INTRODUCTION

Approximately 25 - 30% of all pregnancies end as miscarriages and around 80% of them occur during the first trimester. Besides chromosomal and endocrine abnormalities, immunological risk factors are accountable for 20% of miscarriages, especially for recurrent pregnancy loss. Autoimmune diseases during childbearing age are considered as potential risk factors for reproduction abnormalities in females from conception till loss of pregnancy.

Among the pregnant females, the frequency of hypothyroidism is around 2 - 3% and autoimmune thyroid diseases account for 50% in such cases. During pregnancy, proper function of thyroid gland plays a vital role for the health of both mother and developing fetus. Up to 12 weeks of gestation, fetus is completely dependent on maternal thyroid hormone for optimal development. During pregnancy, autoimmune hypothyroidism is found to be associated with impaired fetal brain development as well as increased risk of loss of conception.

For the laboratory diagnosis of subclinical autoimmune hypothyroidism, antibodies against thyroid gland are critical to measure. In such subjects, slightly higher values of thyroid stimulating hormone with normal thyroid hormone levels are usually observed. However; thyroid antibodies have been found in 90% of patients with subclinical autoimmune hypothyroidism. Moreover, elevated level of thyroid peroxidase antibody (TPO Ab) is regarded as very early and sensitive marker in the course of developing hypothyroidism secondary to Hashimoto thyroiditis. Laboratory evaluation of thyroid antibodies, such as thyroglobulin and thyroid peroxidase, is also used to diagnose the autoimmune thyroid disease in euthyroid patients and considered as a part of diagnostic workup in patients with recurrent pregnancy loss. In Pakistan, very limited data is available for autoimmune thyroid illness during pregnancy and its association with adverse pregnancy outcomes. The rationale of this study was to establish the frequency of unknown autoimmune hypothyroidism and to look at the outcome of pregnancy in subjects with thyroid antibodies in a group of Pakistani patients.

The objectives of this study were to measure the frequency of TPO Ab among clinically euthyroid pregnant women during first trimester and to examine the association of TPO Ab with the pregnancy outcome as miscarriage or live birth.

METHODOLOGY

This was a cohort study carried out at the section of Chemical Pathology, Department of Pathology and Laboratory Medicine, The Aga Khan University Hospital, Karachi.
Karachi, Pakistan. A number of 1050 first trimester pregnant women were recruited from Gynaecology and Obstetric outpatient clinics from July to December 2012. Only those clinically euthyroid pregnant women were included who had no personal or family history of thyroid disorder. All those subjects were excluded who had any history of diabetes mellitus, hypertension, antenatal infection and any other known autoimmune disease. Those subjects were also excluded who had history of medications such as lithium, interferon alpha and amiodarone. The demographic details of patients were collected as a part of routine antenatal care and recorded in questionnaire. The demographic details included name, age, height and weight of the patient. Past obstetrical history of miscarriages was also recorded from multiparous women. An informed written consent was taken by all participants. The study was approved by institutional ethical committee.

Subjects were followed till the outcome of pregnancy. The numbers of subjects from enrolment till follow-up or dropout are illustrated as flow diagram (Figure 1). Information of pregnancy outcome was collected either from labour room, operation theatre or patient medical records by principal investigator. At least 5 ml of venous blood was taken from each subject and separated serum was stored at -70°C till analysis of TPO Ab.

TPO Ab levels were measured by automated quantitative micro particle enzyme immunoassay, on AxSym (Abbott Laboratories, USA). Reagents, calibrators and controls of same manufacturer were used. Results of TPO Ab, ≥ 25 IU/ml were interpreted as positive. Analytical sensitivity and intra-assay imprecision of TPO Ab was found 1 IU/ml and 6.8%, respectively.

Data was entered using Epi Info (version 6.04) and then compared for data entry errors. SPSS version 19 was used to analyse the data. Shapiro Wilks test was used to find the type of data distribution. Median (IQR), mode and range were calculated for age of all subjects. Median (IQR), mode and range were calculated for body mass index (BMI) of all subjects. BMI was calculated from weight and height of subjects. Similarly, median (IQR), mode and range were calculated for parity of subjects. Frequencies and percentages were calculated for gravidity (number of pregnancies) and previous miscarriages in multigravida. TPO Ab status was determined in subjects with positive and negative results. Frequency and percentage of TPO Ab was calculated and relative risk of miscarriages was estimated by Cox regression model to examine the association of TPO Ab with miscarriages or live births.

**RESULTS**

The median (IQR), mode and ranges for age, body mass index and parity of a cohort of 943 subjects are given in Table I. Among all subjects, 381 (40.4%) were primigravida and 562 (59.6%) were multigravida. Among the multigravida, 230 (40.9%) and 155 (27.6%) women were experiencing second and third pregnancies, respectively. Remaining 177 (31.5%) women were gravid more than three times. A number of 87 (9.2%) women had history of previous miscarriages. From a cohort of 943 subjects, 127 (13.5%) were found positive for TPO Ab. Miscarriages were observed in subjects with positive and negative TPO Ab status (Table II). A 2.03% increase risk of miscarriages was found in TPO Ab positive than TPO Ab negative pregnant women (Table II).

**DISCUSSION**

Limited data is available for the frequency of TPO Ab among euthyroid pregnant women in Pakistan. In a study from Pakistan, Ghafoor et al. reported the frequency of TPO Ab 11.2%, in pregnant women with no clinical manifestation of thyroid disease.11 In this study, the frequency was 13.5%, a little higher than that reported by Ghafoor et al. For Asian pregnant women, variable frequencies of TPO Ab were observed in different studies, ranged from 5 to 15%. From India,
frequencies of TPO Ab were reported 7% to 18% from south and north region, respectively.\textsuperscript{4,12} Further, frequencies of 5.1% and 13.9% were reported for TPO Ab in Chinese and Korean pregnant women respectively.\textsuperscript{13,14} In European countries, frequency of TPO Ab in euthyroid pregnant women is also studied and observed from 7 to 33%.\textsuperscript{15,16} Literature showed that presence of TPO Ab in euthyroid pregnant women is not uncommon. Prevalence of TPO Ab during gestation is dependent on different factors including genetic makeup, environmental conditions and demographic characteristics of population.\textsuperscript{17}

Association of autoimmune hypothyroidism and adverse pregnancy outcomes is the matter of great attention for researchers. Controversies were found in literature for the association of miscarriages and TPO Ab among pregnant population. Some studies did not support any associated risk of miscarriages in TPO Ab positive pregnant women.\textsuperscript{16,18} However, in some studies, a strong correlation of miscarriages in TPO Ab positive pregnant women has also been reported.\textsuperscript{15,19}

In this study, higher risk of miscarriages (2.03%) was found in TPO Ab positive subjects. This result is supporting the finding of Ghafoor et al., who found the risk of miscarriage higher (O.R 49.2) in pregnant women with positive TPO Ab. Iravani et al. also found the risk of miscarriage 2.2% higher in Irani pregnant women with thyroid autoimmune disease.\textsuperscript{19}

In cases of hypothyroid autoimmune disease, hypothyroidism is postulated as a cause of miscarriages in pregnant women. In such patients, Levothyroxine treatment during pregnancy is found effective to reduce the adverse pregnancy outcomes.\textsuperscript{20} However, in euthyroid pregnant women with autoimmune thyroid disease, autoimmunity is hypothesised as independent risk of miscarriage. TPO Ab has been appeared as a sole risk factor for miscarriages (10%) without any thyroid dysfunction.\textsuperscript{21}

The pathophysiology of loss of pregnancy due to thyroid antibodies is not extensively studied. However, some studies found generalized activation of the immune system, supported by the presence of high levels of tumor necrosis factor-alpha (TNF alpha) in TPO Ab subjects.\textsuperscript{22,23}

TPO Ab is also considered as peripheral markers for T lymphocyte defects, which may be responsible for pregnancy loss in subjects with autoimmune thyroid disease.\textsuperscript{24} Though in one study by Herman et al., no statistically meaningful relation was observed between TNF alpha and miscarriages in pregnant women with TPO Ab.\textsuperscript{25}

In this study, the authors did not evaluate the cause of miscarriages, however, the risk of miscarriages was found higher in TPO Ab positive pregnant subjects. Future studies are required to find the cause and effect relationship of TPO Ab and associated risk of miscarriages.

**CONCLUSION**

There were 13.5% clinically euthyroid pregnant women with TPO Ab. The risk of miscarriage was found 2.03% more in subjects with TPO Ab, suggesting an association of TPO Ab with miscarriages.

**REFERENCES**


