

Serum Leptin Levels in Women with Immunological Recurrent Abortion

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Abstract

Introduction: Recurrent abortion (RA) may be a consequence of aberrant expression of immunological factors during pregnancy. Although the relative importance of immunological factors in human reproduction remains controversial, substantial evidence suggests that autoantibodies contribute to reproductive failure. Production of such antibodies is under the control of cytokines; and leptin, besides its role in reproductive success, has a profound effect on directing the cytokine profile toward Th₁ (cellular) pattern. Therefore, the present study was performed to assess serum leptin levels in women with immunological recurrent abortion.

Materials and Methods: In this prospective study, 250 women who attended Avicenna Infertility Clinic with RA were screened for known causes of abortion from July to December 2008 in Tehran, Iran. Eighty-one patients with normal karyotypes and hormonal profile with normal ovaries and uterus and no signs of infection were categorized as patients with immunological (IRA, n = 39) or unexplained (URA, n = 42) recurrent abortion based on presence or absence of autoantibodies. After blood sampling, levels of anti-nuclear antibody (ANA), anti-double stranded DNA antibody (anti-dsDNA), lupus anti-coagulant antibody (LACAb), anti-phospholipid antibody (APA), anti-cardiolipin antibody (ACA), anti-thyroglobulin antibody (TgAb), anti-thyroperoxidase antibody (TPOAb) and anti-thrombin III antibody (ATIIIAb) were measured by enzyme-linked immunosorbent assay (ELISA) or chemiluminescent enzyme immunoassay (CLEIA).

Results: In IRA group, 9 (23.1%), 24 (61.5%), 25(64.1%) and 1 (2.6%) women were above the normal cut-off point for ANA, TgAbs, TPOAbs and AT-III Abs, respectively. IRA patients had normal values of LACAbs, APA and ACA. With normal level of fasting blood sugar (FBS), IRA and URA groups had similar serum leptin levels (23.7 ± 13.2 ng/ml vs. 22.7 ± 12.5 ng/ml, respectively). Serum leptin concentrations showed a positive correlation with weight and BMI in both groups.

Conclusion: This study suggests that serum leptin levels are higher in IRA and URA patients than normal women. The findings of this study suggest the need for a more comprehensive study and comparison of leptin levels in IRA and URA patients to women with no history of miscarriages.

Keywords: Autoantibodies, Immunological, Leptin, Pregnancy, Recurrent abortion, Spontaneous abortion.

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Recurrent abortion (RA) is a growing problem all over the world (1). RA occurs in both

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natural and in-vitro fertilization (IVF)-mediated pregnancies, and there is increasing evidence that

immunological factors play an important role in the failure of these pregnancies (2-4). These factors include various humoral abnormalities such as anti-nuclear antibodies (ANA), anti-double stranded DNA antibodies (anti-dsDNA), lupus anti-coagulant (LACAb), anti-phospholipid (APA), anti-cardiolipin (ACA), anti-thyroglobulin (TgAb), anti-thyroperoxidase (TPOAb) and anti-thrombin III antibodies (ATIIIAb). The association of recurrent losses of natural or artificial pregnancies with immunological abnormalities has been termed as immunological recurrent abortion (IRA) when other factors such as anatomical abnormalities, endocrine disorders and abnormal karyotypes have been ruled out (5).

It has been reported that presence of some types of autoantibodies are associated with Th₁ cytokine profile; which among them are ANA in patients with Sjögren's syndrome (6), rheumatic diseases (7) and multiple sclerosis (8), anti-dsDNA in NZB/W F1 lupus-prone mice (9), APA in recurrent spontaneous abortions (10), TgAbs in Graves' disease (11), TPOAbs in Hashimoto's thyroiditis (12) and thyroid-associated ophthalmopathy (13) and AT-III Abs in spontaneous abortion (14).

Leptin, a 167-amino acid poly peptide, is secreted mainly by adipocytes. It is involved in signaling the amount of body fat to hypothalamic nuclei, leading to body weight homeostasis (15). Leptin is thought to regulate reproductive functions, as *ob^{-/-}/ob^{-/-}* mutant mice which lack leptin are infertile and administration of recombinant leptin to these animals corrects the reproductive defect (16).

During gestation, leptin is produced and secreted by trophoblastic cells (17) and its maternal serum levels increase progressively, reaching a peak during the second trimester and remain relatively constant thereafter (18). There is a prompt fall in its concentration after parturition, indicating that placental leptin may represent a significant source of maternal leptin (19).

In addition to its critical involvement in physiological functions, leptin has been increasingly recognized as a cytokine-like hormone with pleiotropic actions in modulating immune responses (20). Moreover, leptin can activate and stimulate monocytes, dendritic cells (DC) and macrophages to produce Th₁ type cytokines (21). Importantly,

leptin has been shown to modulate the adaptive immunity *via* enhancing T cell survival and stimulating their production of pro-inflammatory cytokines such as IFN- γ and IL-2 (22).

All the studies reported so far, have mainly focused on the role of leptin and abortion during the course of pregnancy and / or abortion (23-28). However, the present study aims at studying the role of leptin in women with RA beyond pregnancy.

Considering the profound effects of leptin on immune system functions, the present study was undertaken to investigate the serum levels of leptin in women with immunological recurrent abortions.

Materials and Methods

Patients: The study population comprised of women with a history of two or more miscarriages during natural or IVF-mediated pregnancies. Anatomic, infectious, genetic and hormonal causes for RA were excluded. Among 250 women with RA who attended Avicenna Infertility Clinic in Tehran, Iran, 81 patients who had normal karyotypes and hormonal profile and did not have anatomical abnormalities in the reproductive organs or any signs of infectious diseases were categorized as patients with immunological (IRA, n = 39) or unexplained (URA, n = 42) causes of recurrent abortion. The patients were screened by a battery of immunological tests including ANA, anti-dsDNA, LACAbs, APA, ACA, TgAbs, TPOAbs and AT-III Abs and cases with one or more positive results for these autoantibodies were categorized as the IRA group. Women with negative findings for the aforesaid autoantibodies were grouped as URA cases.

Study design: This prospective study was conducted in Avicenna Infertility Clinic in Tehran, Iran from July to December 2008. When patients signed a written informed consent, blood samples were obtained and the sera were stored at -20 °C. The levels of leptin and autoantibodies were measured by enzyme-linked immunosorbent assay (ELISA) or chemiluminescence enzyme immunoassay (CLEIA) methods.

The study protocol was approved by Avicenna Research Institute's Ethics Committee and it was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Serological Evaluations: Serum levels of leptin and autoantibodies were measured by ELISA and CLEIA methods according to the manufacturer's instructions. Kits used in this study were purchased from the following companies: Leptin ELISA (BioVendor, Czech Republic), APA and ACA ELISA (Orgentech Diagnostica GmbH, Mainz, Germany), ANA and anti-dsDNA ELISA (Aesku Diagnostics, Wendelsheim, Germany), TgAbs and TPOAbs CLEIA (DiaSorin, Antony, France), AT-III Abs, and LACAbs CLEIA (Diagnostica Stago, Asnieres, France).

Minimal detection levels of commercial kits for Leptin, anti-dsDNA, LACAbs, APA, ACA, Tg Abs, TPOAbs and AT-III Abs were 0.17 ng/ml, 1 U/ml, 3 sec, 0.5 IU/ml, 1 IU/ml, 0.8 IU/mL, 6 IU/mL and 9% activity, respectively.

Statistical Analyses: Two-tailed statistical analyses were performed using SPSS software, version 13 (SPSS Inc., Chicago, Illinois, U.S.A.). The proportion of women with antibodies above the defined threshold, as suggested by the ELISA and CLEIA kits manufacturers, were computed

and the Spearman rank correlation coefficient was employed to investigate the correlation of the variables. Differences among groups in variables were determined by using the Mann–Whitney U test. P-values less than 0.05 were considered statistically significant.

Results

Study Population: In this study, 39 (48%) patient had abnormal results for one or more immunologic tests (IRA group). However, URA group consisted of 42 (52%) women with normal results for autoantibodies. All the patients were compared in terms of height, weight, BMI (body mass index), FBS (fasting blood sugar) and age with no significant differences being found between the two groups (Table 1).

Serum levels of leptin and autoantibodies: All 39 women with IRA had normal values of anti-dsDNA (cut-off point >16U/ml), LACAbs (cut-off point >50 seconds), APA (cut-off point >10 IU/ml) and ACA (cut-off point >10 IU/ml), but one case (2.6%) showed an increased level of AT-

Table 1. Summary of demographic and clinical characteristics of the women with immunologic recurrent abortion

Variables	Immunologic recurrent abortion	Unexplained recurrent abortion	P-value*	Normal range
	M ± SD	M ± SD		
Age (year)	32 ± 6	31 ± 6	0.44	ND
Weight (Kg)	62 ± 9	63 ± 6	0.51	ND
Height (m)	1.61 ± 0.05	1.59 ± 0.05	0.16	ND
Body mass index (Kg/m ²)	24.2 ± 3.6	25.0 ± 3.2	0.06	ND
Anti-nuclear antibodies (U/ml)	1.18 ± 3.83	0.25 ± 0.21	0.007	< 0.8
anti-double stranded DNA antibodies (U/ml)	2.5 ± 5.1	3.3 ± 6.3	0.71	< 16
Lupus anti-coagulant antibodies (Second)	37.5 ± 5.2	37.8 ± 4.3	0.58	30-50
Anti-phospholipid antibodies (IU/ml)	3.8 ± 2.0	3.9 ± 2.0	0.86	< 10
Anti-cardiolipin antibodies (IU/ml)	3.2 ± 2.2	3.1 ± 2.3	0.69	< 10
Anti-thyroglobulin antibodies (IU/ml)	234 ± 290	13.6 ± 13.3	< 0.001	5-100
Anti-thyroperoxidase antibodies (IU/ml)	140 ± 210	2.6 ± 2.8	< 0.001	1-16
Anti-trombin III (% Activity)	94 ± 10	95 ± 11	0.64	80-100
FBS (mg/dL)	85 ± 7	83 ± 8	0.90	70-105
Leptin (ng/ml)	23.7 ± 13.2	22.7 ± 12.5	0.75	ND

ND: Not defined, * Mann–Whitney U test

III Abs (cut-off point >120% activity). Nine women (23.1%) had antibody levels above the defined threshold for ANA (cut-off point >1.2 U/ml), 24(61.5%) for TgAbs and 25 (64.1%) for TPOAbs.

Since serum levels of leptin is highly dependent on serum sugar concentration (29), fasting blood sugar (FBS) was first measured in all women and it was shown to be within normal ranges in IRA (mean 85 ± 7 mg/dL) and URA (Mean 83 ± 8 mg/dL) cases. Serum levels of leptin were higher in 31(79.5%) (Mean 23.7 ± 13.2 ng/ml) IRA and 33 (78.6%) (mean 22.7 ± 12.5 ng/ml) URA patients relative to the considered cut-off point. At the present time there is no valid cut-off point for serum leptin levels for the Iranian population, therefore a cut-off point which had been reported by Nystrom et al was employed (30). Currently, no international cut-off point for serum leptin levels has been defined yet. Serum leptin concentration showed positive correlations with weight ($r = 0.613$, $p = 0.001$) and BMI ($r = 0.621$, $p = 0.001$) in IRA, as it did in URA patients, respectively ($r = 0.565$, $p < 0.001$) and ($r = 0.554$, $p < 0.001$).

Discussion

Various immunological abnormalities that interfere with a successful pregnancy (31) have been identified. A considerable portion of immunological abnormalities appear to be caused by a shift in the immune response away from the so called Th₂ (humoral) pattern toward the so called Th₁ (cellular) profile that is deleterious to pregnancy outcomes (31). Likewise, leptin has been shown to have a profound effect on reproduction. Leptin deficient homozygote *ob^{-/-}* mice remain in a prepubertal stage despite a normal early sexual differentiation (32). These findings further support the idea that leptin is possibly involved in the control of fertility, but a definitive proof was obtained when it was shown that administration of leptin could restore fertility in *ob^{-/-} / ob^{-/-}* mice (16, 33, 34) by allowing them to achieve pubertal maturity.

Lage and colleagues conducted the first study on the role of leptin in spontaneous abortion in 1999 (23). In that study, serum leptin levels were measured in 29 women with spontaneous abortion, 24 hours after the incident and showed

lower leptin levels in RA patients compared to normal women (23). In another study by Laired and collages measured serum leptin levels in 53 women with a history of RA at 5-6th and 6-8th weeks into gestation. They also reported lower leptin levels in RA patient who underwent miscarriage in the aforementioned intervals than these who managed to continue their pregnancies to full term (26). Interestingly, anti-progesterone medications (Mifepristone) has been shown to significantly decrease serum leptin levels leading to abortion two days after the drug administration in candidates for induced abortion (25). However, the existing information on the effects of serum leptin on pregnancy outcome is controversial as evidenced in the study by Thommaselli and colleagues. They showed similar leptin levels in the first trimester of women who had normal full term deliveries as compared to those who aborted the course of pregnancy in the first trimester (28).

To our knowledge, it seems that no study has been carried out on the effects of serum leptin levels on pregnancy outcome in women with RA in periods well beyond the pregnancy period and the present study maybe the first to serve this aim. In addition, the existing data is lacking regarding the effects of leptin levels on specified groups of RA patients. The present study is also aiming at analyzing serum leptin levels in women with IRA.

Leptin is a well-defined agent that can enhance Th₁ immunity (21). Since Th₂ cytokine profile is dominant during most of gestational period and Th₁ cytokines are reportedly incompatible with successful pregnancy, we hypothesized that overproduction of leptin in women with RSA may lead to the production of Th₁-associated autoantibodies and indirectly trigger fetal loss. Based on the fact that, serum leptin concentration is profoundly dependent on FBS levels (29), the latter was measured in all patients and it was found to be within normal ranges. No significant differences in serum leptin levels were found between IRA and URA groups. In line with our findings, it has recently been reported that serum leptin levels in the first trimester of pregnancy may not be the primary indicator of miscarriage in cases of threatened abortion (28). In women with a history of recurrent miscarriage the same conclusion has been outlined (26).

Importantly, a significant correlation between

BMI and serum leptin levels was found in the experimental groups which is in line with what Nystrom et al have reported (30).

Conclusion

This study suggests that serum leptin levels are higher in IRA and URA patients compare to those of normal women. It seems that separating known causes of recurrent abortion (genetic, infectious, anatomical and hormonal) from immunological causes is necessary to have a better understanding about mechanisms leading to miscarriage.

Lack of a statistically significant difference between serum leptin levels in RA and URA patients might be due to the low number of samples in each group, therefore, a more comprehensive study is suggested for comparing serum leptin levels in IRA and URA patients with those in normal women without a history of miscarriage.

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