Does Low Molecular Weight Heparin Influence the Triple Test Result in Pregnant Women with Thrombophilia?

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ABSTRACT:
Background: The triple test serum markers for Down's syndrome screening may be altered because of various conditions other than chromosomal trisomies.
Objectives: To assess the profile of mid-trimester triple test serum markers in a cohort of women treated with low molecular weight heparin (LMWH) for thrombophilia since the first trimester.
Methods: Women with inherited or acquired thrombophilia treated with LMWH prior to 12 weeks gestation were followed between October 2006 and September 2009 at our obstetric outpatient clinic. The second-trimester screening test for Down syndrome was calculated from the combination of triple serum markers and maternal age, and expressed as a multiple of the gestation-specific normal median (MoM). Reference MoM values were calculated from the local population. Data on pregnancy outcome were obtained from patient records.
Results: The median human chorionic gonadotropin (hCG) level of women with inherited thrombophilia was 0.87 MoM, compared to 0.99 MoM in controls ($P = 0.038$) and compared to 1.355 MoM in women with acquired thrombophilia ($P = 0.034$). In contrast, alpha-fetoprotein MoMs did not differ significantly between women with inherited and women with acquired thrombophilia (0.88 vs. 0.99 MoM, $P = 0.403$).
Conclusions: The triple test serum markers may be altered in thrombophilia patients treated with LMWH. Clinicians should consider offering these patients the first-trimester nuchal translucency test and other sonographic markers that are probably unaffected by the underlying maternal disease and/or treatment modality.

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KEY WORDS: acquired thrombophilia, hereditary thrombophilia, low molecular weight heparin (LMWH), pregnancy, triple test

Thrombophilia is a complex condition that has serious implications for the pregnant woman and her fetus. About half the women who suffer thrombotic events during pregnancy have underlying hereditary or acquired thrombophilia [1], yet the exact mechanisms underlying placental dysfunction in these women are not fully understood.

In a study of women with preeclampsia, intrauterine growth restriction, unexplained stillbirth or placental abruption, 65% had a form of heritable or acquired thrombophilia [2]. The most common hereditary thrombophilias in the Caucasian population are the factor V Leiden mutation and the prothrombin gene mutation G20210A, with prevalence rates of 5% and 2%, respectively, in this population [1,3].

Antiphospholipid syndrome is an acquired thrombophilia phenomenon associated with arterial and venous thrombosis and elevated levels of circulating aPL antibodies [4]. Obstetric complications that have been associated with aPL syndrome include miscarriage, IUGR, stillbirth and early severe preeclampsia [5]. One half the patients with systemic lupus erythematosus test positive for aPL antibodies [6].

The maternal serum markers evaluated in the second-trimester screening test are maternal serum human chorionic gonadotropin or its free beta-subunit, alpha-fetoprotein and unconjugated estriol. Pregnancies with fetal Down syndrome are characterized by low maternal serum AFP and uE3 levels and high levels of hCG. The most common second-trimester screening protocol – the triple screen – is based on a composite likelihood ratio determined by levels of all three of the above analytes. The maternal age-related risk is then multiplied by this ratio.

Altered levels of mid-gestation hCG/AFP markers have been detected in pregnant women with SLE/aPL syndrome [7-9]. However, whether these patients were treated with low molecular weight heparin, and if so, from which gestational age, has not been well documented.

Since the presence of hereditary or acquired thrombophilia may affect the interpretation of screening tests for Down syndrome, we investigated the effect of LMWH prophylactic treatment during pregnancy on the triple test results. The aim of the current study was to assess the profile of mid-gestation triple

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aPL = antiphospholipid
IUGR = intrauterine growth restriction
AFP = alpha-fetoprotein
uE3 = unconjugated estriol
hCG = human chorionic gonadotropin
SLE = systemic lupus erythematosus
LMWH = low molecular weight heparin

test serum markers in a cohort of patients with thrombophilia treated with LMWH prior to 12 weeks gestation. To the best of our knowledge this effect on the triple test serum markers has not been previously reported.

PATIENTS AND METHODS

This retrospective study was conducted between October 2006 and September 2009 at our obstetric outpatient clinic. It included pregnant women with hereditary or acquired thrombophilia treated with LMWH prior to 12 weeks gestation. Only singleton pregnancies were included. Pregnant women who did not undergo second-trimester screening or those without proven thrombophilia were excluded from the study.

According to Rand's criteria [10], aPL syndrome was considered present if at least one biological criterion and one clinical event were met. Clinical criteria for aPL syndrome were arterial, venous or small vessel thrombosis, pregnancy morbidity (fetal death after 10 weeks of gestation), prematurity due to severe preeclampsia or placental insufficiency, or three or more unexplained consecutive spontaneous abortions before 10 weeks [11]. The diagnostic laboratory tests for acquired thrombophilia include lupus anticoagulant, anti-cardiolipin antibody and anti-beta2-glycoprotein 1 antibody. The clinical and laboratory methods used to diagnose aPL syndrome were adopted from Brochet et al. [8]. Diagnostic tests for inherited thrombophilia include activated protein C resistance, factor V Leiden mutation, prothrombin G20210A mutation and basal homocysteine levels, and deficiencies of protein S, protein C and antithrombin 3 [12]. We accessed data on the second-trimester Down syndrome screening tests, pregnancy outcome and the dose and duration of LMWH treatment. All women were treated with a prophylactic (40 mg/day), high prophylactic (60 mg/day) or full treatment dose (1 mg/kg every 12 hours) of enoxaparin. The study was approved by the ethics committee for research at our institution.

SCREENING TESTS

The second-trimester screening test was derived from the combination of triple serum markers and maternal age, and was calculated using commercial software. The maternal serum markers evaluated in this study were maternal serum hCG or its free beta-subunit (FβhCG), AFP and uE3.

The serum samples were tested in a routine analytical run together with regular maternal serum samples, all in the same prenatal Down syndrome screening program at Zer Medical Laboratories (ISO 9002 UK, certified and authorized by the Ministry of Health, Israel). Testing was carried out in a manner that was blind to group classification, that is, samples from our thrombophilia study cases and those of other pregnant women were assessed in the same laboratory during the same period. The measured marker levels were expressed as multiples of the gestation-specific normal medians. Median values for each serum analyte were calculated against completed menstrual weeks and adjusted for maternal weight. We compared results with reference MoM values that were calculated from our own local population as established at Zer Medical Laboratories [13].

STATISTICAL ANALYSIS

Standardized kurtosis showed that the data were derived from a normal distribution and were expressed as mean and standard deviation. Frequencies were expressed as percentages. Student's t-test was used to compare the second-trimester marker between hereditary and acquired thrombophilia. AFP and hCG concentrations were logarithm transformed to follow normal distributions; uE3 showed normal distribution.

One sample t-test was applied to compare each type of thrombophilia to normal population values. A P value < 0.05 was considered significant. Statistical analysis was performed by the Tel Aviv University statistical department using Statistics Package for Social Sciences software.

RESULTS

Data were accessed from 72 pregnancies of 69 pregnant women, whose mean age was 34.6 ± 4 years. Forty women (58%) had inherited thrombophilias and 29 (42%) acquired thrombophilias. The main clinical presentations that led to the thrombophilia workup were habitual abortions and thrombotic events (deep vein thrombosis, pulmonary embolism, transient ischemic attack, ovarian vein thrombosis), each manifesting in about 30.4% of the women [Figure 1].

Fifty percent of the women (n=33) delivered vaginally and 50% by cesarean section; information on the mode of delivery was not available for three women. Down syndrome screening test samples were analyzed together with samples unrelated to our study. Laboratory technicians were blinded

MoM = multiples of the median

Figure 1. Initial clinical presentation of women with thrombophilia (percentage)
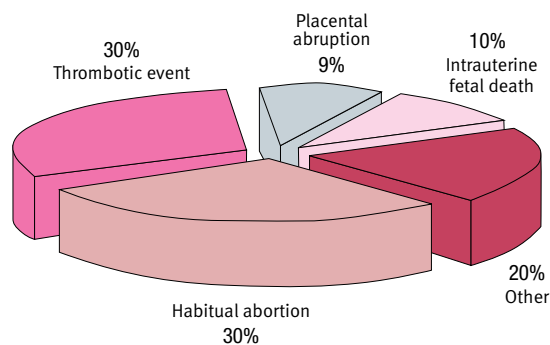
to which samples belonged to the study. The median AFP level in the control samples was 1.01 MoM.

Median hCG, AFP and uE3 levels are presented in Table 1. The median hCG level of women with inherited thrombophilias who were treated with LMWH prior to 12 weeks of gestation was 0.87 MoM, compared to 0.99 MoM for controls, $P = 0.038$ (95% confidence interval of the difference -0.171 to -0.0054). The median hCG level was lower in women with inherited thrombophilia treated with LMWH than in those with acquired thrombophilias (0.87 and 1.355 MoM respectively, $P = 0.034$) In contrast, AFP MoMs were not significantly different between these two groups (0.88 vs. 0.99 MoM, $P = 0.403$). Similarly, uE3 MoMs did not differ significantly between these two groups (1.00 vs. 0.97 MoM, $P = 0.58$)

DISCUSSION

The current study showed significantly reduced hCG values in the inherited thrombophilia population treated with LMWH, compared to both reference values and to levels in women with acquired thrombophilia. The median hCG level in patients with acquired thrombophilia was not significantly increased compared to the reference group. Similarly, Maymon and colleagues [9] reported elevated hCG levels in women with SLE and in those with primary APL syndrome (though there was no statistical significance). Treatment with LMWH was not recorded in that study. In support of the results of the current study, Brochet et al. [8] did not find significant differences between hCG MoM levels in aPL patients who received low doses of aspirin and/or LMWH to prevent thrombotic events and in controls.

We did not observe a significant difference between AFP levels of pregnant women with thrombophilia treated with LMWH and the reference group. This contrasts with Brochet et al. [8] who found elevated AFP values among women with APL syndrome who received low doses of aspirin and/or LMWH.

Several mechanisms could account for our findings. For one, lupus anticoagulant causes placental vasculitis, leading to reduced perfusion and local hypoxia in the intervillous circulation; this could result in elevated hCG levels in women with thrombophilia who were not treated with LMWH. LMWH treatment may diminish the hypoxic effect and increase perfusion in the intervillous circulation, which may result in significantly reduced hCG levels in inherited thrombophilia, and levels that are not elevated in acquired thrombophilia. LMWH treatment may improve perfusion more efficiently in inherited thrombophilia than in acquired thrombophilia.

Many studies of patients with thrombophilia have evaluated the efficacy of LMWH for prevention of adverse pregnancy outcomes as their primary endpoint; enoxaparin is the LMWH most studied. Brenner and co-authors [14] noted that live births occur in 75% of pregnancies in women with

Table 1. Comparison of triple test screening markers between acquired and inherited thrombophilia, with reference values

	AFP* (median MoM)	uE3** (median MoM)	hCG* (median MoM)
Reference laboratory	1.01	0.98	0.99a
Inherited thrombophilia (33 cases)	0.88	1	0.87b
Acquired thrombophilia (18 cases)	0.99	0.965	1.355c

* Comparison of mean log$_{10}$ for each marker.
** Comparison of the mean.
P value between a and b = 0.038
P value between b and c = 0.034
P value between a and c = 0.069
AFP = alpha-fetoprotein, uE3 = unconjugated estriol, hCG = human chorionic gonadotropin

thrombophilia who were treated throughout gestation and postpartum with enoxaparin, compared to 20% of previous pregnancies without prophylaxis in the same women. This improvement in pregnancy outcome could be attributed to better placentation with LMWH treatment.

Aharon and team [15] investigated local placental hemostatic balance in women with gestational vascular complications and their potential modulation by LMWH. They hypothesized that enoxaparin exerts its effect in pregnant women with thrombophilia by modulating local hemostasis on the placental syncytiotrophoblast surface [15].

Di Simone et al. [16] investigated the hypothesis that LMWH might regulate in vitro trophoblast invasiveness and placental production of matrix metalloproteinases and tissue inhibitors. Their finding that increasing doses of LMWH inhibited the production of tissue inhibitors suggests that heparin regulates the degradative capacity of trophoblast cells. The investigators concluded that LMWH is capable of upregulating several specific proteins that can promote trophoblast invasion, and that the decline in tissue inhibitor expression, induced by LMWH, might eliminate the inhibitory influence on matrix metalloproteinase activity [16].

Another possible explanation for our findings is that LMWH treatment initiated in thrombophilia patients early in pregnancy may lead to successful implantation and ongoing pregnancy. Kupferminc and collaborators [17] reported the use of LMWH in the prevention of recurrent adverse pregnancy outcomes. Women with established thrombophilia and a history of severe preeclampsia, abruption placenta, IUGR or stillbirth who were treated with LMWH from 8 to 12 weeks gestation showed significantly increased birth weights and mean gestational ages at delivery, as well as fewer pregnancy complications. In contrast, a recent randomly controlled trial of 364 women with a history of unexplained recurrent miscarriage revealed that neither aspirin combined with heparin nor aspirin alone improved the live birth rate compared with a placebo [18].

The retrospective design and small sample size are the main limitations of this study, precluding analysis by such subgroup characteristics as indications for thrombophilia

workup. We were not able to determine whether the abnormal hCG values observed resulted from the underlying thrombophilia, the LMWH treatment, or both. The optimal study that will control for the effect of thrombophilia should compare the use of LMWH treatment in patients with thrombophilia to that of women treated by LMWH without thrombophilia. We did not have available for investigation women treated with LMWH for other indications, such as prostatic heart valve. Smoking, diabetes and assisted conception are among the conditions that may alter some of the triple test serum markers, in which case the use of a correction equation is preferable to omitting a marker. If our findings are validated in additional studies, a correction equation should be used. A larger study may elucidate whether the effect of LMWH treatment in pregnant women with thrombophilia on triple test serum markers is dose dependent. The national program in Israel for the prevention of Down syndrome includes two components: screening of young women using the triple test, and free invasive diagnostic tests early in pregnancy for women older than 35 years. Free prenatal diagnostic testing is offered to all other women with a risk higher than 1/380 for Down syndrome in a newborn [19].

We encourage other medical centers to validate our data and to report their experience with regard to the best approach for detection of Down syndrome among pregnant women with thrombophilia treated with LMWH.

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