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PREGNANCY AND DISEASE

Miscarriage, and TNF-α and osteopontin relationship in women patients with Hashimoto's thyroiditis

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***Objective*: Infertility and reproductive impairment can be compromised by abnormalities in both endocrine and immune system. TNF-α promotes apoptotic cell death in fetal membrane tissues and pro-inflammatory, proapoptotic, and procoagulant properties of TNF-α probably contribute to widely accepted abortogenic profile of this cytokine. The aim of this study was to assess the alteration in the levels of TSH, FT3, FT4, TNF-α, osteopontin in pregnant and controls. *Methods*: Study subjects were 28 pregnant women, 28 non-pregnant women, and 28 healthy controls. All subjects underwent venous blood drawing for levels of TNF-α, osteopontin, and also hormonal assays including the levels of anti-TPO, anti-TG antibodies, TSH, FT3, FT4. *Results*: Both patient and control groups are similar in terms of age. Pregnancy age in conceived patients is 23.64 ± 2.040. No statistically meaningful relation was found in correlation analysis between TNF-α and osteopontin among the groups ($p = 0.963$). Anti-thyroglobuline antibody and anti-microsomal antibody levels were found to be higher in patients with non-pregnant patients with Hashimoto thyroiditis than the control group ($p < 0.001$). No statistically meaningful relation was found in terms of TNF-α ($p = 0.66$) and osteopontin serum levels ($p = 0.50$) in patient groups with or without miscarriage history. *Conclusions*: In our study, no statistically meaningful relation was found in terms of TNF-α and osteopontin serum levels in patient groups with and without miscarriage history.**

Keywords: Hashimoto's thyroiditis, miscarriage, osteopontin, TNF-α

Introduction

Infertility is defined as the inability to conceive after one year of regular intercourse without contraception. Among the factors that may negatively influence normal fertility, immunologic factors are known to play an important role in the reproduction processes of fertilization, implantation and early development of an embryo. Different reports support the association between reproductive failure and autoimmune diseases, including anti-phospholipids, anti-nuclear antibodies and organ specific autoimmunity, among which the occurrence of anti-thyroid antibodies takes place [1,2].

With regard to thyroid dysfunction, clinical hypothyroidism is clearly associated with female infertility, and autoimmune thyroid disease (AITD) is undoubtedly the most common cause of hypothyroidism in women in their reproductive age. The association between sub-clinical hypothyroidism (SCH) and infertility has been evaluated in different studies, but most of them are retrospective and uncontrolled [3].

Infertility and reproductive impairment can be compromised by abnormalities in both endocrine and immune system. The cytokine network has been suggested to be involved with positive or negative evolution of ongoing pregnancies [4,5].

TNF-α promotes apoptotic cell death in fetal membrane tissues and pro-inflammatory, proapoptotic, and procoagulant properties of TNF-α probably contribute to widely accepted abortogenic profile of this cytokine [6]. Osteopontin (OPN) is a phosphorylated acidic glycoprotein that has been implicated in a number of physiological and pathological events, including maintenance or reconfiguration of tissue integrity during inflammatory processes [7,8]. Cell-cell adhesion, communication, and migration are possibly important in the interface between uterus and placenta throughout pregnancy [9].

The aim of this study was to assess the alteration in the levels of anti-TPO, anti-TG antibodies, TSH, FT3, FT4, TNF-α, osteopontin in pregnant, non-pregnant women, and healthy controls, and to correlate these parameters with clinical situations.

Materials and methods

This prospective study was conducted between September 2007 and January 2008 at the University hospital in Turkey. Study subjects were 28 pregnant women (pregnancy age in conceived patients is 23.64 ± 2.040, weeks), 28 non-pregnant women, and 28 healthy controls. Informed consent was obtained from 56 patients and 28 healthy controls. In this study, we have accepted a history of with/without abortion, pregnant/non-pregnant with Hashimoto's thyroiditis patients and healthy controls.

At each appointment, venous blood samples were collected into vacutainer tubes in order to test for thyroid function. Thyroid function was assessed by measuring the concentration of thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine (FT4) by using enzyme immunoassay method (Beckman coulter, Unicel DxI 800, 2003, USA).

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TPO-Ab and Tg-Ab were measured in an IMMULITE auto-analyzer. Assay designs' human Osteopontin (OPN) TiterZyme Enzyme immunometric Assay (EIA) kit was used for quantitative determination of OPN in plasma samples of 28 pregnant, 28 non-pregnant, and 28 healthy control groups. The kit uses a monoclonal antibody to human OPN immobilized on a microtiter plate for binding human OPN in the standards or samples. A recombinant human OPN Standard was provided with the kit. After a short incubation, excess or standard sample was washed out and a biotinylated monoclonal antibody was added to human OPN. This antibody binds to the human OPN captured on the plate. After a short incubation, excess antibody was washed out and Streptavidin conjugated to alkaline phosphatase was added, which binds to biotinylated monoclonal human OPN antibody. Excess conjugate was washed out and substrate was added. After a short incubation, enzyme reaction was stopped and generated color was read at 405 nm. Measured optical density was directly proportional to the concentration of human OPN in either standards or samples.

For determination of TNF-α by enzyme-linked immunosorbent assay (ELISA), sera samples were collected from groups consisting of 56 patients and 20 healthy controls. Sera samples were stored in the deep freeze (−80°C) until usage. To reduce inter-assay variance, all samples were analyzed in one assay. Repeated freeze-thaw cycles were avoided. Mean value of triplicate readings for each standard, patients with adrenal mass, and control group were calculated.

Quantitative detection of Human Tumor Necrosis Factor- α (TNF-α) was determined by using an enzyme-linked immunosorbent ELISA method (Bender MedSystems) in the sera samples of 50 patients with adrenal mass and the group consisting of 30 controls. In this method, microwell plates were coated with TNF-α monoclonal antibody. TNF-α present in test or standard sample binds to antibodies adsorbed into the microwells. A biotin-conjugated TNF-α antibody was added for detecting TNF-α captured by the first coated antibody. Following incubation, unbound biotin-conjugated anti-TNF-α was removed during a wash step. Streptavidin–HRP (Horseradish peroxidase) was added for binding biotin-conjugated anti-TNF-α. Following incubation, unbound Streptavidin–HRP was removed during a subsequent wash step. Substrate solution reactive with HRP was finally added to the wells. A colored product was formed in proportion to the amount of TNF-α present in the sample. The reaction was terminated by adding phosphoric acid and absorbance of each microwell was measured on a spectrophotometer using 450 nm as primary wave length. Absorbance of both, the samples and TNF-α standard were determined. A standard curve was prepared from seven geometric TNF-α standard dilution to have it extrapolated to test samples.

Statistical analysis

Statistical analysis was performed by using an SPSS (SPSS, Inc., Chicago, IL) program. Differences in proportions were tested for significance by means of Fisher's exact test. Correlations between variables were assessed by using Spearman's test, and differences between mean values were determined by the Mann–Whitney *U*-test. All statistical tests were considered to be statistically significant whenever $p < 0.05$.

Results

Twenty-eight pregnant, 28 non-pregnant, and 28 control women cases were taken into this study. Characteristics of pregnant, non-pregnant patients with Hashimoto thyroiditis and the control group participating in the study are given in Table I. Both patient and control groups are similar in terms of age. Pregnancy age in conceived patients is 23.64 ± 2.040. No statistically meaningful relation was found in correlation analysis between TNF-α and osteopontin among the groups ($p = 0.963$). No statistically meaningful relation was found in correlation analysis between TNF-α ($p = 0.503$) and osteopontin ($p = 0.911$) and the number of miscarriage. No statistically meaningful relation was found between TNF-α level and osteopontin levels among (pregnant and non-pregnant) patients with Hashimato thyroiditis and the control group. Anti-thyroglobuline antibody and anti-microsomal antibody levels were found to be higher in patients with non-pregnant patients with Hashimoto thyroiditis than the control group ($p < 0.001$). No statistically meaningful difference was found between pregnant, non-pregnant patients with Hashimoto thyroiditis and the control group in terms of f-T3, f-T4, and TSH levels. No statistically meaningful difference was found in terms of previous miscarriage number between pregnant and non-pregnant patients with Hashimoto thyroiditis.

Pregnant and non-pregnant groups among patients with Hashimoto thyroiditis were analyzed according to thyroid antibody positiveness; the ratio of antibody positive patients in non-pregnant group was determined to be higher (Table II).

Relation of TSH level and antithyroid antibody positiveness, miscarriage history with serum osteopontin and TNF-α levels are shown in Figure 1. No statistically meaningful relation was found in terms of TNF-α ($p = 0.66$) and osteopontin serum levels ($p = 0.50$) in patient groups with or without miscarriage history. No statistically meaningful relation was found in anti-thyroid positive and negative patient groups in terms of TNF-α ($p = 0.53$) and osteopontin serum levels ($p = 0.39$). When grouped according to TSH levels, no statistically meaningful relation was also found in terms of TNF-α ($p = 0.38$) and osteopontin serum levels ($p = 0.47$)

Table I. Clinic and metabolic characteristics of pregnant and non-pregnant patient groups.

	Non pregnant group ($n = 28$)	Pregnant group ($n = 28$)	Control ($n = 28$)	p value
Mean age	32.78 ± 4.93	32.92 ± 3.73	29.75 ± 6.69	0.22
TNF-α	2.61 ± 3.73	2.65 ± 4.85	5.05 ± 2.73	0.21
Osteopontin	4.22 ± 0.96	4.83 ± 1.46	4.06 ± 0.94	0.18
Anti Tg	346.02 ± 671.28	64.80 ± 121.86	14.9 ± 1.6	0.001
Anti TPO	432.17 ± 605.54	68.43 ± 140.00	8.9 ± 1.23	0.001
F-T3	2.93 ± 0.33	2.80 ± 0.35	2.91 ± 0.19	0.278
F-T4	0.80 ± 0.19	0.57 ± 0.06	0.99 ± 0.088	0.376
TSH	2.91 ± 1.27	2.60 ± 1.02	1.34 ± 0.48	0.25
Number of miscarriage	1.04 ± 0.99	1.46 ± 1.20	–	0.252

Table II. Thyroid antibody positiveness in pregnant and non pregnant patient groups.

	Non pregnant group ($n = 28$)	Pregnant group ($n = 28$)	Total ($n = 56$)	p value
Anti thyroid antibody positive patients	85.7% ($n = 24$)	35.7% ($n = 10$)	60.7% ($n = 34$)	0.001
Anti thyroid antibody negative patients	14.3% ($n = 4$)	64.3% ($n = 18$)	39.3% ($n = 22$)	

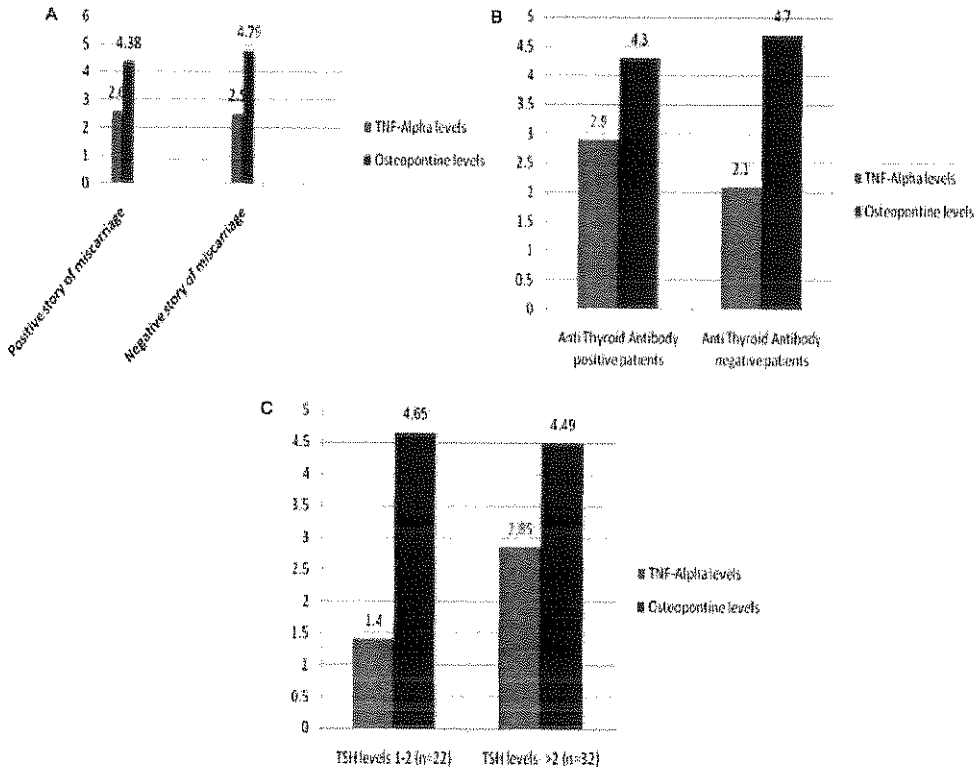

Figure 1. Miscarriage history of serum osteopontin and TNF-α levels, TSH level and its relation with anti-thyroid antibody positiveness. (a) No statistically meaningful relation was found in terms of TNF-α ($p = 0.66$) and osteopontin serum levels ($p = 0.50$) in patient groups with and without miscarriage history. (b) No statistically meaningful relation was found in terms of TNF-α ($p = 0.53$) and osteopontin serum levels ($p = 0.39$) in anti-thyroid positive and negative patient groups. (c) No statistically meaningful relation was also found in terms of TNF-α ($p = 0.38$) and osteopontin serum levels ($p = 0.47$) between the group whose TSH level is between 1–2 and the group whose TSH level is between 2–4 when grouped according to TSH levels. Pregnancy age is 23.64 ± 2.040 in conceived patients. No statistically meaningful relation was found in correlation analyses between TNF-α and osteopontin ($p = 0.963$). No statistically meaningful relation was found in correlation analysis between TNF-α ($p = 0.503$) and osteopontin ($p = 0.911$).

between the group whose TSH level is between 1 and 2 and the group whose TSH level is within 2–4.

Discussion

Different reasons were described in the past for the pathogenesis of RM, including genetic anomalies, hormonal disorders, genital abnormalities, infections, and autoimmune situations. In the literature, many reports have linked thyroid autoimmunity (TA) with recurrent miscarriages, and it has been suggested that anti-thyroid antibodies and thyroid hormone status may be the indicator for miscarriage risks [10].

A number of studies have linked TA with recurrent abortions, although related mechanism is unknown. Autoimmunity of thyroid proposes that the presence of thyroid autoantibodies reflects the activation of the immune system and a generally sensitive autoimmune attack across the feto-placental unit. In a study, Stagnaro-Green et al. found higher miscarriage risk in thyroid antibody positive patients than antibody negative controls [11].

Different diseases of the thyroid gland, such as hypothyroidism and hyperthyroidism, are associated with fetal loss [12,13]. Negro et al found that the miscarriage ratio was 13.8% in the thyroid autoantibody positive group and 2.4% in the antibody negative group ($p < 0.05$) [14]. Relation with the increase in miscarriage ratio in women who were antibody positive in the first trimester when compared to antibody negative women [15,16] was found in previous studies.

However, no statistically meaningful relation was determined when the number of miscarriage in pregnant and non-pregnant group was evaluated according to TSH and anti-thyroid antibody positiveness in our study. But, it was determined to be more accumulated in non-pregnant group when evaluated according to positiveness of anti-thyroid antibody levels in the group. In healthy pregnant women, TNF-α is thought to modulate growth and invasion of trophoblasts in maternal spiral arteries. But when produced in excess, TNF-α acts as a potent proinflammatory cytokine with primary biological activities in inflammation and endothelial cell activation [17].

Different mechanisms were proposed for pro-abortogenic effects of TNF-α as well as trophoblast invasion and placentation [18] and stimulation of the expression of pro-apoptotic genes in fetal membranes [19]. In our study, no correlation was found in statistical analysis between the number of miscarriage and TNF-α level. OPN fragments are known to initiate cell proliferation and migration, and may be involved in cytoskeletal reorganization for placentation. Consequently, OPN may prevent the miscarriage and serve to stabilize cells at uterine-placental interface [20].

In our study, no statistically meaningful relation was found in terms of TNF-α ($p = 0.66$) and osteopontin serum levels ($p = 0.50$) in patient groups with and without miscarriage history. In a study, serum concentrations of TNF-α in the patients with hypothyroidism were found to be significantly higher than those in controls [21].

However, we didn't determine a statistically meaningful relation between serum TSH levels and TNF-α levels in our study.

As a result, no meaningful relation of miscarriage ratio with serum osteopontin and TNF-α levels determining miscarriage risk in pregnant and non-pregnant patients with Hashimoto thyroiditis was found.

Declaration of Interest: The authors declare no conflict of interest.

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