turn result in infertility. Its relevance has been increased recently attributed to the delayed age of motherhood in developed countries [1]. It is typically accompanied with primary or secondary amenorrhea for at least four months in women younger than 40 years of age with menopausal serum FSH levels > 40 IU/L obtained on two intervals at least one month apart and estradiol (E2) levels < 50 pg/ml [2]. Even though serum levels of anti-Müllerian hormone can serve as an indicator of POI since it reflects of the state of follicular senescence, there are no single screening tests that can predict a woman's reproductive lifespan at the moment [3, 4]. Due to the continuum of impairment of ovarian function, with no specific endpoint for this process, use of the term POI seems to be more appropriate than the term premature ovarian failure [5]. Actually, hypergonadotropic hypogonadism, premature ovarian failure, and ovarian dysgenesis can be included within the context of POI.

The incidence of POI for women younger than 20 years of age is 1:10,000 whereas it rises to 1:1,000 in women younger than 30 years and 1:100 in women before 40 years [6]. Therefore, POI is claimed to be one of the leading causes of female infertility [1]. POI presents with a variety of symptoms due to low levels of estroid hormones and secondary to diminution of ovarian function. Thus, symptoms including hot flashes, sleep disturbances, decreased mental concentration, loss of sexual desire, and vaginal dryness can be seen. Moreover, long-term consequences of hypoestrogenism including higher risk for osteoporosis or cardiovascular disease can occur [7]. In addition to hormone replacement therapy, professional and family support is necessary to eliminate the adverse effects of POI on emotional health, stress, social life, and profession. Etiology of POI may be linked with iatrogenic reasons, environmental factors, viral infections, metabolic factors, autoimmune diseases, and genetic alterations [8]. Mostly, the origin is idiopathic and it occurs without warning symptoms in many cases [9].

The role of autoimmunity in POI and the presence of autoimmune oophoritis has been shown in POI patients with adrenal autoimmunity. Such autoimmunity may occur due to antigens common to both organs composed of steroidogenic cells. The mechanism that causes and induces ovar-

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ian autoimmunity and inflammation is still obscure. Nevertheless, POI may be associated with ovarian tissue damage attributed to viral infection or other injury, which may in turn induce the ovary to become antigenic [10].

The systemic inflammatory response can be evaluated with the neutrophil to lymphocyte ratio (NLR) which has been suggested as an inexpensive and widely available marker [11]. Yildirim *et al*. suggested that NLR, which is an inexpensive and readily available marker, could have a promising role as a marker for POI [12].

Growth differentiation factor 15 (GDF-15), is a member of the human transforming growth factor-α superfamily. The placenta is the only tissue that expresses a substantial amount of GDF-15 under physiological circumstances and GDF-15 presumably plays a role at the maternal-fetal interface. It is supposed to promote fetal survival via suppression of the production of proinflammatory cytokines within the uterus. Notably, GDF-15 displayed immunosuppressive effects via inhibition of the proliferation of peripheral blood mononuclear cells [13].

As far as we know, any association between POI and levels of GDF-15 has not been investigated in the medical literature yet. The aim of the current study was to evaluate levels of GDF-15 in POI and to investigate the hormonal, inflammatory and biochemical correlates.

Materials and Methods

Study design

This cross-sectional, comparative trial was implemented in the Obstetrics and Gynecology Department of the present institution. Before the study, approval of local Institutional Review Board and written informed consent of all participants were obtained.

Thirty healthy controls and 30 women diagnosed with POI were recruited. Participants in these two groups were matched for age, gravidity, and body-mass index (BMI). Women with primary or secondary amenorrhea before 40 years of age, a normal karyotype of 46XX, and follicle stimulating hormone (FSH) levels > 40 IU/L in at least two consecutive measurements were selected. Patients with secondary causes of POI, such as surgery, chemotherapy or radiotherapy, and chromosomal abnormalities were excluded from the study. Women in the control group reported to no pre-existing medical or obstetric conditions, such as chronic or acute inflammatory diseases, such as collagen vascular diseases, infections, cardiovascular diseases, diabetes mellitus or renal diseases. Descriptive data including age, BMI, smoking habit, and marital status were recorded.

Serum studies

Peripheral venous blood samples from drawn from antecubital veins after bed rest in semirecumbent position for one hour subsequent to an overnight fasting period. All of the collected blood samples were centrifuged at 4,000 rpm and +4°C for ten minutes and they were transferred into Eppendorf tubes. After storage of samples at room temperature for an hour, samples were kept at -80°C in deep freeze until analysis was carried out. Complete blood count, serum glucose level, lipid profile (including total cholesterol and triglycerides), and levels of thyroid stimulating hormone (TSH) were studied. NLR was calculated for POI and control groups.

Table 1. — *Comparative overview of demographic, biochemical, hormonal, and inflammatory markers in primary ovarian insufficiency and control groups.*

Variable	Control group	POI group	*p*-value
Age (years)	29.2±5.0	28.9±6.8	0.864
BMI (kg/m^2)	24.1±4.2	25.9±4.1	0.094
Marital status (M/S)	26/4	22/8	0.197
Smoking habit (Y/N)	10/20	11/19	0.787
Gravidity	1.0-2.3	1.0-2.0	0.412
Glucose (mg/dl)	89.8±18.0	96.3±17.5	0.165
Total cholesterol (mg/dl)	170.6±46.9	184.5±25.4	0.159
Triglycerides (mg/dl)	110.9±37.1	124.4±25.6	0.106
FSH (IU/L)	5.98±1.95	60.09±20.01	<0.001*
LH (IU/L)	6.35±3.72	38.07±21.21	<0.001*
E2 (pg/ml)	52.69±39.98	33.75±39.70	0.071
PRL (ng/ml)	14.15-7.49	14.00-9.45	0.594
TSH (mIU/L)	1.60±0.83	2.41±1.63	0.020*
GDF-15 (ng/L)	371.19±333.59	531.17±393.14	0.142
NLR	1.84-1.65	7.60-8.65	<0.001*

POI: primary ovarian insufficiency; BMI:body-mass index; M: married; S: single;Y: yes; N: no; NLR: neutrophil-lymphocyte ratio; FSH: follicle stimulating hormone; LH: luteinizing hormone; E2: estradiol; PRL: prolactin; TSH: thyroid stimulating hormone; GDF-15: growth differentiation factor-15; *statistically significant.

Biochemical parameters were analyzed by using an autoanalyzer. Plasma glucose levels were measured using the glucose oxidase method. Prolactin (4–15.2 ng/ml), FSH (1–8 IU/L), luteinizing hormone (LH) (1–12 IU/L), TSH (0.3–4 mIU/ml), and E2 (7.63–42.6 pg/ml) levels were analyzed by an immunoassay analyzer.

Growth differentiation factor 15 immunoassay

In accordance with the method described by Kempf *et al.*, GDF-15 level in plasma was measured by an immunoradiometric sandwich assay by using a polyclonal, affinity chromatography-purified goat anti-human GDF-15 IgG antibody [14]. All analyses were performed in duplicate and clinical data were blinded to the laboratory. The detection limit of the assay was 20 ng/L, intra-assay imprecision was 10.6% or less, and inter-assay imprecision was 12.2% or more [14].

Statistical analysis

Data was analysed by means of "SPSS Statistics 20" program. Normal distribution of variables was assessed with Kolmogorov-Smirnov test and parametric tests were used for variables with normal distribution, while non-parametric tests were utilized for variables without normal distribution. Two dependent groups were compared with Independent-Samples *t*- and Mann-Whitney U-tests. Correlation between variables with normal distribution was evaluated with Pearson Correlation test, while Spearman's Rho test was used for assessment of variables that do not display normal distribution. Pearson Chi-square test was used for comparison of categorical variables. Quantitative variables were expressed as mean, standard deviation, median, and interquartile range. Confidence interval was 95% and a *p*-value < 0.05 was accepted as statistically significant.

Table 2. — *Correlation of GDF-15 and NLR to demographic, biochemical, hormonal and inflammatory markers in primary ovarian insufficiency and control groups.*

Variable	Control group				POI group			
	GDF-15		NLR		GDF-15		NLR	
	r-value	*p*-value	*r*-value	*p*-value	*r*-value	*p*-value	*r*-value	*p*-value
Age (years)	-0.329	0.224	-0.176	0.353	0.160	0.398	-0.075	0.696
BMI (kg/m^2)	-0.133	0.483	0.088	0.644	0.103	0.590	0.141	0.498
Gravidity	-0.080	0.673	0.124	0.515	0.281	0.133	-0.054	0.777
Glucose (mg/dl)	-0.019	0.921	-0.083	0.665	-0.043	0.820	-0.142	0.453
Total cholesterol (mg/dl)	-0.199	0.292	-0.273	0.145	0.188	0.319	0.215	0.253
Triglycerides (mg/dl)	-0.290	0.120	-0.030	0.877	0.115	0.546	-0.019	0.921
FSH (IU/L)	-0.048	0.800	-0.211	0.263	0.195	0.303	-0.108	0.570
LH (IU/L)	-0.070	0.715	0.011	0.953	-0.101	0.595	0.031	0.871
E2 (pg/ml)	-0.121	0.524	0.308	0.098	-0.058	0.760	0.084	0.658
PRL (ng/ml)	-0.022	0.910	0.190	0.315	0.362	0.049*	-0.160	0.397
TSH (mIU/L)	0.222	0.238	0.225	0.232	-0.113	0.553	0.154	0.417

POI: primary ovarian insufficiency; NLR: neutrophil-lymphocyte ratio; BMI: body-mass index; FSH: follicle stimulating hormone; LH: luteinizing hormone; E2: estradiol; PRL: prolactin; TSH: thyroid stimulating hormone; GDF-15: growth differentiation factor-15; * statistically significant.

Results

Demographic and laboratory data derived from control and POI groups are demonstrated in Table 1. As can be seen, no significant differences were detected between two groups in terms of age, gravidity, smoking habit, BMI, marital status, as well as serum levels of glucose, cholesterol, triglycerides, prolactin (PRL) and GDF-15. Notably, serum levels of FSH ($p < 0.001$), LH ($p < 0.001$), NLR ($p < 0.001$) and TSH ($p = 0.020$) were increased significantly in POI group. However, serum TSH levels were within normal levels in both groups.

As presented in Table 2, results of the correlation analysis yielded that there was no correlation between any of the variables under investigation and levels of GDF-15 and NLR. In POI patients, a correlation was detected between levels of GDF-15 levels and PRL ($p = 0.049$). However, no correlation could be established between NLR and other parameters. In POI group, patients with smoking habit had remarkably higher levels of GDF-15 ($p = 0.037$).

In the control group, no difference was detected between smokers and non-smokers in terms of NLR ($p = 0.355$). Similarly, there was no difference between smokers and non-smokers in POI group with respect to NLR ($p = 0.683$).

Discussion

The current study was implemented to assess NLR and serum levels of GDF-15 in patients with POI and to evaluate the inflammatory, biochemical, and hormonal correlates. The present results imply that GDF-15 levels were not altered in POI but NLR can be a useful marker for diagnosis and follow-up.

POI has been associated with three potential mechanisms including a congenital decrease in primordial follicles, accelerated follicular atresia, and an inability to recruit primordial follicles. However, etiology underlying POI remains unexplained for the vast majority of cases. Potential etiologies for POI can be divided into genetic, autoimmune, metabolic dysfunction, infectious, and iatrogenic categories. The most common autoimmune disorder linked with POI is thyroiditis and a strong association was suggested between POI and autoimmune polyendocrine syndrome [15]. Inflammatory basis for POI has been recently investigated by Yildirim *et al.* and they suggested that NLR may be a significant promising marker before presentation or in the early stages of POI and may be useful for developing appropriate fertility treatment options [11]. The present results are consistent with their data indicating that NLR may have a potential as a marker for diagnosis and screening of POI. However, they found that NLR was lower in POI patients compared to controls. Controversially, the present authors found that NLR was higher in POI. The reason for this difference may be either linked with genetic or environmental conditions or may be attributed to the distinct types of inflammation that may be involved in POI. Remembering that there are currently no standardized tests for identification of POI, NLR may constitute a practical and inexpensive alternative tool for diseases related to chronic low-grade inflammation [16].

Biomarkers can aid in exploration of new targets for therapy and may define risk groups for individualized therapy. GDF-15 increases in cancer as well as in acute inflammation and it is induced by the tumor suppressor gene p53. Therefore, it may be a downstream target of pathways regulating cell cycle arrest and apoptosis and thus important for proliferation, invasion, metastases, and treatment resistance in cancer [17]. Inflammation is recognized as a hallmark of cancer and owing to the finding that NLR was claimed as a discriminator between myomas and sarcomas [18].

To the best of the present authors' knowledge, this is the first report focussing on the levels of GDF-15 in POI. Although the present authors could not demonstrate any association between POI and GDF-15, the complex process of inflammation and interaction with many variables hinder

making straightforward conclusions. Further trials investigating molecular and inflammatory basis of diseases should be designed in a multi-centric fashion on larger series to achieve more accurate outcomes.

The present authors did not observe any difference between controls and POI patients in terms of GDF-15, however, interestingly POI patients with smoking habit had higher levels of GDF-15 compared to POI patients that do not smoke. Therefore, GDF-15 may be involved in processes linked with carcinogenesis rather than the type of inflammation involved in pathogenesis of POI. Elucidation of the precise association between GDF-15 and inflammation cascade warrants further randomized, controlled trials on larger series.

The present results remind that early diagnosis and treatment of POI require further investigation and role of inflammatory process in pathogenesis of POI may extend beyond ovarian autoimmunity. Understanding the molecular and inflammatory basis of POI is crucial for development of appropriate anti-inflammatory treatment, which may provide preservation of ovarian function. Not only autoimmunity, but also infectious or other types of ovarian injury may result in insufficiency of immune regulation and give rise to loss of tolerance to components of ovarian tissue [12].

As a recently described marker of systemic inflammation, NLR is used for diagnosis and follow-up of malignancies in gynecological practice [11, 16]. It has been demonstrated that increased NLR was associated with greater pathology [19]. Although previous publications has shown that lymphocytosis was linked with chronic inflammation and autoimmunity [20], the present authors noted that NLR was increased in POI representing a relative dominance of neutrophils over lymphocytes. Miyake *et al.* have shown that CD8 T lymphocytes were diminished and total lymphocyte count was increased in POI [21]. Although the present authors have not analyzed lymphocyte subset counts, overt diminution of CD8 T lymphocytes may be one of the explanations of increased NLR in POI. Furthermore, this finding may remind a possible role of infectious injury in development of POI.

Lack of analysis for lymphocyte subgroups, small sample size, cross-sectional study design, and data derived form the experience of a single institution comprise the main limitations of the current study. Thus, associations and interpretations must be made with caution.

To conclude, results of the current study indicate that NLR can serve as a promising marker for diagnosis and follow-up of POI, whereas GDF-15 seems not to have such a potential. Understanding the molecular and inflammatory basis of POI is mandatory for development of more effective modes of diagnosis and treatment.

References

[1] Fortuño C., Labarta E.: "Genetics of primary ovarian insufficiency: a review". *J. Assist. Reprod. Genet.,* 2014, *31,* 1573.

[2] Nelson L.M.: "Clinical practice. Primary ovarian insufficiency". *N. Engl. J. Med.*, 2009, *360,* 606.

[3] Visser J.A., Schipper I., Laven J.S., Themmen AP.: "Anti-Mullerian hormone: an ovarian reserve marker in primary ovarian insufficiency". *Nat. Rev. Endocrinol.*, 2012, *8,* 331.

[4] De Vos M., Devroey P., Fauser B.C.: "Primary ovarian insufficiency". *Lancet*, 2010, *376,* 911.

[5] Welt C.K.: "Primary ovarian insufficiency: a more accurate term for premature ovarian failure." *Clin. Endocrinol. (Oxf.)*, 2008, *68,* 499.

[6] Coulam C.B., Adamson S.C., Annegers J.F.: "Incidence of premature ovarian failure". *Obstet. Gynecol.,* 1986, *67,* 604.

[7] Aubuchon M., Santoro N.: "Lessons learned from the WHI: HRT requires a cautious and individualized approach". *Geriatrics,* 2004, *59,* 22.

[8] van Kasteren Y.M., Hundscheid R.D., Smits A.P., Cremers F.P., van Zonneveld P., Braat D.D.: "Familial idiopathic premature ovarian failure: an overrated and underestimated genetic disease?" *Hum. Reprod.,* 1999, *14,* 2455.

[9] Beck-Peccoz P, Persani L.: "Premature ovarian failure". *Orphanet J Rare Dis.,* 2006, *1,* 9.

[10] Hoek A., Schoemaker J, Drexhage H.A.: "Premature ovarian failure and ovarian autoimmunity". Endocr. Rev., 1997, *18*, 107.

[11] Ilhan M., Ilhan G., Gök A.F., Bademler S., Verit Atmaca F., Ertekin C.: "Evaluation of neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and red blood cell distribution width-platelet ratio as early predictor of acute pancreatitis in pregnancy". *J. Matern. Fetal Neonatal Med.,* 2015, Jun 24, 1. [Epub ahead of print]

[12] Yildirim G., Tokmak A., Kokanal M.K., Sarkaya E., Zungun C., Inal H.A., Yilmaz F.M., Ylmaz N.: "Association between some inflammatory markers and primary ovarian insufficiency". *Menopause,* 2015, *22,* 1000.

[13] Corre J., Hébraud B., Bourin P.: "Concise review: growth differentiation factor 15 in pathology: a clinical role?" *Stem Cells Transl. Med.,* 2013, *2,* 946.

[14] Kempf T., Horn-Wichmann R., Brabant G., Peter T., Allhoff T., Klein G., *et al.*: "Circulating concentrations of growth-differentiation factor 15 in apparently healthy elderly individuals and patients with chronic heart failure as assessed by a new immunoradiometric sandwich assay". *Clin. Chem.,* 2007, *2,* 284.

[15] Cox L., Liu J.H.: "Primary ovarian insufficiency: an update". *Int. J. Womens Health,* 2014, *6,* 235.

[16] Kirbas A., Biberoglu E., Daglar K., Iskender C., Erkaya S., Dede H., Uygur D., Danisman N.: "Neutrophil-to-lymphocyte ratio as a diagnostic marker of intrahepatic cholestasis of pregnancy". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2014, *180,* 12.

[17] Trovik J., Salvesen H.B., Cuppens T., Amant F., Staff A.C.: "Growth differentiation factor-15 as biomarker in uterine sarcomas". *Int. J. Gynecol. Cancer*, 2014, *24,* 252.

[18] Kim H.S., Han K.H., Chung H.H., Kim J.W., Park N.H., *et al.*: "Neutrophil to lymphocyte ratio for preoperative diagnosis of uterine sarcomas: a case-matched comparison". *Eur. J. Surg. Oncol.*, 2010, *36,* 691.

[19] Keskin Kurt R., Okyay A.G., Hakverdi A.U., Gungoren A., Dolapcioglu K.S., Karateke A., Dogan M.O.: "The effect of obesity on inflammatory markers in patients with PCOS: a BMI-matched casecontrol study". *Arch. Gynecol. Obstet*., 2014, *290,* 315.

[20] Shah A., Diehl L.F., St Clair E.W.: "T cell large granular lymphocyte leukemia associated with rheumatoid arthritis and neutropenia". *Clin. Immunol*., 2009, *132,* 145.

[21] Miyake T., Sato Y., Takeuchi S.: "Implications of circulating autoantibodies and peripheral blood lymphocyte subsets for the genesis of premature ovarian failure". *J. Reprod. Immunol*., 1987, *12,* 163.

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