Evaluation of serum levels of interleukin-10, interleukin-11 and leukemia inhibitory factor in differentiation of eutopic and tubal ectopic pregnancies

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Summary

Purpose of study: To investigate whether serum levels of leukemia inhibitory factor (LIF), interleukin 10 (IL-10) and interleukin 11 (IL-11) are different in reference to the site of implantation. *Methods:* Seventeen patients with laparoscopic diagnoses of tubal ectopic pregnancy (EP) and 19 patients with intrauterine pregnancy delivering healthy term neonates (IUP) were prospectively evaluated for LIF, IL-10 and IL-11 levels. The data were compared by using the Student's t-test, chi-square test, Kruskal-Wallis and the Mann-Whitney U test with Bonferroni's correction ($p < 0.05$) as appropriate. *Results:* A statistically significant difference was observed in serum LIF levels between the EP and IUP groups ($p = 0.002$). Ranges of LIF were 15-300 and 70-1200 ng/ml for the IUP and EP groups, respectively. There were no significant differences between groups in terms of IL-10 and IL-11 levels. *Conclusion:* LIF, but not IL-10 or IL-11, levels may be increased in early tubal ectopic pregnancies when compared to normal intrauterine pregnancies.

Key words: Cytokines; Ectopic pregnancy; Fallopian tube; Interleukin 10 (IL-10); Interleukin 11 (IL-11); Leukemia inhibitory factor (LIF).

Introduction

Despite early intervention, ectopic pregnancy (EP) remains a major cause of maternal morbidity and mortality in the first trimester. Transvaginal ultrasound and serial measurements of sensitive β-human chorionic gonadotropin (β-hCG) levels have both assisted in diagnosis and lowered morbidity and mortality. This strategy may not always accurately separate an early normal intrauterine pregnancy (IUP) from an ectopic one because there is no consistent β-hCG pattern that characterizes EP [1]. Therefore, recent research has converged on finding candidate markers to reliably diagnose EP with a single serum measurement, especially to be used in emergency settings.

Although the role of cytokines is well documented in immune reactions like inflammation, their roles in pregnancy are unknown [2]. Their involvement especially in EP has not been clearly defined. Some investigators have published conflicting reports on the association of leukemia inhibitory factor (LIF), a cytokine of the interleukin 6 (IL-6) family, with EP [3-5]. Another IL-6-type cytokine, interleukin 11 (IL-11), has been shown to be involved in regulation of trophoblast invasion [6]. In addition, its serum levels are decreased in women with spontaneous abortion [2].

The aim of the present study was to investigate whether serum levels of cytokines are different in reference to the site of implantation. We have compared serum LIF, IL-11 and interleukin 10 (IL-10) (an anti-inflammatory cytokine which has not been previously studied) levels in IUP and EP groups in an effort to help in predicting the site of implantation and to suggest a possible role in immune-regulatory mechanisms of early normal and ectopic pregnancy.

Materials and Methods

Subjects

In this prospective study, a total of 41 consecutive patients admitted to at our clinic with a diagnosis of either possible EP or early intrauterine pregnancy were enrolled. All women presented with delay of menses, abdominal pain or abnormal bleeding. All women had a gynecologic examination and a transvaginal ultrasound. None of the patients had a history of pelvic inflammatory disease.

Of the 20 patients with a presumptive diagnosis of EP, none had signs of an intrauterine pregnancy, but rather an adnexal mass with free fluid. Only 17 patients with an accurate gestational age who had been diagnosed as having a tubal ectopic pregnancy during laparoscopy were included in the study. One patient was left out due to cervical pregnancy and another two patients were treated by methotrexate only so they had no surgical confirmation of their diagnosis and were left out of the study.

Of the 21 patients who were diagnosed with early IUP, only 19 patients who had an uneventful pregnancy and delivered a healthy term infant were included in the analysis. One woman had a miscarriage and the other had an immature delivery at the 20th gestational week and both were left out of the study.

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Therefore a total of 36 patients were included in the final analysis of the data: the EP group (n = 17) and the IUP group (n = 19). All blood samples, one from each patient, were collected by peripheral venous puncture upon admission. The sera were stored at -80ºC until assays were performed in batches.

The study is accordance with the 1975 Helsinki Declaration on Human Experimentation. It was approved by the local ethics committee and informed consent was given by all participants.

Cytokine assays

The plasma concentration of LIF (in nanograms per milliliter), IL-10 (in nanograms per milliliter) and IL-11 (in picograms per milliliter) were determined by commercially available enzyme linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions. LIF/HILDA (Biosource, Belgium) was a solid phase enzyme amplified sensitivity immunoassay performed on a microtiter plate. IL-10 (RnDSystems, USA) and IL-11 (RayBio, USA) were both ELISAs for the quantitative measurement of cytokine in serum.

Statistical analysis

The data on patient characteristics were compared by using the Student's t-test and chi-square test as appropriate. The data on cytokine levels are given as minimum, maximum and the median. They were compared by using Kruskal-Wallis and the Mann-Whitney U test with Bonferroni's correction.

Results were considered significant when p was < 0.05. All statistical analysis was carried out by using SPSSr12 software package.

Results

Age, parity and estimated gestational age of patients in the EP and IUP groups are presented in Table 1. All parameters were similar between the groups. The range of β-hCG levels for both groups was inbetween 297 and 11,106 mIU/ml.

Table 2 gives the median serum markers for IL-10, IL-11 and LIF along with their corresponding minimum and maximum values. IL-11 levels are expressed in pg/ml, while those of IL-10 and LIF are in ng/ml. There were no significant differences between EP and IUP groups in terms of IL-10 and IL-11 levels.

A statistically significant difference was observed in serum LIF levels between the EP and IUP groups (p = 0.002). The median level for LIF in the EP group was calculated to be higher than in the IUP group (120 ng/ml vs 80 ng/ml). The range of LIF levels in IUP was only between 15 and 300 ng/ml, while the measured LIF range spanned from 70 to 1200 ng/ml in the EP group. The scatter diagram of each measured LIF variable is detailed and plotted in Figure 1. A threshold for diagnosis of EP according to LIF levels was not estimated because of the small size of the study group.

Table 1. — *Patient characteristics according to ectopic and normal intrauterine pregnancy groups. Statistically* p *value is considered significant if less than 0.05. NS: not significant; S: significant. †Minimum and maximum parity is given in parenthesis.*

Characteritcs	IUP (Intrauterine pregnancy)	EP (Ectopic pregnancy)	p value
Age (years)	26.8 ± 4.6	28.7 ± 5.9	NS
Parity	0.89 (0-6)†	0.82 (0-4)†	NS
Estimated gestational age (days)	52.7 ± 15.2	44.9 ± 11.0	NS

Table 2. — *IL-10, IL-11 and LIF serum measurements for ectopic and normal intrauterine pregnancy.*

Cytokine and p levels	Group	n	Median	Minimum	Maximum
IL-10 (ng/ml)	EP	17	5	2	38
ns	IUP	19	5	3	14
IL-11(pg/ml)	EP	17	1.8	0.7	85
ns	IUP	19	2.3	0.3	45
LIF (ng/ml)	EP	17	120	70	1200
p = 0.002	IUP	19	80	15	300

ns = not significant.

Discussion

The exact pathophysiologic mechanism underlying the ectopic implantation process is not clear. This lack of knowledge prevents the development of a certain molecular marker (or markers) to differentiate an ectopic pregnancy from a normal intrauterine one with precision. We examined the hypothesis of whether differences in cytokine levels between women with EP and women with IUP during early first trimester may be related to ectopic implantation and immune mechanisms. We also investigated whether the concentration of one of these cytokines may indicate the place of implantation.

LIF has been proven to have a role in the implantation process and ectopic pregnancy [7, 8]. Keltz *et al*. have shown that the LIF messenger ribonucleic acid and tubal secretion of LIF were markedly increased in ectopic pregnancy [9]. Wegner and Mershon evaluated serum LIF as a marker of ectopic pregnancy and interestingly found that LIF levels were lower in ectopic pregnancies when compared to IUP but were not discriminatory enough [5]. Although Kiran *et al*. demonstrated a tendency for increased concentrations of LIF in tubal extracts of ectopic pregnancies, the difference was not statistically significant [10]. Recently increased production and presence of LIF in tubal ectopic pregnancies were also supported by immunohistochemical and Western blot works from Güney *et al*. and Ji *et al*. [4, 11]. In another research, Daponte failed to show a difference in serum levels of LIF from ectopic and abnormal intrauterine pregnancy patients [3]. Our finding of increased LIF levels in blood samples of patients with EP when compared to normal IUP, lends credit to the findings of Keltz, Güney and Ji and colleagues. Although the number of women enrolled in the study is small, it also supports the original hypothesis of Wegner and Mershon's work that serum LIF would be elevated in patients with tubal ectopic implantation [5]. Randomized controlled studies involving a larger number of patients may be warranted to come up with a better answer.

IL-11 has been shown to be important in embryo

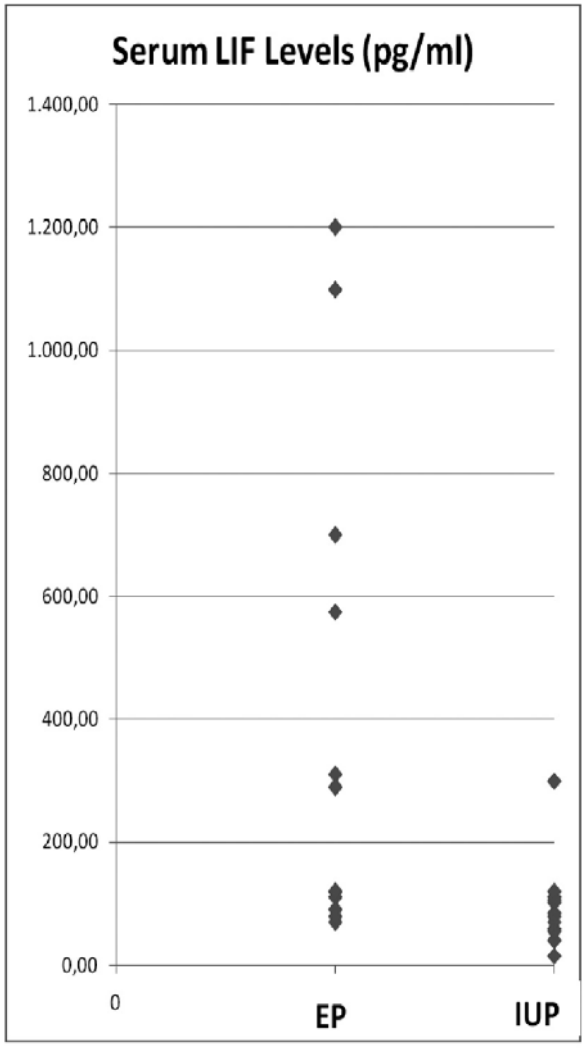

Figure 1. — Scatter diagram of serum LIF levels for ectopic (EP) and intrauterine pregnancies (IUP). No cut-off was calculated due to the small number of cases.

implantation and decidual response in mice and its expression has been shown in eutopic and ectopic human implantation [6, 12]. In addition, IL-11 levels are increased during early normal pregnancy [2]. Although von Rango *et al*. speculated that dysregulated IL-11 expression may be involved in a series of reactions leading to inadequate trophoblast invasion in tubal ectopic pregnancies, their work immunohistochemically showed that in tubal pregnancy staining of IL-11 was similar to the intrauterine situation [6]. Therefore we hypothesized that IL-11 levels should be similar between our study groups, and our results were in concordance with previously published data. IL-11 levels are probably not helpful in distinguishing the site of implantation of a viable pregnancy.

IL-10, the last cytokine we have investigated, has primarily been implicated in limiting and terminating inflammatory responses in the body [13]. Previous research has indicated that IL-10 may be linked with tubal factor infertility due to chlamydial infection and fibrosis [14, 15]. An abnormal implantation of the embryo, e.g. in the tube, unlike the normal one, would evoke an inflammatory response. The hypothesis is that the anti-inflammatory response to EP might lead to increased concentrations of IL-10 in the blood. Our preliminary results showed that this is not the case. The levels of this anti-inflammatory cytokine were similar between women with EP and women with an IUP. Although a conclusion may not be drawn from this data, the anti-inflammation evoked by ectopic and normal intrauterine pregnancy seems to be of similar magnitude and independent of place of implantation.

The major limitation of our investigation was the small number of women enrolled in the research groups, decreasing the power of the study. Another was the absence of serial and time-dependent measurements of the cytokines studied. Whether these concentrations change is not known according to current data.

Conclusion

To summarize, LIF levels seem to be increased in tubal ectopic pregnancies when compared to early normal intrauterine pregnancies. This finding supports the role of LIF in ectopic pregnancy and its possible use in differentiating the site of implantation. Further studies with a greater number of women are needed to establish this role. However, IL-10 and IL-11 levels do not seem to change according to the site of implantation, hence limiting their use in this regard.

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