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Human chitinase-3-like protein: A pathogenic role in recurrent implantation failure?

İnsan kitinaz-3 protein: Tekrarlayan implantasyon başarısızlığında patojenik rolü var mı?

Yaprak ENGİN-ÜSTÜN[1,2], Ayse Seval ÖZGÜ-ERDİNÇ[2], Nafiye YILMAZ[2], Salim ERKAYA[2], Ayla AKTULAY[2], Nilüfer AKGÜN[2], Canan DEMİRTAŞ[3]

ABSTRACT

Human Chitinase-3-like Protein 1 (CHI3L1) (also known as serum human cartilage glycoprotein 39 (YKL-40) is a new biomarker associated with cancer and inflammatory diseases. Hematological parameters also may be called as sensitive markers of inflammation including mean platelet volume (MPV) and systemic inflammatory response (SIR) markers [neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR). In this paper, it was aimed to examine the levels of CHI3L1, MPV, NLR and PLR in patients with recurrent implantation failure (RIF) and compare these values with those of healthy women with proven fertility. The study group consisted of 28 infertile women with RIF characterized by a failure to attain a clinical pregnancy after three consecutive therapeutic cycles of IVF or ICSI. In these processes, first, all embryos must be in good condition and at an appropriate developmental phase-second, the total number of transferred ones should undergo at least four cleavages-stage embryos and minimum two for blastocysts as well. The control group included 28 healthy women with proven fertility that had conceived spontaneously without miscarriages. Hematological parameters were automatically calculated. Irrevelant differences were revealed among the groups with regard to age and body mass indices. Whereas Human Chitinase-3-like Protein 1 levels were similar in RIF (59.6 (38.6-421.2 ng/ml) and control subjects (68.5 (20.6-486.4 ng/ml) (p=0.222). Insignificant differences were found among MPV, NLR and PLR values. Recurrent implantation failure was not associated with altered CHI3L1, MPV, NLR and PLR levels.

Keywords: *Human Chitinase-3-like Protein 1, recurrent implantation failure, neutrophil-lymphocyte ratio, platelet-lymphocyte ratio*

ÖZ

İnsan kitinaz - 3 protein 1 (CHI3L1) (aynı zamanda serum insan kıkırdak 39 glikoprotein olarak bilinir, YKL-40), kanser ve inflamatuvar hastalıklar ile ilgili yeni bir belirteçtir. Bunun yanında inflamasyonun hassas belirteçleri arasında ortalama trombosit volüm (MPV) ve sistemsel inflamatuvar yanıtı (SIR) belirteçleri [nötrofil lenfosit oranı (NLR), trombosit-lenfosit oranı (PLR) sayılabilir. Bu çalışmada, yineleyen implantasyon başarısızlığı (RIF) olan hastalarda CHI3L1, MPV, NLR ve PLR düzeylerini incelemek ve bu değerlerin doğurgan sağlıklı kadınlarla karşılaştırılması amaçlanmıştır. Bu çalışma bir kesitsel çalışma olup, yineleyen implantasyon başarısızlığı olan 28 infertil kadın çalışmaya alındı. RIF arka arkaya üç siklus IVF veya ICSI tedavisi sonucunda klinik gebelik elde edilememesi olarak tanımlandı. Bölünme aşaması embriyolar için en az dört ve blastokistler için en az iki kaliteli ve uygun gelişim düzeyinde embriyonun verilmesi şartı arandı. Kontrol grubuna spontan gebe kalmış ve abort yapmamış 28 sağlıklı kadın dahil edildi. Hematolojik parametreler otomatik olarak hesaplandı. İnsan serumu kitinaz-3-protein 1 düzeyleri, üreticinin talimatlarına göre bir ticari kolorimetrik kiti (Elabscience Biyoteknoloji Ltd, Pekin) kullanılarak hesaplandı. Yaş ve vücut kitle indeksi için gruplar arasında anlamlı fark bulunmadı. İnsan kitinaz-3-protein 1 düzeyleri RIF (59.6 (38,6-421,2 ng/ml)) ve kontrol grubunda (68.5 (20,6-486,4 ng/ml) benzerdi. (p=0.222). MPV, NLR ve PLR açısından anlamlı farklılık bulunamadı. Yineleyen implantasyon başarısızlığında CHI3L1, MPV, NLR ve PLR kan düzeyleri ile ilişki saptanmadı.

Anahtar kelimeler: *İnsan Kitinaz-3-benzeri protein 1 (YKL-40/ CHI3L1), yineleyen implantasyon başarısızlığı, nötrofil lenfosit oranı, trombosit-lenfosit oranı*

INTRODUCTION

Recurrent implantation failure (RIF) as a subgroup of recurrent failed IVF processes may be prevented in sequence transferring of minimum 4 embryos in good condition, essentially three fresh or frozen cycles particularly in females under the the age of 40 years in order to achieve a clinical pregnancy[1]. In

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[1]Bozok University Faculty of Medicine, Department of Gynecology and Obstetrics, Yozgat, Turkey
[2]Zekai Tahir Burak Education and Research Hospital, Reproductive Endocrinology Unit, Ankara, Turkey
[3]Gazi University, Department of Biochemistry, Ankara, Turkey
Yazışma adresi: Nilüfer Akgün, Ataköy 3-4-11. Mahalle, Kilitbahir Sok. Begonia Blokları No: A Kapısı/4, Bakırköy, İstanbul, Turkey
e-mail: niluferakgun80@hotmail.com

nearly 10%-15% of all infertile couples RIF has been detected despite being various attempts of in vitro fertilization and embryo transfer (IVF/ET) cycles[1,2]. Genetic, hormonal, anatomic, thrombophilic conditions and inflammatory factors are responsible for the etiology of RIF[1].

Particularly the immunological causes may be among the most important factors that adversely effect endometrial receptivity and embryo implantation[3]. The endometrium allows implantation mediated by several factors such as immune cells growth factors, chemokins, cytokines and adhesion molecules at implantation window.

Human Chitinase-3-like Protein 1 (CHI3L1) is a new biomarker of tissue inflammation, which is recognized as glycoprotein produced by various cell types of the immune system epithelial cells, differentiated vascular endothelial cells and smooth muscle cells as well. On the other hand, CHI3L1 has some roles like antipathogen responses, injury, repair and angiogenesis hereby they lead to dysregulation and result in the development of atherosclerotic cardiovascular diseases, cancer and inflammatory diseases[4,5].

Experiments also revealed that the level of expression of the chitinase- like protein CHI3L1 was elevated during Th2-type anti-inflammatory response[6] that promotes migration of vascular endothelial cell secreted by activated macrophages during late stages of differentiation and the remodeling of extracellular matrix[7,8].

Mean platelet volume (MPV) is one of the marker among hematological parameters as well as systemic inflammatory response (SIR) [i.e. neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR)] are also known as sensitive markers of inflammation since they have diagnostic value in certain pathologies like diabetes mellitus, coronary artery disease[9,10]. For clinical applications, cheap, efficient and simple immunosensing SIR markers are readily available and their levels can be calculated easily.

The purpose of this article was to examine the levels of CHI3L1, MPV, NLR and PLR in patients especially regarding RIF and compare them with those of women with proven fertility.

MATERIALS and METHODS

The study group consisted of 28 infertile women with RIF characterized by failed attempts to obtain a clinical pregnancy after three consecutive cycles of IVF or ICSI. While the total count of processed embryos should be either at least four for cleavage-stage embryos or minimum two for blastocysts, two embryos transferred, all embryos are fresh transfer, whole embryos were in good shape and phase. The control group included 28 healthy women with proven fertility that had conceived spontaneously without any miscarriage.

Subsequent to IVF-ET process, blood samples were retrieved from 28 women with proven fertility and RIF presented to Center for Reproductive Medicine of Zekai Tahir Burak Education and Research Hospital, Reproductive Endocrinology Unit, from June 2015 to June 2016. Blood samples were drawn from an antecubital vein (3 mL), and pipetted into sterile heparinized tubes on 12-14th days of the menstrual cycle before gonadotropin stimulation to be performed for the IVF/ICSI-ET procedures.

Hematological parameters were automatically calculated. Human serum chitinase-3-like Protein 1 levels (CHI3L1) were analyzed using a commercial colorimetric test kit (Elabscience Biotechnology Co., Ltd., Beijing, PRC) according to the manufacturer's instructions.

The method Sandwich-ELISA is used by ELISA kit in which the micro- ELISA plate pre-coated with an antibody specific to CHI3L1 was delivered. The accumulated samples on correct micro-ELISA plate wells was integrated with the specific antibody. Afterwards, firstly to ensure incubation, a biotinylated detection antibody specific for CHI3L1 and Avidin-Horseradish Peroxidase (HRP) conjugate was transferred onto every microplate well. Next, free components were cleared away and subsequently every well was filled

up with the substrate solution. As a consequence, biotinylated detection antibody and Avidin-HRP conjugate wells solely containing CHI3L1 were turned blue. Also, the addition of a sulphuric acid solution changed the color of the substrate from blue to yellow due to termination of enzyme-substrate reaction. Therefore, spectrophotometrically the optical density (OD) wavelength of 450 nm ± 2 nm was detected while the OD value depends on the concentration of CHI3L1. Since the OD value is connected with the concentration of CHI3L1, we adapted OD of the samples to the standard curve to obtain the concentration of CHI3L1. Results were expressed as pg/mL protein.

The studied hematological parameters could easily be affected by the health status of women (i.e. any infectious disease or drug use). The study included untreated healthy women between the ages of 25 to 38 without any systemic comorbidities, and taking only iron and multivitamin preparations. The RIF group having hematological disorders, systemic problems such as hypertension, diabetes, previous autoimmune or thyroid disease, receiving blood transfusion specified medications, existing or previous uterine malformation, current or previous ultrasonographic evidence of a hydrosalpinx and those aged > 40 years were excluded from the study.

All responder women had follicle-stimulating hormone (FSH) < 10 mIU/mL or estradiol levels on day 3. No statistically significant difference was revealed between the groups with respect to the patient characteristics, age, day 3 FSH, LH and estradiol levels and number of antral follicles. Approval of local ethics committee was received.

The software package SPSS designed for windows 11.0 was used for statistical analysis of the data (Statistical package for social sciences; SPSS Inc. Chicago, IL). The mean ± standard deviation for normally distributed data or median and range for non-normally distributed data was calculated. With the help of non-parametric tests (Kruskal-Wallis and Mann-Whitney U tests), statistical disparity between the collected data was approximated. Pearson correlation coefficient was calculated to investigate the relationship between CRP and ferritin, and P<0.05 was acknowledged as a cut-off value for statistical significance.

RESULTS

The mean age of the RIF group was 31.6±5.1 (range: 25-43) years, and the mean age of the control group was 30.36±4.21 (range: 28-38) years. The two parameters ie. age and body mass index slightly differed

Table 1. Demographic data of groups.

	Age (years) (mean±SD)	BMI (mean±SD)	FSH (Median (minumum-maximum)	LH Median (minumum-maximum)	Estradiol Median (minumum-maximum)	AFC (L) Median (minumum-maximum)	AFC (R) Median (minumum-maximum)
Normal Fertility	30.36±4.21	25. 6±4.6	6.4 (2-10)	3.3 (2,1-8,9)	32.1 (15,6-51)	7 (3-12)	8 (4-12)
RIF (n=28)	31.6±5.1	26.1±4.8	8 (2-10)	3.5 (1,9-9,4)	33 (8-65)	6.5 (2-12)	8 (2-12)
p value (n=28)	0.340	0.776	0.750	0.860	0.924	0.672	0.774

Table 2. Characteristics of the two groups.

	Recurrent implantation failure (n=28)	Control group (n=28)	p value
Neutrophil / lymphocyte ratio	2.0 (1.3-3.7)	1.8 (0.0-4.8)	0.902
Platelet / lymphocyte ratio	138 (84-212)	122 (4-452)	0.711
MPV	9.8 (8-11.8)	9.1 (0.2-11.5)	0.217
CHI3L1 (ng/mL)	59.6 (38.6-421.2)	68.5 (20.6-486.4)	0.222

between groups (Table 1). Human Chitinase-3-like Protein 1 levels were similar in RIF (59.6 (38.6-421.2 ng/ml)) and control subjects (68.5 (20.6-486.4 ng/ml)) (p=0.222). There were also no significant intergroup differences regarding MPV, NLR and PLR (Table 2).

DISCUSSION

The aim of this study was to examine the possible correlation among the levels of CHI3L1, MPV, NLR and PLR in patients with recurrent implantation failure (RIF) and compare these parametres with those of healthy women with proven fertility. The principal aim of this study was to compare peripheral blood CHI3L1 levels of women with , and without RIF and to find out whether there are any other parameters in the peripheral blood samples were effective on RIF. Similarly, we found no correlation between the NLR ratios, PLR ratios, MPV among the groups in RIF and healthy women.

Diverse replaceable factors were implicated for implantation failure especially maternal factors as uterine abnormalities, hormonal or metabolic disorders, infections, thrombophilias, immunological factors with potential embryonal or endometrial origins[2].

If good quality embryos have been supplied on each IVF treatment, the probable cause of failure is an abnormal endometrium which is not conducive to the establishment of the implantation process. CHI3L1 correlates inversely with endothelial function regulated vascular endothelial cell morphology, also including cell migration and attachment chemotaxis[8]. Cytokines are produced by desidua and trophoblasts and uterine natural killer cells may affect their growth and function playing potential roles in implantation failure[3].

Although numerous cytokines have been studied in cases with recurrent abortion, much less has been reported on their role in infertility with or without failed IVF. Lee et al.[11] recently published an investigation that studied pro-inflammatory cytokines (TNF-a, IFN-c, and IL-17), anti-inflammatory cytokine IL-10, and Foxp3 in the peripheral blood mononuclear cells of idiopathic women with RIF. Mariee et al.[12] revealed an altered expression of leukaemia inhibitory factor and IL-15 in the endometrium of women with RIF. In the study of Yang et al.[13] women with RIF have elevated T cell activation in peripheral blood lymphocytes, and T cell suppressor activation seems to be related with decreased Th1 immunity. Lédée-Bataille et al.[14] tried to document the correlation among the interleukin (IL)-12, -15, and -18 mRNA in the endometrium and the relation between cytokine levels, vascular status, and endometrial natural killer (NK) cell count in patients with recurrent implantation failures. They showed that the assessment of the tripod IL-12/-15/-18 revealed distinct immune-related mechanisms. Although there are many studies on the role of cytokines in implantation failures related to peripheral blood Th1 /Th2-cells, various results have been obtained and no concensus exists between studies[15].

Many studies have been leaning on the function of inflammation in RIF, hence strong affilations were obtained between various inflammatory markers and RIF process. Neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) in peripheral blood are associated with increased inflammation, health condition and apoptosis due to physiological stress[16,17].

NLR values change in preeclampsia, gestational diabetes, intrahepatic cholestasis, premature rupture of membranes. The studies of Sahbaz A and Kurtoglu E et al.[18,19] concluded that NLR was a useful marker for predicting inflamatuar process. Sarraf et al.[20] found that systemic inflammation measured by NLR has significant association with prevalent chronic condition. PLR values also reflect inflammation, thrombotic events and malignancies. The study of Toprak E et al.[21] has shown that apparent association exists between gestational diabetes, acute pancreatitis, preeclampsia and premature rupture of membranes.

In this study, we studied systemic inflammatory response (SIR) markers in RIF patients and fertile women but unlike other studies the inflammation markers CHI3L1, MPV, NLR and PLR were not significantly different between the groups (p=0.222). The reason for that result is deriving from our limitations of low

number of cases and controls. Although we studied circulating immunological markers in the peripheral blood, cytokines were considered to have local effects. It is probable that they were rapidly inactivated in the plasma or diluted in the systemic blood and they may not reflect levels of cytokine at the local site in the uterus. The other problem was related to collection time of the blood samples of the groups. The cyclical function of the endometrium and some factors including cytokines change rapidly, and precise timing is not known as well. As it is extremely difficult to study embryo implantation in vivo in women, the evaluation of immunological status in the endometrium may be statistically significant in the context of CHI3L1, MPV, NLR and PLR.

Since RIF is likely a limited inflammatory process which does not induce reticulo-endothelial responses, they can be determined by a simple blood count. In our study RIF was not associated with altered CHI3L1, MPV, NLR and PLR levels. For more clarification of this issue, further randomized prospective studies are required.

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