ABSTRACT — OBJECTIVE: Endocrine and metabolic functions are altered during pregnancy to provide for the demands of the fetus, leading to an unbalance of the normal biochemical values. Subclinical hypothyroidism (SCH) is a common pregnancy-related thyroid disorder, in which fluctuations of thyroid-stimulating hormone (TSH) are recorded over the upper limit. The aim of this study was to evaluate the subclinical fluctuations of thyroid hormones throughout gestation and to maintain the euthyroid state in all pregnant women, as recommended by the ATA/AACE Guidelines.

PATIENTS AND METHODS: 33 pregnant women meeting the inclusion criteria were included in the study and divided in 2 groups: one group following a treatment with MI + Se from 1st visit throughout pregnancy and the 2nd group in which no treatment was prescribed. Primary outcome was serum TSH and second outcomes were fT3, fT4 levels, type of delivery and neonatal complications.

RESULTS: Normal thyroid hormones values were maintained in 94.1% of pregnant women in the treated group, compared to 68.7% in the control group.

CONCLUSIONS: The present study corroborates our previous finding, proving that, the combined treatment, MI + Se is effective in maintaining the values of TSH, fT3 and fT4, thus preventing SCH.

KEYWORDS
Subclinical Hypothyroidism, Myo-inositol, Inositol, Selenium, TSH, Thyroid hormones, Pregnancy.

INTRODUCTION
Different physiological changes occur throughout pregnancy, that supports the proper growth and development of fetus. Endocrine and metabolic functions are altered to provide for the demands of the fetus, leading to an impairment of the normal biochemical values. During the first trimester, women go through high concentrations of serum human chorionic gonadotropin (hCG) accompanied by reduced circulating thyroid-stimulating hormone (TSH) levels. Clinical evidence suggests that hCG has a thyrotrophic activity, and it can bind to the TSH receptor, due to their structural similarity, stimulating the synthesis and secretion of free thyroxine (fT4)1-3. This leads, through the negative feedback, to a reduction of TSH levels. Furthermore, an augment of the thyroid binding globulin (TBG) as well as the total triiodothyronine (T3) and T4, is observed in early pregnancy. Thyroid hormones are essential for the fetus, which during the first trimester depends completely on the transplacental passage of maternal thyroid hormones4. In particular, maternal thyroid hormones play a physiological role especially in the fetus neurodevelopment5. While normalization of TSH occur by the second trimester, the free T3 (fT3) and fT4 levels remain slightly lower until the second and third trimesters. Regarding the diagnosis and management of thyroid disease in pregnancy, the American Thyroid Association (ATA) 2011 recommends 2.5 μIU/ml as the upper limit of normal for TSH in the first trimester, 3.0 μIU/ml in the second trimester, and 3.5 μIU/ml in the third trimester6. An increase of TSH level above these values, although maintaining normal fT4, is defined subclinical hypothyroidism (SCH), which should be accurately man-
aged. A correlation between SCH during pregnancy and preterm delivery has been reported, however medical evidences are still controversial. A recent meta-analysis confirmed the increased risk of adverse pregnancy outcomes in pregnant women with SCH compared to euthyroid pregnant women, such as pregnancy loss, placental abruption, premature rupture of membranes, and neonatal death. Higher miscarriage rate was observed in another study in women with anti-thyroid peroxidase antibodies (TPOAb) negative and TSH levels between 2.5 and 5.0 μIU/ml compared with those with TSH levels below 2.5 μIU/ml. The same authors previously observed in euthyroid women with thyroid antibody-positive that TSH levels increased progressively as pregnancy progressed, from a mean of 1.7 μIU/ml in the first trimester to 3.5 μIU/ml in the third trimester, with approximately 19% of women having a TSH value above the limit at delivery. Approximately 2-2.5% of healthy pregnant women would develop SCH but this range would be higher in areas of iodine insufficiency. Furthermore, prevalence of SCH may vary by obesity and gestational age. Indeed, it was observed a 13.7% prevalence of SCH in morbidly obese women (body mass index 40 kg/m²) and almost 7% in women aged 35-44 years. Regarding this topic, another very important aspect has been highlighted: neurocognitive deficits in the developing fetus has been reported from untreated SCH. It was shown a reduction in intelligence quotient (IQ) accompanied by delays in motor, language, and attention in the offspring of untreated hypothyroid women when compared with euthyroid controls. Therefore, the prevention of maternal thyroid diseases is essential not only to avoid correlated risks for the mother but also for optimizing the perinatal outcomes. The aim of this study was to examine the subclinical fluctuations in biochemical values of thyroid hormones, measured at up to three time points throughout gestation, in order to maintain the euthyroid state in all pregnant women. We took cues from previous case report in which a treatment with myo-inositol (MI) and selenium (Se) maintained stable the serum TSH, fT3, and fT4 levels during the 9 months of pregnancy.

PATIENTS AND METHODS

All pregnant women who attended our outpatient unit for first prenatal checkup were screened for thyroid hormones. Enrollment was carried out between November 2015 and January 2017. Initially, all women were asked for any history of thyroid dysfunction and/or use of thyroid hormone (LT4) or anti-thyroid medications (carbimazole, methimazole, or propylthiouracil). Women aged 18-40 years, with a body mass index between 19 and 25 kg/m², a singleton

LABORATORY AND TECHNICAL INVESTIGATIONS

Blood samples were drawn from all women at each timepoint (T0, T1 and T2). Serum TSH, fT4, fT3 levels were measured by electro-chemiluminescence immunoassay (ECLIA) (Roche Diagnostics Ltd., Basel, Switzerland).

<table>
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<tr>
<td>fT3 (pg/ml)</td>
<td>2.97 ± 0.41</td>
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Myo-inositol and selenium prevent subclinical hypothyroidism during pregnancy: an observational study

Clinical characteristics of patients by group at baseline are illustrated in Table 1. At first visit both groups were comparable for age and thyroid hormones. One dropout was recorded in the treated group because TSH levels raised up to 3.00 μIU/ml in the second trimester, and initiated treatment with Levothyroxine. All the other 16 patients continued the treatment until delivery. In the control group, 3 dropouts were observed at the second trimester and started treatment with Levothyroxine, and 2 patients showed high levels of TSH in the third trimester. TSH levels remained almost unchanged in the treated group throughout pregnancy. Levels slightly fluctuated from 1.99 ± 0.40 μIU/ml at T0, to 2.01 ± 0.57 μIU/ml at T1 and 2.05 ± 0.54 μIU/ml at T2. There was a significant increase of mean TSH concentrations in the control group from baseline to T1 and T2 (2.03 ± 0.37 μIU/ml to 2.59 ± 0.43 μIU/ml and 2.93 ± 0.47 μIU/ml, respectively, p < 0.001). Inter-group analysis has shown a statistical difference between groups in either T1 (p ≤ 0.05) and T2 (p < 0.001) timepoints (Figure 1).

Free-triiodothyronine (fT₃) fluctuated from 2.74 ± 0.67 pg/ml to 2.54 ± 0.63 pg/ml, and 2.79 ± 0.47 pg/ml, in the treated group and from 2.97 ± 0.41 pg/ml to 3.11 ± 0.45 pg/ml, and 3.46 ± 0.43 pg/ml, in the control group (p < 0.0001) (Figure 2A). Inter-group analysis has shown a statistical difference between groups in either T1 (p ≤ 0.05) and T2 (p < 0.01) timepoints (Figure 2A). Free-thyroxine (fT₄) oscillated from 1.25 ± 0.38 ng/dl to 1.32 ± 0.46 ng/dl, and 1.20 ± 0.29 ng/dl throughout pregnancy, in the treated group and from 1.26 ± 0.29 ng/dl to 1.21 ± 0.30 ng/dl, and 1.16 ± 0.27 ng/dl, in the control group (Figure 2B). Inter-group analysis has shown non-significant difference between groups (Figure 2). All patients had a natural delivery after the 37th week of gestation and none had fetal-maternal medical complications.

DISCUSSION

Our results suggest that subclinical alterations in individual maternal thyroid hormones may be prevented by supplementation of MI + Se. Indeed, in this study the levels of TSH, fT₃ and fT₄ remained

Statistical analysis was performed by using unpaired t-test (2018 GraphPad Software, La Jolla, CA, USA), when comparing two groups, with results being expressed as mean ± SD. Comparisons for repeated measures was assessed for intragroup analysis by one-way ANOVA. Statistical significance was accepted at the level of p-value ≤ 0.05.

RESULTS

We enrolled 33 age-matched pregnant women. Clinical characteristics of patients by group at baseline are illustrated in Table 1. At first visit both groups were comparable for age and thyroid hormones. One dropout was recorded in the treated group because TSH levels raised up to 3.00 μIU/ml in the second trimester, and initiated treatment with Levothyroxine. All the other 16 patients continued the treatment until delivery. In the control group, 3 dropouts were observed at the second trimester and started treatment with Levothyroxine, and 2 patients showed high levels of TSH in the third trimester. TSH levels remained almost unchanged in the treated group throughout pregnancy. Levels slightly fluctuated from 1.99 ± 0.40 μIU/ml at T0, to 2.01 ± 0.57 μIU/ml at T1 and 2.05 ± 0.54 μIU/ml at T2. There was a significant increase of mean TSH concentrations in the control group from baseline to T1 and T2 (2.03 ± 0.37 μIU/ml to 2.59 ± 0.43 μIU/ml and 2.93 ± 0.47 μIU/ml, respectively, p < 0.001). Inter-group analysis has shown a statistical difference between groups in either T1 (p ≤ 0.05) and T2 (p < 0.001) timepoints (Figure 1).

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stable and in the range of normality throughout the whole gestation in those women undergoing MI + Se treatment. No significative changes were observed in this group and only one dropout was reported. Instead, in the control group significative changes were recorded (although remaining in the normal range): 3 women left the trial after the second visit and 2 had THS above the limit at the third visit. Therefore, we evinced that normal thyroid hormones values were maintained in 94.1% of pregnant women in the treated group, compared to 68.7% in the control group. These findings are perfectly in line with previous clinical evidences that highlighted the beneficial effect of the supplementation of MI + Se in restoring the euthyroid state in patients diagnosed with SCH. MI is a carbocyclic polyol, belonging to the Inositol family, and is the most distributed form naturally occurring. It can be found in many foods such as fruits, beans, grains, and nuts, but it is also synthesized endogenously in the cells. It is involved in cell signaling and many biochemical pathways regulating glucose metabolism, cell proliferation and morphogenesis. It is a precursor of phosphatidyl-inositol phosphate cascade (PIP2) and it activates the cAMP cascade and the Ca²⁺ phosphatidyl-inositol phosphate cascade (PIP2). In follicular cells, TSH stimulates the aCAMP cascade and the Ca²⁺-mediated iodination. In follicular cells, TSH, follicle-stimulating hormone (FSH) and insulin activate TSH, follicle-stimulating hormone (FSH) and insulin. Indeed, in TSH signal cascade, inositol regulates hydrogen peroxide-mediated iodination. In follicular cells, TSH activates the aCAMP cascade and the Ca²⁺-mediated iodination and it seems that the increase of MI availability at cellular level ameliorates TSH sensitivity of the thyroid follicular cell. Therefore, this might explain the effect of MI in maintaining the TSH levels at normal values throughout patient’s pregnancy. MI safety during pregnancy has been fairly confirmed and has been widely used for other pathologies. Se is a trace element that can be found in many different chemical forms in biological materials as selenomethionine, dimethylselenide, selenites or selenates. In food, Se is predominantly present as selenomethionine, and is an important source of dietary essential for the well-functioning of thyroid. Indeed, it plays important role in the metabolism of thyroid and in antioxidant selenoproteins for protection against reactive oxygen species and reactive nitrogen species. Se has been shown to reduce the antibody titer in patients with autoimmune thyroiditis associated to SCH. In this control study we monitored the temporal parameters of thyroid function across gestation. We evaluated whether the supplementation of MI + Se prevent the subclinical thyroid hormonal fluctuations in order to maintain the euthyroid state in all pregnant women. This result would help pregnant women to avoid the correlated-complications, either for mother and fetus.

CONCLUSIONS

Pregnancy might progress in a trend towards an increase of TSH resulting in SCH or fetal-maternal related-complications. Thus, prevention undoubtedly remains the primary concern in attempting to reduce the prevalence of SCH during gestation. The present study corroborates our previous result, proving that, the combined treatment, MI + Se is effective in maintaining the values of TSH, fT₃ and fT₄, and thus preventing SCH. Therefore, our experience would encourage gynecologists to prescribe a safe and effective supplementation with MI + Se, when at first visit a pregnant woman presents borderline TSH levels. Pregnant women should also be educated to enhance awareness of SCH-related symptoms and complications. However, we believe that further controlled studies, with larger randomized cohort and different ethnic groups, might be required to embrace these encouraging results.

CONFLICTS OF INTEREST:
The Authors declare that there are no conflicts of interest.

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