

The interaction between menstrual cycle, Tumour Necrosis Factor alpha receptors and sex hormones in healthy non-obese women – results from an observational study

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Rzym ski P, Wilczak M, Malinger A, Włoszczak-Szubzda A, Jarosz MJ, Opala T. The interaction between menstrual cycle, Tumour Necrosis Factor alpha receptors and sex hormones in healthy non-obese women – results from an observational study. *Ann Agric Environ Med.* 2014; 21(3): 571–575. doi: 10.5604/12321966.1120604

Abstract

There is growing evidence that TNF-alpha and its two receptors play an important role in hormonal regulation, metabolism, inflammation and cancer. The biological effects of TNF-alpha are mediated by two receptors, p55 and p75. The aim of this study was to analyze serum concentrations of p55 and p75 and hormonal status in healthy women during the normal menstrual cycle. Eight women aged 20–22 with regular menstrual cycles were scheduled for examination on 3rd, 8th, 14th and 25th day of their menstrual cycle. We only observed a positive correlation of p75 subunit with prolactin level (correlation coefficient 0.417; $p=0.0116$) and negative correlation with insulin level (correlation coefficient -0.35; $p=0.032$) and HOMA_{IR} insulin resistance index correlation coefficient 0.39; $p=0.0185$). Furthermore, a negative correlation of p55/p75 ratio with prolactin (correlation coefficient -0.42; $p=0.0101$) and a positive correlations of p55/p75 ratio with insulin level (correlation coefficient 0.43; $p=0.008$) and HOMA_{IR} insulin resistance factor correlation coefficient 0.45; $p=0.0065$) were found.

Key words

TNF- α , menstrual cycle, estradiol, gonadotropins, insulin, prolactin

INTRODUCTION

Sex and gender differences in prevalence of different disease, pathogenesis and modulation have been frequently reported. The menstrual cycle represents one of most important factors which should be considered during study of the physiological effect of hormonal fluctuations and the immune function, as well as chronic disease modulation. The effect of the cycle on immune cell numbers and activity fluctuations have not often been reported in studies, but recent publications demonstrate an increasing interest in the subject. The menstrual cycle might affect immune cell numbers and modulate their activity throughout the 4-week cycle, as demonstrated in the case of regulatory T cells. The importance of these fluctuations are particularly relevant in the chronic diseases affecting women of reproductive age. The inflammation and immune cell activation in association with other mechanisms, such as regulation of receptor expression, modulation of muscular contraction and behavioural aspects might explain the menstrual-associated fluctuations known in many diseases. The modulatory effects of the menstrual cycle on both immune cells and systemic

diseases, such as autoimmune diseases, asthma, diabetes, cardiac arrhythmia and schizophrenia, has been reported. Most of these diseases display worsening of symptoms premenstrually or during menses due to physiologic effects on the target tissue mediated by progesterone and estrogen fluctuations and, thus, display important changes potentially relevant to numerous other conditions [1]. Menstrual cycles act as evident indicators of underlying reproductive health, i.e. infertility and increases future risk of various chronic diseases. Dysfunctional menstrual cycles can begin from adolescence and persist for many years and throughout reproductive life, causing physical, psychological, and economical strains on a women's life. Menstrual length has been used as good marker of ovarian steroid production and its regularity is a marker of smooth function of hypothalamus pituitary ovarian axis. The average menstrual bleeding is about 7 days, and the range could be between 3–10 days. Many environmental factors may affect the characteristics of the menstrual cycle including workplace, caffeine consumption, smoking, occupation, physical activity, diet, age, weight, exposure to organic solvent, medical conditions, and lifestyle factors. Shayesteh found that both irregular menstruation and abnormal menstrual amount were connected with environmental effects. The heritability value for menstrual characteristic were less than 0.1% for interval, and were about 2% for duration; further analysis suggested that 95% of non-shared environmental effect for interval and 67% of shared environmental effect for duration [2].

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Received: 08 February 2013; accepted: 17 May 2013

The menstrual cycle is a series of cyclic physiological changes that occur in the fertile human female as well as in some primate females. It is a highly complex process involving close hormonal interaction between the hypothalamus, the hypophysis and the ovary. Within the menstrual cycle, ovarian, endometrial and cervical cycle can also be distinguished. In addition to hormonal feedback mechanisms, there is also a variety of cytokines and growth factors that regulate ovarian and endometrial changes on the cellular level [3]. Among these substances, Tumour Necrosis Factor- α (TNF- α) has become of special interest.

TNF- α is a well known member of TNF superfamily involved in numerous cellular processes, such as regulation of cell survival, apoptosis, migration and differentiation. Numerous, both physiological and pathological functions of TNF- α , have been described. In the field of gynaecology, numerous authors describe its pivotal role in the physiology of the ovary and endometrial function [4, 5]. TNF- α is also involved in several gynaecological pathologies, such as ovarian and endometrial cancer, endometriosis and infertility [4, 6, 7, 8, 9].

The biological effects of TNF- α are mediated by its two receptors, namely p55 (CD120a; TNFR1) and p75 (CD120b; TNFR2). Upon binding to these receptors the signal is transferred into the cell to target transcription proteins: Nuclear Factor Kappa B (NF κ B) and c-Jun, which initiate transcriptional changes [4]. This results in activation of DNA regions responsible for cell growth, death and inflammatory response [9]. Both receptors are present on virtually any cell of higher mammals. Current data indicate that TNFR1 signals for all known activities of TNF- α , including apoptosis, differentiation and proliferation, while TNFR2 seems to signal rather metabolic actions. Relative expression and concentration of selected receptors might also shape the final response to TNF- α [10].

Cell-bound TNF- α receptors are able to dissociate from the cell surface and become active in the serum [11]. These soluble receptors (sTNFRs) are able to bind TNF- α in the blood stream, thereby blocking its biological effects. On the other hand, sTNFRs stabilize TNF- α activity by creating a slow release reservoir for TNF- α by impeding spontaneous denaturation of the cytokine [12]. Although the exact role of sTNFRs remains unknown, several studies, among them also ours, describe varying concentrations of both soluble and cell bound p55 and p75 concentrations in patients with benign and malignant ovarian tumours [13, 14, 15, 16, 17] or infertility [18]. Furthermore, sTNFRs are more stable and easier to measure than TNF- α , thereby becoming attractive subjects for diagnostic analysis [12].

TNF- α is also critically involved in the physiology of menstrual cycle [4]. The concentration of TNF- α mRNA in endometrial tissue varies across the cycle and peaks in the proliferative phase [13,20]. Additionally, local expression of its receptors varies across the cycle. TNFR1 peaks in the late secretory phase [13]. TNFR2 has a slightly different expression pattern with highest levels in the late proliferative and late secretory phases [19]. To the best of our knowledge, there is no available data concerning changes of serum free TNFRs in the menstrual cycle. The aim of this study, therefore, was to analyze serum concentrations of p55 and p75 and hormonal status in healthy women during the normal menstrual cycle.

MATERIALS AND METHOD

The study was performed between 1 November – 20 December 2009. Eight healthy Caucasian female volunteers aged 20–22 (BMI 20.2–25.4 kg/m²) were enrolled in the study. The inclusion criteria consisted of regular menstrual cycles (27–29 days), negative history of menstrual or hormonal disorders or therapies, no actual contraception and no serious disease for at least 3 months before the beginning of the study. All females were nulliparous, with no medical chronic disease or neoplasm in anamnesis. All patients were scheduled for 4 visits dated exactly on 3rd, 8th, 14th and 25th day of their menstrual cycle. The days were chosen based on physiological changes: early and late follicular phase, paraovulatory time and luteal phase. During every follow-up visit blood was collected and routine hormonal analysis performed for follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17 β -estradiol (E2), testosterone, sex hormone binding globulin (SHBG), DHEA-S, Thyroxine-stimulating hormone (TSH), free thyroxin and triiodothyronine (fT3, fT4) and prolactin. All hormones were estimated by electrochemiluminescence (ECLIA) on Roche C6000 (Hitachi, Japan) with maximum coefficients of 3.7% variations (reagents in routine laboratory processes). Based on glucose and insulin levels, the Insulin Resistance was calculated according to the HOMA (Homeostasis Mode Assessment) formula: $HOMA_{IR} = \text{Insulin } (\mu\text{U/ml}) \times \text{Glucose (mmol/l)} / 22.5$. Additionally, 5ml of blood was centrifuged for 5 min at 3,000 rpm to separate serum from morphotic elements. The serum was then stored at -20°C until whole material was obtained. The concentrations of TNF- α , p55 and p75 were assayed with commercially available kits (Quantakine, R and D Systems, USA) in duplicate. The extinction was read on Dynex MRX Endpoint 1.33 (UK). According to the manufacturer, the sensitivity of the p55 and p75 tests were 3.0 pg/ml and 1.0 pg/ml, respectively. The intraassay precision did not exceed 8.2%.

Statistical analysis was performed using SigmaStat v3.1 software (Systat Inc., USA). For repeated measures, Friedmann Repeated Measures Analysis of Variance was used because the Kolgomorov-Smirnoff test failed to discover normal distribution. Data are presented as means \pm Standard Deviation. Correlations were evaluated with Spearman's test (Rs). P value less than 0.05 was considered significant.

The study was approved by the Local Bioethics Committee.

RESULTS

The mean concentrations of TNF- α receptors on days 3, 8, 14 and 25 are shown in Table 1. There were no statistically significant fluctuations of the p55 and p75 receptor concentrations during the menstrual cycle. Also, the p55/p75 ratio did not change significantly throughout the cycle. The changes in hormonal levels are presented in Table 2.

Although no fluctuations of p55 and p75 receptors in a single menstrual cycle were found, the correlation between TNF- α receptors and selected serum biochemical parameters were evaluated. Almost no statistically significant correlation was found between either p55 or p75 or p55/p75 ratio and hormones (Tab. 3). Only a positive correlation of p75 subunit with prolactin level (Rs=0.417, p=0.0116) was observed, and

Table 1. Serum concentrations of p55 and p75 receptors during the menstrual cycle

parameter	3 rd day of menstrual cycle	8 th day of menstrual cycle	14 th day of menstrual cycle	25 th day of menstrual cycle	Friedman's test
P55 (pg/ml)	2.43 ± 1.41	1.78±0.98	2.58±1.36	2.06±0.94	p=0.53
P75 (pg/ml)	20.20±20.60	13.50±17.93	14.95±16.44	15.13±16.14	p=0.064
P55/p75 ratio	0.27±0.23	0.51±0.41	0.51±0.56	0.33±0.32	p=0.59

Table 2. serum concentrations of hormones

parameter	3 rd day of menstrual cycle	8 th day of menstrual cycle	14 th day of menstrual cycle	25 th day of menstrual cycle	Friedman's test
TSH (uIU/ml)	1.56±0.51	2.03±1.02	1.43±0.67	1.94±0.68	p=0.028
Free T3 (pg/mL)	2.87±0.43	3.27±0.70	3.07±0.38	3.07±0.63	p=0.104
Free T4 (ng/dL)	1.19±0.21	1.31±0.13	1.22±0.11	1.14±0.07	p=0.026
FSH (mIU/ml)	6.00±1.59	5.66±1.16	6.96±3.90	3.31±1.39	p=0.016
LH (mIU/ml)	4.90±1.69	5.96±1.99	16.08±11.93	4.22±1.29	p=0.026
Estradiol (pg/ml)	43.70±11.28	75.88±33.65	199.87±156.81	187.5±95.37	p<0.001
Progesterone (ng/mL)	0.57±0.21	1.52±2.51	2.60±4.97	6.44±5.36	p=0.010
Prolactin (ng/ml)	9.39±3.29	10.25±4.80	10.41±2.97	15.57±10.44	p=0.046
Testosterone (ng/ml)	0.50±0.15	0.56±0.18	0.65±0.24	0.50±0.12	p=0.145
Insulin (mU/mL)	14.92±8.33	16.84±11.64	17.56±13.38	12.11±11.30	p=0.866
Glucose (mg/dL)	89.7±6.4	87.1±6.8	85.8±7.1	89.07±8.26	p=0.435
HOMA _{IR}	0.94±2.02	1.34±1.87	1.28±2.18	1.64±1.81	p=0.954

a negative correlation with insulin level ($R_s = -0.35$; $p = 0.032$) and HOMA_{IR} insulin resistance index ($R_s = -0.39$; $p = 0.0185$) (Tab. 2). Finally, a negative correlation of p55/p75 ratio with prolactin ($R_s = -0.42$; $p = 0.0101$), as well as a positive correlation of p55/p75 ratio with insulin level ($R_s = 0.43$; $p = 0.008$) and HOMA_{IR} insulin resistance index ($R_s = 0.45$; $p = 0.0065$) were found (Tab. 3).

Table 3. Correlation between p55 and p75 TNF-receptor subunits and selected serum parameters

TNF receptors parameter	receptor p55	receptor p75	p55/p75
TSH	$R_s = -0.07$, $p = 0.673$	$R_s = 0.03$, $p = 0.838$	$R_s = -0.10$, $p = 0.567$
free T3	$R_s = 0.05$, $p = 0.770$	$R_s = 0.07$, $p = 0.698$	$R_s = -0.06$, $p = 0.732$
free T4	$R_s = -0.32$, $p = 0.0545$	$R_s = -0.22$, $p = 0.196$	$R_s = 0.11$, $p = 0.531$
FSH	$R_s = -0.08$, $p = 0.619$	$R_s = 0.20$, $p = 0.241$	$R_s = -0.27$, $p = 0.109$
LH	$R_s = -0.17$, $p = 0.323$	$R_s = -0.25$, $p = 0.136$	$R_s = 0.22$, $p = 0.201$
Estradiol	$R_s = 0.06$, $p = 0.716$	$R_s = -0.17$, $p = 0.329$	$R_s = 0.18$, $p = 0.284$
Progesterone	$R_s = -0.01$, $p = 0.954$	$R_s = 0.004$, $p = 0.979$	$R_s = -0.05$, $p = 0.782$
Prolactin	$R_s = 0.16$, $p = 0.351$	$R_s = 0.41$, $p = 0.011$	$R_s = -0.42$, $p = 0.010$
Testosterone	$R_s = 0.29$, $p = 0.082$	$R_s = 0.20$, $p = 0.235$	$R_s = -0.17$, $p = 0.316$
Glucose	$R_s = -0.24$, $p = 0.151$	$R_s = -0.20$, $p = 0.244$	$R_s = 0.19$, $p = 0.263$
Insulin	$R_s = 0.02$, $p = 0.98$	$R_s = -0.35$, $p = 0.032$	$R_s = 0.43$, $p = 0.008$
HOMA _{IR}	$R_s = -0.04$, $p = 0.79$	$R_s = -0.39$, $p = 0.018$	$R_s = 0.45$, $p = 0.006$

DISCUSSION

In the presented study, fluctuations of serum-free TNFRs across the female menstrual cycle were evaluated. According to this research, there were no changes in serum TNFR1 level and TNFR1/TNFR2 ratio across the menstrual cycle. Only TNFR2 changes reached borderline significance. Interestingly, Fernandez-Real et al. described somewhat contradictory results, with sTNFRs levels changing during the menstrual cycle in most of lean women. Furthermore, the levels of these molecules remained unaltered in overweight women [21]. The changes of sTNFRs were observed in parallel to changes in leptin levels, which might indicate some

correlation between TNF-signaling, obesity, and menstrual cycle.

This concept is particularly interesting in the context of our further biochemical analysis. A correlation between serum TNFR1 concentration and serum insulin levels, and HOMA-IR was found. The p55/p75 ratio also correlated positively with serum insulin levels and the HOMA-IR index. There are several factors that influence glucose homeostasis. In one aspect, insulin sensitivity depends on the phase of the cycle. There is a decrease in insulin sensitivity in the luteal phase [22]. More detailed studies revealed a positive correlation of estrogen and progesterone with HOMA-IR across the menstrual cycle [23]. The presented data might indicate additional involvement of TNF-signaling in this phenomenon. In various studies, TNF- α was found to be crucial in the pathogenesis of insulin resistance [24, 25]. In overweight patients, TNF- α is upregulated in adipose and muscle tissue [26, 27]. Furthermore, treatment of some rodent diabetes models with soluble TNF- α receptor-Ig fusion protein led to reduction of insulin resistance [28]. Finally, various states characterized by increased levels of circulating TNF- α , such as infection or inflammatory diseases, also coincide with insulin resistance [29]. Upregulation of TNF- α was also found in gestational diabetes mellitus patients [30]. On the molecular level, TNF- α downregulates GLUT4 glucose transporter in adipocytes and myocytes [31]. Additionally, TNF produces serine phosphorylation of insulin receptor substrate-1, decreasing the tyrosine kinase activity of the insulin receptor [32]. The role of sTNFRs in the development of insulin resistance is not entirely clear. The presented study shows a slight negative correlation between sTNFR2 and insulin and insulin resistance index. In contrast, sTNFR1 did not correlate with either of these parameters. Data currently available in the literature support the presented observation [33]. Furthermore, sTNFR2 correlated with body-mass index (BMI), waist-hip ratio and fat-free mass – well-known cofounders in the evaluation of insulin sensitivity [34]. The presented findings and the findings of other authors support the involvement of TNF and its receptor systems in the regulation of glucose homeostasis, even in non-obese patients. But the current study focused on changes during the menstrual cycle; thus, the bias of obesity and insulin

resistance could be minimal regarding fluctuations in follow-up subjects.

Another issue is the positive correlation between p75 and negative between p55/p75 ratio with prolactin levels observed in the presented study. Prolactin is a peptide hormone secreted mainly by the anterior pituitary. It is considered a reproductive hormone, responsive mainly for milk production, but might also play an immunomodulatory role in inflammation [34]. Studies on the effect of TNF on the secretion of prolactin are conflicting. In animal experiments, some authors, e.g. Friedrichsen and Koike showed an increase [35, 36], while Gaillard showed a decrease [37] or no effect (Milenkovic) [38] in PRL secretion upon treatment with TNF. In the current literature, however, there is some evidence concerning the link between prolactin regulation, estrogen and TNF signaling. *In vivo*, pituitary prolactin expression is regulated by dopamine and hypothalamic factors, such as TRH and plenty of intrapituitary factors [39]. In addition, estrogen is considered a major activator of prolactin synthesis and lactotroph proliferation [40]. Interestingly, the effects of estradiol on hPrl prolactin promoter gene are significantly amplified by TNF- α via the NF κ B signaling pathway [35, 41]. Furthermore, estradiol increases expression of TNF- α and TNFR1 in lactotrope cells of the anterior pituitary [42]. All these data indicate a close relationship between estrogen, prolactin and TNF signaling pathways. Our data suggest that serum-free TNF receptors might also be involved in the regulation of prolactin secretion.

CONCLUSIONS

The levels of serum-free TNF receptors and p55/p75 ratio do not fluctuate significantly across the menstrual cycle. However, there is a correlation of p75 TNF receptor and p55/p75 ratio with insulin and prolactin levels and insulin resistance, which might indicate a new role of soluble TNF receptors in the human physiology. The limitation of the presented study is the small number of subjects. Some differences may not occur or are of borderline significance and need to be checked in further research. The potential information from this study is the understanding of TNF alpha receptors in the physiological status, which is the reference for considering pathologies iE ovarian cancer. We proved earlier that there are strong correlations and unspecific neoplastic answers, including TNF alpha receptors [15, 16, 17].

Acknowledgments

The study was financed by Poznań University of Medical Sciences (Grant No. 501-01-4412525-08454).

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