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Thyroid Antibodies in Gestational Diabetes

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Abstract

Objectives- To compare the prevalence of thyroid antibodies in pregnant women with and those without GDM, determine the thyroid antibodies in women with abnormal thyroid hormones and the risk factors for thyroid antibodies in GDM.

Materials And Methods- This was a comparative cross-sectional study conducted at the Jos University Teaching Hospital, Jos, Nigeria. In this study 140 participants comprising 70 women with GDM and 70 normoglycemic pregnant women were recruited by simple consecutive sampling between July 2020 and January 2021. The serum anti-TPO, anti-Tg, TSH, fT3 and fT4 were analysed with Enzyme-Linked Immunosorbent Assay (ELISA) kits and then interpreted. The data were analysed using Statistical Package for Social Sciences (IBM-SPSS) software version 26.

Results- The prevalence of anti-TPO was higher in the GDM group (4.3%) than the control group (1.4%) while the prevalence of anti-Tg was the same (4.3%) in both groups, none of these differences were statistically significant. Subclinical hypothyroidism accounted for 34.3% of thyroid hormone disorder among women with GDM. Among GDM

patients with subclinical hypothyroidism anti-TPO was present in 8.3%. There was no predisposing factor to thyroid antibodies that was significantly different when the two groups were compared.

Conclusion- The prevalence of thyroid antibodies was higher in women GDM compared with normoglycemic women but the finding was not statistically significant. Subclinical hypothyroidism was the commonest thyroid hormone disorder in GDM and had the highest prevalence of thyroid antibodies. There were no exposure indices for thyroid antibodies that showed significantly higher odds of predisposing to thyroid antibodies

Keywords: Thyroid antibodies, anti-thyroglobulin, anti-thyroperoxidase, gestational diabetes, normoglycaemic women, Pregnancy.

Introduction

Thyroid disorders and Gestational Diabetes Mellitus (GDM) are common endocrinopathies in pregnancy (1). They coexist in pregnancy and have been referred to as a "dual gestational endocrinopathy" (2). Gestational diabetes is characterized by varying degrees of glucose intolerance that is recognized for the first time in pregnancy (3). Thyroid antibodies may result in hypothyroidism, hyperthyroidism or the patient may remain euthyroid. Thyroid antibodies may be directed at cytoplasmic antigens-Thyroid Peroxidase Antibody (TPOAb) and Thyroglobulin antibody (TgAb) and those directed to the TSH Receptor (TSHRAb)(4).

There is a pathophysiologic association between GDM and thyroid autoimmunity and this has clinically relevant implication for adequate management(5). To ensure a healthy balance between the mother and the fetus, physiologic adaptations occurs in multiple organ systems(6,7). These organ-systems include glucose metabolism, thyroid physiology and the immunologic system(4,6).

Cells and molecules in the maternal immune system act in such a way as to prevent the rejection of the semiallogenic fetus so as to support the growth and development of the fetus. This is achieved by cytokine shift, immunomodulation of estrogen and progesterone and unique HLA expression by the trophoblast(8). Gestational diabetes mellitus arises from dysfunction of beta cells of the pancreas that results in the inability of the pancreas to compensate for the physiologic insulin resistance of pregnancy (6). The defective pancreatic beta-cell function may be associated with insulin resistance, monogenetic factors and autoimmune disease (9). There is an inextricable link of autoantibodies to thyroid disorders. Microchimerism resulting from trafficking of fetal cells to maternal circulation, HLA compatibility, immune stimulatory effect of female hormones, environmental factors and chromosomal differences have been implicated in thyroid autoimmunity (10).

There have been reports on the association of GDM with thyroid autoimmunity (10). There is a link between inflammation and insulin resistance on one side and thyroid autoimmunity and inflammation on the other (11). Thyroid autoimmunity has been demonstrated to be associated with insulin resistance because thyroid antibodies are related to hyperglycemia and hyperinsulinaemia (12). Autoimmune gestational diabetes as a clinical entity has been described (13). Autoimmunity has been found to be associated with β-cell dysfunction with consequent disturbance of glucose haemostasis. Hyperglycemia may result in an elevated expression of several β-cell antigens such as Glutamic Acid Decarboxylase (GAD). This makes these β-cells vulnerable to GAD antibodies (14). Islet Cell Antibodies (ICSAb), Glutamic Acid Decarboxylase (GAD) antibody and Insulin Autoantibodies have been demonstrated in women with GDM (15). The prevalence of GAD antibody is higher in AITD than the general population (16).

Studies have found a high prevalence of thyroid antibodies in women with GDM compared to healthy controls (10,12). The need to monitor women with GDM for autoimmune antibodies has been emphasized (7). International Federation of Gynaecology and Obstetrics (FIGO) initiative on GDM recommends that "all pregnant women should be tested for hyperglycemia during pregnancy using a one-step procedure" (17). There has however not been any guideline on the screening of thyroid antibodies in pregnancy. Because of the prevalence of thyroid antibodies in women with GDM, screening for thyroid antibodies have been suggested (18).

Euthyroidism is important in pregnancy because prior to the 20th week of gestation the mother is the unique

source of thyroid hormones. Impaired cognitive development is a recognized risk in children of women with thyroid disorders (19). It has also been noted that perinatal morbidity and mortality is increased in thyroid antibody positive pregnant women. This could result from prematurity, congenital anomalies or neonatal jaundice, pregnancy induced hypertension, placenta abruption, anemia etc (20). The aim of this study was to determine the prevalence of thyroid antibodies in pregnant women with and those without gestational diabetes, determine the thyroid antibodies in women with abnormal thyroid hormones and the risk factors for thyroid antibodies in GDM. There is paucity of data in our environment on the prevalence of thyroid antibodies in GDM or an association between GDM and thyroid autoimmunity. This study will help to determine the prevalence of thyroid antibodies in women with GDM and establish if there is an association. This study can serve as a basis for recommendation for screening for thyroid antibodies in GDM and also the formulation of a guideline for management of thyroid disorders in GDM. Furthermore, findings will serve as source of secondary data for future research on similar study.

Materials And Methods

Study Setting and Design: This was a cross-sectional comparative study conducted in the antenatal care clinic of the Jos University Teaching Hospital (JUTH), Jos, Nigeria.

Study Population- Between July 2020 and January 2021 we recruited gestational diabetics and apparently healthy normoglycemic pregnant women from a population of women who had done Oral Glucose Tolerance Test (OGTT) between 24-28 weeks of gestation. In this study, blood glucose values recommended by IADPSG were used for the interpretation of OGTT results in pregnancy (21).

Exclusion Criteria- Patients with suspected or established thyroid disorders, type 1 or 2 diabetes mellitus, multiple gestations and women who were on drugs that could affect thyroid function e.g. iodine, amiodarone were excluded from the study.

Sample Size determination- The sample size was determined using the sample size for comparing proportions (22)-

$$n = \frac{r+1}{r}(P^*)(1-P^*)\{Z_{\alpha}+Z_{\beta}\}^2$$

$$r \quad (P_1-P_2)^2$$

Confidence interval was set at 95%, Z_{β} is standard normal distribution was for a significance level of 0.05 and desired level of power of 80%. P_1 and P_2 is the reference prevalence of thyroid antibodies in GDM and normoglycemic pregnant women derived from a similar study was 0.1286% and 0.000% respectively (18). P^* is the average proportion for GDM and normoglycaemic pregnant women. A sample size-n of 70 for each group and a total of 140 women was arrived at.

Data Collection- Seventy women with GDM (cases) and 70 apparently healthy normoglycemic pregnant women (controls) were consecutively recruited. The controls were matched with cases for age and gestational age. In doing this, it was ensured that for every case selected the difference in age for the selected control was ±2 years and the difference in gestational age was ±2 weeks and vice versa. Using the proforma that was formulated, a history was taken and recorded with regards to age, parity, and gestational age at screening and recruitment. Also past obstetric history, past history of thyroid disorder, history of head and neck irradiation, use of iodized salt and whether anterior neck swelling was common in her environment were recorded. Blood pressure of each participant was measured in the sitting position using Accoson mercury sphygmomanometer. Needle and syringe was used to collect 5mls of venous blood which was transferred into an anticoagulant-free plain bottle. The blood was allowed to clot for a minimum of 30minutes before centrifugation. The serum was separated into 1ml aliquots in 2 cryovials and immediately stored at -20^{o}C until adequate sample size was reached. Study identification numbers was given to each participant to protect her identity and eliminate bias during laboratory assessment of their thyroid profile.

The frozen serum samples were used for the following assay anti-TPO, anti-Tg, TSH, T3 and T4 using ELISA kit manufactured by Monobind Inc. Lake Forest, CA 92630, USA. The results were interpreted using the trimester specific values of thyroid hormones recommended by ATA and the serum values of thyroid antibodies recommended by the kit manufacturer and recorded (23,24,25).

Statistical Analysis- Data collected were entered into a Microsoft excel data base created. Statistical analysis

was performed using IBM-SPSS software (version 26). Frequencies and percentages were computed for demographic and clinical characteristics of cases and controls and presented in tables, difference in categorical and numerical variables between GDM patients and controls was done using Chi square and students t- test respectively. Multiple logistic regression models were built for obstetric characteristics and exposure indices for thyroid antibodies. In both GDM status was used as a covariate to identify independent predictors of thyroid antibodies among GDM and non-GDM groups. The results are presented as odd ratio (OR) and 95% confidence interval (CI). A p-value of <0.05 was taken as significant.

Ethical Approval-The Jos University Teaching Hospital Health Research ethical committee gave ethical approval for this study. All participants gave and signed an informed consent before enrolment into this study. All participants eventually identified with thyroid hormone disorder were informed about the result and referred for appropriate treatment.

Results

Table 1 showed the demographic, clinical and laboratory characteristics of the study participants. The mean serum level of anti-Tg was higher among the controls (41.76IU/ml) than cases (20.1IU/ml) and this was statistically significant (p= 0.044) when both groups were compared. The mean fT4 was significantly lower among the cases (GDM) than the controls (Normoglycaemia) (1.31ng/dl versus 1.43ng/dl, p= 0.01).

Table 1: Demographic, Clinical and Laboratory variables of study participants

Variables	Cases	Control	χ^2	p-value
Mean Age	33±4.81	32±5.23	1.170	0.244
Mean parity	3±2.27	2±1.84	1.461	0.146
Mean GA(weeks)	30.63±4.17	30.35±3.98	0.421*	0.674
Mean GA at Diagnosis	29.01±4.08	29.51±4.39	0.578*	0.565
Mean SBP(mmHg)	110.25±13.32	108.92±13.04	0.618*	0.538
Mean DBP(mmHg)	65.23±12.55	64.30±11.57	0.470*	0.639
Mean TSH miu/L	2.06±1.44	1.77±0.97	1.432*	0.154
Mean fT4(ng/dl)	1.31±0.51	1.43±0.59	2.606	0.010
Mean fT3(pg/ml)	2.30±0.73	2.21±0.71	0.463	0.644
Mean anti TPO(IU/ml)	3.23±1.17	3.23±1.63	0.002*	0.998
Mean anti Tg(IU/ml)	20.46±4.79	41.76±10.36	1.866*	0.044

GA=Gestational age, SBP=Systolic Blood Pressure, DBP= Diastolic Blood Pressure, TPO= Thyroperoxidase, Tg= thyroglobulin *=t test derived values

Among women with GDM, the prevalence (Table 2) of only anti-TPO and anti-Tg was 4.3% each while it was 1.4% and 4.3% respectively in the control group. None of these was statistically significant (p=0.310 and 1.000) when the two groups were compared. Both antibodies (anti-TPO+ anti-Tg) was present in 8.6% and 4.3% of women with GDM (cases) and control group respectively, none of these was statistically significant (p=0.301).

Table 2: Prevalence of thyroid antibodies among cases and controls

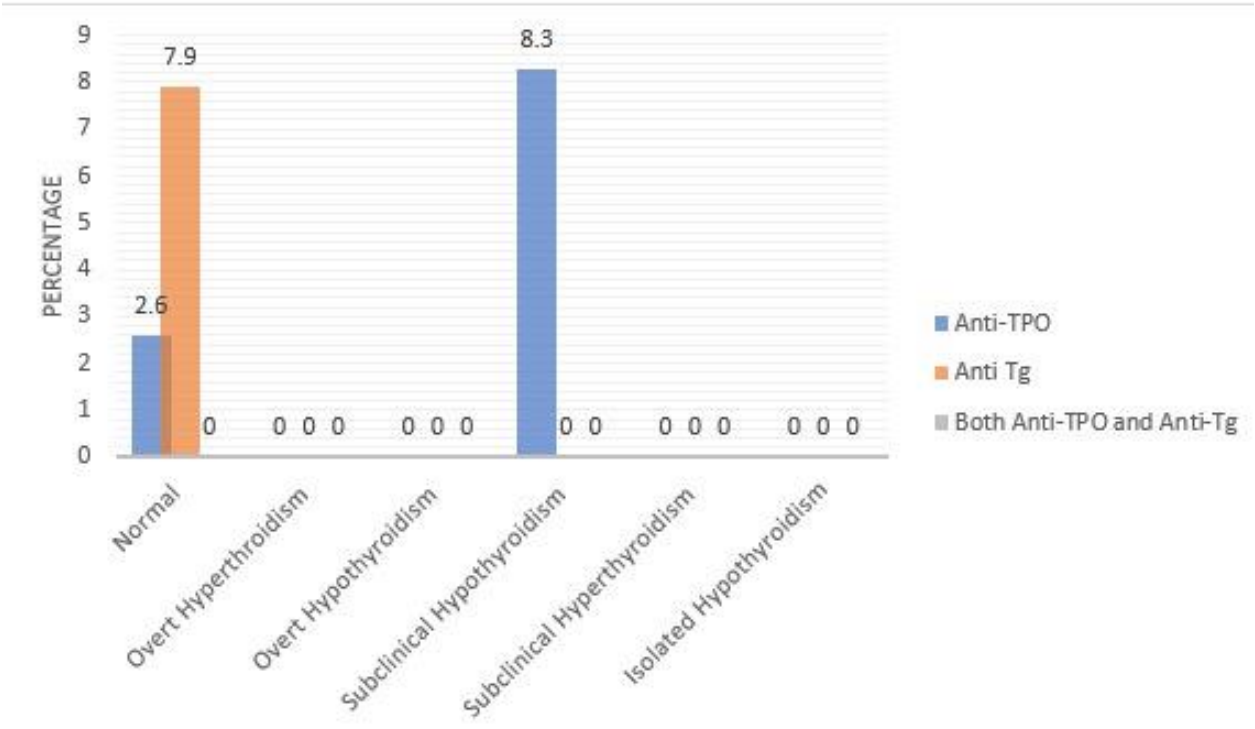
Parameter	Cases n(%)	Controls n(%)	χ^2	p-value
Anti-TPO positive	3(4.3)	1(1.4)	1.029	0.310
Anti-Tg positive	3(4.3)	3(4.3)	0.000	1.000
Both anti-TPO and Tg positive	2(2.9)	3(4.3)	0.040	0.841

anti TPO=Thyroperoxidase antibody, anti Tg= Thyroglobulin antibody

The prevalence of thyroid hormone disorders was higher in the GDM group when compared with the non-GDM group (45.7% versus 28.8%). Subclinical hypothyroidism was the most common type of thyroid hormone disorder among women with GDM accounting for 34.3% of all types of thyroid hormone disorders, this was higher and statistically significant (p=0.001) when compared with the prevalence among the non-GDM group (1.4%). Among the non-GDM group, subclinical hyperthyroidism accounted for 18.6%, this was higher and statistically significant (0.005) when compared with the GDM group (2.9%).

From Figure 1 below, GDM patients with subclinical hypothyroidism anti-TPO was present in 8.3%, no thyroid antibody was demonstrated in other thyroid hormone disorders among women with GDM. In 7.9% euthyroid GDM patients, anti-Tg was demonstrated in 7.9% while anti TPO was found in 2.6%. Normoglycemic women with subclinical hyperthyroidism had the highest (7.7%) prevalence of thyroid antibodies as demonstrated by the presence of anti-Tg. Also, among the control group that were euthyroid, anti-Tg was found in 4.0% and anti-TPO was present in 2%.

Figure 1: Prevalence of thyroid antibodies among cases with thyroid hormone disorder



TPO= anti Thyroperoxidase antibody, anti Tg= Thyroglobulin antibody

There was a negative correlation between mean anti-TPO and mean FBG and 2HPP, this was not statistically significant (p=0.568 and 0.980). The correlation between FBG and 2HPG and anti-Tg was positive, mean FBG showed a weak positive correlation while mean 2HPG showed a strong positive correlation, none of these was statistically significant (p>0.005). The other correlations are shown in Table 3.

Table 3: Correlation between mean blood sugar and mean thyroid antibodies

	Mean anti-TPO		Mean anti-Tg	
Parameter	**Correlation coefficient**	**p-value**	**Correlation coefficient**	**p-value**
Mean FBG	-0.296	0.568	0.200	0.704
Mean 2HPG	-0.016	0.980	0.619	0.265

FBG=Fasting Blood Glucose, 2HPD= 2 Hours Post prandial Glucose

There was no obstetric characteristic with a significantly high odd of predisposing to the presence of thyroid antibodies when the two groups were compared.

Though not statistically significant, odds of thyroid antibodies being present was 2 times higher in gestational diabetics with head and neck irradiation and family history of thyroid disease when compared with normoglycemic women.

Discussion

The prevalence of anti-TPO was higher in the GDM group (4.3%) than the control group (1.4%) while the prevalence of anti-Tg was the same (4.3%) in both groups, none of this differences was statistically significant. The finding in this study is similar to a report by Esra et al. that compared the thyroid function of diabetic pregnant women including women with GDM and normoglycemic pregnant women, of the 40 women in the GDM arm 15% were anti TPO positive while in the control arm comprising 40 participant 10% were positive for anti-TPO and this was not of statistical significance (26). In a cross sectional prospective study that compared the thyroid function test of 185 normoglycemic women with 45 women with GDM there was no woman that was anti-Tg positive among the experimental group while 9.7% of the controls were positive. In the same study, among the cases and controls the prevalence of anti-TPO positivity was 6.7% and 8.6% respectively (27). None of the findings in the study above demonstrated statistical significance. The similarity of the findings in our study with the studies above may be due to the small sample size used for the studies just like the index study.

The finding in this study differs from a similar study conducted by Safian et al. compared the thyroid function test of 252 women with GDM with 252 pregnant women that were normoglycemic. There was a significantly higher (18.6%) prevalence of anti-TPO among cases than controls (10.3%) (28). The significant difference reported in the study above may have been possibly due to the larger sample size used in the study, regional/racial and iodine consumption differences. It is believed that the consumption of excess iodine may increase thyroid autoimmunity and cause a rise in serum antibody levels (23). From this study the prevalence of anti-Tg in women with GDM and normoglycaemic women was the same (4.3%). Inacil et al in a prospective cross sectional study among 185 normoglyceamic women and 45 GDM patients however reported a higher (9.7%) anti-Tg positivity among normoglycemic women than in GDM, this was however not statistically significant. Though not significant, this study revealed that more women (4.3%) in the control group were positive for both anti-Tg and anti-TPO compared to the cases (2.9%). Inacil et al reported that 3.8% of the normoglycaemic women were positive for both anti-TPO and anti-Tg but none of the women with GDM was positive for both anti-TPO and anti-Tg, there was no significant difference when the two groups were compared (27).

The prevalence of thyroid hormone disorder in women with GDM was 45.7%, this was higher when compared with the controls (28.6%). Subclinical hypothyroidism was the most common (34.3%) type of thyroid hormone disorder among women with GDM followed by overt hyperthyroidism (7.1%). Subclinical hyperthyroidism was the most common form of thyroid hormone disorder in the control group. The findings in this study are similar to findings in a study by Perham et al where thyroid disorders were assessed in 105 women with and 105 women without GDM. Perham et al using the trimester specific values for thyroid function test recommended by ATA just like in this study found out that thyroid disorders mostly subclinical hypothyroidism was higher (17.1%) among the cases than controls (30). Safian et al also compared the thyroid function test of 252 women

with GDM and 252 health pregnant controls with similar findings. They found out that there was a significant difference in the prevalence of subclinical hypothyroidism in the experimental group (38.4%) and control group (14.06%) (31).

There was no significant difference found in the prevalence of thyroid hormone disorders in a study conducted among 35 normoglycemic pregnant controls and 61 cases that included 22 GDM and 39 pregestational diabetic patients (29). In a study to assess the association between subclinical hypothyroidism and GDM among 68 GDM patients and 74 normoglycemic pregnant women, there was no significant difference in terms of the prevalence between both groups (32). The difference observed may be due to the difference in the study populations as well as the sample size used for the study. Also it appeared there was no matching of the groups in the study with the GDM group. The reference ranges used for these studies like that by Shahbazian et al (29) differ from the reference ranges by ATA which was used in this study. The serum iodine levels of the population and the variations in the interpretation of the results from the various kits used and different geographical regions.

Among the experimental group, women with subclinical hypothyroidism recorded the highest (8.3%) rate of thyroid antibody positivity represented by anti-TPO. In the control group the participants with subclinical hyperthyroidism had the highest (7.7%) prevalence of antibody positivity represented by anti-Tg. In a cross sectional retrospective study, it was noted that among hypothyroid women that had GDM, 21.9% were positive for anti-TPO while 16.0% were positive for anti-Tg (33). However, it has been reported in a cross sectional study comprising 382 antenatal women majority (28.2%) of GDM patients with subclinical hypothyroidism did not have positive anti-TPO (34). It had been reported that the level of iodine consumed by a population could affect the serum levels of thyroid hormones. Also the timing during pregnancy when thyroid antibodies are assessed could also affect the prevalence of thyroid antibodies as they have been observed to decline with advancing gestation (35).

There was a positive but statistically insignificant correlation between FBG, 2HPG and anti-Tg. The correlation between FBG, 2HPG and anti-TPO was negative and insignificant. A study in a Chinese population did not find a correlation between GDM and positive anti-TPO and concluded that there was no evidence that supports a correlation between thyroid disease as a whole and GDM (36). It has been stated that there is a positive correlation between anti-TG and anti-TPO and insulin resistance- which characterises GDM (37). It is thought that increased interferon-gamma and tissue necrosis factor-alpha may be responsible for the relationship between insulin resistance and thyroid antibodies (6).

The odds of having thyroid antibodies in women with previous irradiations, family history of thyroid disease and use of iodised salt was higher than women without the characteristics but statistically insignificant. In a study conducted in a general antenatal population to evaluate risk factor based screening for thyroid peroxidase antibody positivity in pregnancy among 546 participants' family history of thyroid disease stood out as a significant risk factor for the presence of thyroid antibodies with an odd ratio of 2.6. Just like in this study, no participant had history of exposure to head and neck irradiation (38).

The strengths of the study include the following: Firstly, it was a comparative study that included women with GDM and normoglycemic pregnant women that were matched for age and gestational age. The trimester specific reference ranges for thyroid hormones recommended by ATA were used for interpretation of results. Secondly, confounding effects were avoided using a regression model, as a result independent associations of thyroid antibodies in GDM were identified

Limitations exist in this study that merit careful consideration. The local trimester specific reference ranges of thyroid function parameters for the general population were not used as it was not available. Also there was no follow-up to determine fetomaternal effects of abnormal levels of thyroid antibodies and thyroid hormones.

In conclusion, the prevalence of thyroid antibodies was higher in women GDM compared with normoglycemic women but the finding was not statistically significant. Subclinical hypothyroidism is the commonest thyroid hormone disorder in GDM and has the highest prevalence of thyroid antibodies. There were no exposure indices for thyroid antibodies that showed

significantly higher odds of predisposing to thyroid antibodies when the two groups were compared. We recommend a larger multi-centre study with long follow up period to determine the effect on the mother, fetus and neonate to further substantiate the findings in this study. There is need to determine local trimester specific values for thyroid hormones and also normal serum levels of thyroid antibodies for each population because racial, ethnic or geographical variability may affect interpretation of results.

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