Thyrotropin and Thyroid Antibodies in Sudanese Women with Recurrent Miscarriage

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Background: Recurrent spontaneous miscarriage (RSM) is defined classically as three or more consecutive pregnancy losses prior to the 20th week of gestation. The etiology of recurrent spontaneous miscarriage is often unclear and has much controversy regarding diagnosis and treatment.

Objectives: To monitor the effect of TSH level on pregnancy outcome and to determine the effect of positive thyroid antibody among miscarriage women.

Materials and methods: A cross-sectional case-control hospital-based study included patients attending the Obstetrics and Gynecology Unit at Khartoum Teaching Hospital and Omdurman New Hospital with recurrent miscarriage during the period from June 2008 to Jan 2009. Thyrotropin and thyroid antibodies (TSH, TPO-Ab and Tg-Ab) were measured for the two groups using radioimmunoassay (RIA) technique.

Results: There was a significant elevation in the concentration of TSH in the target group (p-value = 0.0001). Seventy percent (70%) of the control group had TSH levels below normal. The presence of positive TPO-Ab in the target and control groups was 26.66% and 42.5% respectively. The presence of positive Tg-Ab in the target group was (3.33%). The study observed that the TSH concentration was increased in women with positive antibodies in the patients group.

Conclusion: Thyroid antibodies could be detected in pregnant women and still they can reach term and have babies when the concentration of TSH is low during the first trimester, but the risk of miscarriage could be high in positive thyroid antibodies pregnant women when the TSH level is relatively normal in the first trimester.

Introduction:

Recurrent spontaneous miscarriage (RSM) is defined classically as three or more consecutive pregnancy losses prior to the 20th week of gestation [(1,2)]. It has been estimated that 0.5% – 1% of women suffer from recurrent miscarriages. More recently, investigations and management similar to that for recurrent miscarriages have been started for women who suffer from two consecutive miscarriages. This latter group would include a larger percentage (2%) in fertile couples[(3,4,5)]. The etiology of recurrent spontaneous miscarriage is often unclear and with much controversy regarding diagnosis and treatment. Reasonably accepted etiologic causes include genetics 5%, anatomical abnormalities 10%, hormonal problems; infections 5%. These are responsible for sporadic miscarriage rather than consecutive miscarriage[(6)]. Anti-phospholipids antibody (APA) 7% to 25%, certain coagulation and immunoregulatory protein defects cause miscarriage in the second or third trimester[(7,8)]. Smoking and alcohol consumption, exposure to environmental factors, psychological trauma and stressful life event are also causes of miscarriage. However, the majority (60%) of cases of RSM remain unexplained[(9,10)].

It is estimated that approximately 8 to 12% of all pregnancy losses are the result of endocrine factors. Several endocrinological abnormalities such as thyroid disease, hypoparathyroidism, uncontrolled diabetes, and decreased ovarian reserve have been implicated as etiologic factors for recurrent pregnancy loss[9,11]. Thyroid disorders may not only be the cause of infertility, but also increases the incidence of miscarriages and the morbidity of the pregnancies[12]. In Sudan no data have been reported relating miscarriages and thyroid status. This study explores the effect of thyrotropin and thyroid antibodies among recurrent spontaneous miscarriage as a base line data that may add more information in this field of study.

Materials and Methods:

Informed consent was obtained from all patients and controls who agreed to participate in the study. Sixty, apparently healthy pregnant women with history of unexplained recurrent miscarriages during the first trimester were selected as target group. Their ages ranged between 20 and 45 years. Gestational age was estimated from the date of last menstrual cycle. Forty healthy, normal pregnant women of same gestational age and known to reach term and had healthy labor were selected; Their ages ranged between 17 and 41 years. The two groups were physically examined and their thyroid glands were examined by the doctor in charge. A structured questionnaire was completed for all subjects. Those excluded: uterine myoma, malnourished, with negative Rhesus factor or exposed to toxic substances. Five ml of venous blood were collected from selected patients and control subjects. Each specimen of blood was allowed to clot, centrifuged for three minutes at a rate of 3000 rpm, serum was separated and stored at -20 C till analysis. Radioimmunoassay (RIA) is antigen-antibody competitive reaction i.e. competition between radio- labelled (^{125}I) antigen and analyte contained in the standard solutions or in serum samples to be assayed, for a fixed and limited number of antibody binding sites to form antigen-antibody complexes. Separation system is applied to enhance fine separation of the bound complexes. Radioactivity of the bound complex is detected by lithium iodide crystal gamma counter. The amount of ^{125}I labelled antigen bound to the antibody is inversely related to the amount of analyte present in the sample. The concentration of analyte present in the unknown samples can be detected by measuring the proportion of ^{125}I labelled antigen bound in the reference standards containing various known amounts of analyte. On the other hand, Immunoradiometric (IRMA) assay depends on the reaction of analyte present in serum with monoclonal and polyclonal antibody (reagent excess) to form monoclonal antibody-antigen polyclonal antibody complex. The monoclonal antibody is labeled with ^{125}I as tracer and the polyclonal antibody is coupled to magnetic iron oxide particles or cellulose. The sandwich complex is separated from unbound tracer by placing the assay tubes on the magnetic separator or by centrifugation and decanting supernatant. The radioactivity of tracer in the tubes detected by gamma counter instrument is directly proportional to the concentration of analyte in the samples[18]. The equipments used for the assay included polystyrene assay tubes with capacity of 5 ml from Bio-medical laboratory supplies for performing assays with the serum, vortex for mixing reagents standard and samples, adjustable repeating syringes (50µl-2 ml) for adding the reagents, micropipettes (20- 100µl) with disposable tips for standards and samples, Centrifuge Falcon 6/300 MSE. Also incubator is important for performing assays at specific temperature. The reagents of TSH assay consisted of standards, radio-labeled antibody (tracer) and antibody. Standard solutions of TSH were in lyophilized forms with the following concentrations: 0, 0.3, 1.1, 3.2, 10, 21, and 90mlU/L respectively (standard lot: 200807). Radio labeled antibody (tracer) of TSH was a solution of monoclonal antibody labeled with ^{125}I. TSH antibody was a suspension of polyclonal antibody coupled to magnetic iron oxide particles. The normal range of TSH concentrations in Sudanese population was (0.7 - 5.0) mlU/L. Thyroid autoantibody reagents were obtained from Beijing Atom Hightech CO., LTD – China in form of kits. The code number of thyroglobulin antibody

(Tg-Ab) kit is-476 contains lyophilized tracer (^{125}I-Tg), lyophilized normal serum, lyophilized positive serum, buffer solution, tracer diluent and precipitant. The protocol depends on the binding capacity for determination of autoantibody to thyroglobulin in human serum using this equation:

Ab binding (%) = (Counts of positive control or sample – Counts of Zero) /T * 100

Where: T is counts of tracer (^{125}I-Tg); Ab is antibody.

Normal reference value: Tg-Ab ≤ 30%

The code number of thyroid peroxidase antibody (TPO-Ab) RIA kit is IMK471. This kit contains standards which are lyophilized with the following concentrations, 0, 15, 32, 100, 300, 950 and 3000 U/ml , antibody suspension 10 ml, incubation solution, freeze dried tracer (^{125}I-TPO) and tracer diluents. The reference value is ≤15 U/ml for negative TPO-Ab.

Descriptive statistical analysis of each parameter was performed using SPSS programme. The normality for each parameter was checked using the One-sample Kolmogorov-Smirnov test. The TSH was not normally distributed. Student t-test was performed for parameters with normal distribution to test the difference between mean values in target and control groups. Mann-Whitney U test was employed to test the difference between not normally distributed TSH in the target and control groups.

Results

Thyrotropin:

The mean serum concentration of TSH in the control subject (0.542 ± 0.464) was significantly lower than the mean concentration of the target group (1.295 ± 1.216), p-value ≤ 0.0001) as shown in table 1. The normal range of serum TSH concentration in the Sudanese population was (0.7-5.0 mlU/L)[13]. Low levels of serum TSH values were found in 70% of the control group and 33.3% of the target group. The percentage of serum TSH above normal range was found in 1.7% of target group only (Table 2).

The mean serum concentration of TPO-Ab in the control subject (15.09 ± 11.29) was significantly higher than the mean concentration of TPO-Ab in the target group (10.79 ± 9.56), the p-value = 0.048 as shown in table 3. The reference value is ≤15 U/ml for negative TPO-Ab, accordingly sixteen (16) patients out of sixty (60) with positive TPO-Ab. So the percentage of positive TPO-Ab in the target group is (26.66%) and in the control subject is 42.5% as shown in table 4.

Thyroglobulin antibody (Tg-Ab):

The mean serum concentration of Tg-Ab in the control subject (2.460 ± 4.375) was lower than the mean concentration of the target group (3.318 ± 7.082). The difference was not significant (p-value = 0.496) as shown in table 3. The reference value is ≥30% for positive Tg-Ab, only two (2) patients out of sixty (60) were found to have positive Tg-Ab. Therefore the percentage of positive Tg-Ab in the target group is (3.33%) and zero for the control subject as shown in table 4.

TPO-Ab and Tg-Ab:

The percentage of patients having positive TPO-Ab and Tg-Ab in the target is 1.66%.Only one miscarriage woman had both TPO-Ab and Tg-Ab.

Serum TSH in positive thyroid antibodies subjects:

The mean serum concentration of TSH in the control samples with positive antibodies was found to be (0.667 ± 0.532) whereas the mean serum concentration of TSH in the miscarriage women with positive antibodies is (1.142 ± 0.953) as shown in table 4.

Table 1. Concentrations of Thyroid Hormones in Women with recurrent miscarriage and Control Subjects (Mean ± standard deviation SD)

Hormone	Target group (mean ± SD)	Control group (mean ± SD)	P-value
Thyrotropin (TSH) mlU/L	1.295 ± 1.216	0.542 ± 0.464	≤ 0.0001

Table 2. Thyrotropin Status in Women with recurrent miscarriage (Target) and Control Subjects in Percentage

Hormone	Target group (%)	Control group (%)
Above normal TSH	1.7	0
Normal TSH	65	30
Below normal TSH	33.3	70

Table 3. Levels of Thyroid Antibodies in Women with recurrent miscarriage and Control Subjects (Mean ± standard deviation SD)

Antibody	Target group ± SD	Control group ± SD	p-value
TPO-Ab	10.79 ± 9.56	15.09 ± 11.29	0.048
Tg-Ab	3.318 ± 7.08	2.46 ± 4.37	0.496

Table 4.Concentrations of TSH in Women with recurrent miscarriage with Positive TPO-Ab and Tg-Ab (in percentage) and the control Subjects

Parameters / Subjects	TPO-Ab %	Tg-Ab %	TSH mlU/L
Miscarriage women	26.66	3.33	1.142 ± 0.953
Control samples	42.5	0.00	0.667 ± 0.532

Discussion

Depending on the increased necessity of the thyroid gland for normal development, growth and metabolic homeostasis during pregnancy and fetal life, changes associated with pregnancy require an increased availability of thyroid hormones by 40% to 100% in order to meet the needs of mother and fetus during pregnancy[14]. The relation of the thyroid antibodies with miscarriage is an important issue that has attracted the interest of many investigators. A number of researches have been published concerning the relation of thyroid autoimmunity and miscarriage which include healthy women, women with recurrent miscarriage and those undergoing assisted reproductive techniques. All these studies are not easily comparable due to the different selection criteria employed for specific aims for each study, but most studies have shown a significant positive association between the presence of thyroid autoantibodies and miscarriage rate[15]. It was suggested that those autoantibodies, which can also be higher in the euthyroid patients, may produce a threat for miscarriage in the subsequent pregnancy. Thyroid peroxidase antibodies target the thyroid peroxidase enzyme that assists in the production and metabolism of thyroid hormone. Although they are widely seen in autoimmune thyroid disorders, thyroid peroxidase antibodies are not necessarily a sign of disease. In up to 26 percent of healthy women, low levels of TPO antibodies are seen. In the normal healthy population, when TPO antibodies are accompanied by a higher TSH level (> 2.0 mu/L), they suggest an increased risk for developing Hashimoto's thyroiditis[16]. However, the results of the present study were trying to evaluate both thytropin hormone and thyroid antibodies as causative agents for miscarriage. The higher TSH values (Table 1) that was reported in the recurrent miscarriage group means that the TH in blood circulation is not high enough to stop the stimulation of the hypothalamus to release TRH to the anterior pituitary to inhibit the stimulation of the synthesis and secretion of TSH. Consequently the TH is not sufficient to meet the needs of both mother and fetus resulting in termination of the

pregnancy; This finding contrasts with Zigman and Glinoer[17,18]. Most miscarriage women are euthyroid (Table 2) according to the evaluation strategy of low serum TSH level[19], this finding agreed with Kasper[20]. This may be due to either IDD which diminishes the biosynthesis of thyroid hormones or to the lack of response of estrogen which affects the level of TBG, that is, the H-P-T axis work as if the women are not pregnant. Euthyroid women in early gestational stages tend to have a reduced thyroid functional reserve and they have an increased risk for obstetrical complications such as miscarriage and premature delivery[21]. The present study is indicating positive thyroid antibodies in both groups (Table 3). Smyth et al found that the prevalence of both antibodies in the pregnancy study group showed a progressive decline compared to non-pregnant controls throughout gestation which becomes undetectable in the third trimester[22]. The mean serum TSH value in the miscarriage group was significantly higher than in the control (Table 4), this finding agreed with that reported by Bagis et al[23]. The control pregnant women with positive thyroid antibodies can reach term and have babies due to the suppression in the level of TSH through the feedback mechanism. Only one case in the target group had elevated level of TSH (> 2mU/L), TPO-Ab and Tg-Ab. This patient may be at risk of developing Hashimoto's thyroiditis; this agreed with Basal and Hayman[16]. The significant relationship between TSH and Tg-Ab is due to Tg is glycoprotein and precursor for synthesis and storage of thyroid hormones, small damage to this protein may affect the synthesis of these hormones.

Conclusion:

TSH levels were normal in miscarriage women while these were very low in healthy pregnant women. The feedback mechanism of thyroid-pituitary glands is not properly working in recurrent miscarriage women. Thyroid antibodies can only affect pregnant women when their serum TSH level is relatively within the actual normal range.

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Reference:

1. Li T.C.,Makris M, Tomsu M, Tuckerman E ,Laird S. Recurrent miscarriage: aetiology, management and prognosis. *Human Reproduction Update* 2002; 8: 463 – 481.

2. Rai R., Regan L. Recurrent miscarriage. *Lancet* 2006; 368: 601-11.

3. Porter TF, Scott JR. Evidence-based care of recurrent miscarriage. *Best Pract Res Clin Obstet Gynaecol* 2005; 19:85-101.

4. Jauniaux E, Farquharson RG, Christiansen OB, Exalto N. Evidence-based Guidelines for the investigation and medical treatment of recurrent miscarriage. *Hum Reprod* 2006; 21:2216–2222.

5. Rull K., Nagirnaja L., Ulander V-M., et al. Chorionic Gonadotropin β-Gene Variants Are Associated with Recurrent Miscarriage in Two European Populations. *J Clin Endocrinol Metab* 2008; 93:4697–4706.

6. Kiwi R. Recurrent pregnancy loss: Evaluation and discussion of the causes and their management. *Cleveland Clinic Journal of Medicine* 2006; 73: 913-921.

7. Drakeley AJ, Quenby S, Farquharson RG. Mid-trimester loss ± appraisal of a screening protocol. *Hum Reprod* 1998; 13:1975-80.

8. Empson M, Lassere M, Craig JC, Scott JR. Recurrent pregnancy loss with antiphospholipid antibody: a systematic review of therapeutic

trials. *Obstet Gynecol* 2002; 99:135–144.

9. Raghupathy R. The immunology of unexplained recurrent spontaneous abortion: Cytokines as key mediators. *J. Bulletin of the Kuwait Institute for Medical Specialization* 2003; 2:32-38.

10. Pandey M. K, Rani R ,Agrawal S. An update in recurrent spontaneous abortion. *Arch Gynecol Obstet* 2005; 272: 95–108.

11. Arredondo, F. Noble L. S. Endocrinology of Recurrent Pregnancy Loss. *Seminars in reproductive medicine* 2006; 24: 33-39.

12. Gärtner R. Thyroid disorders during pregnancy. Endokrinologie, *GeburtshilfeDtsch med Wochenschr Thieme Journals* 2009; 134: 83-86.

13. Hassan A. M. Studies on simple goiter epidemiology and an etiology in a thesis submitted to the Sudan Academy of Sciences in fulfillment of the requirements for the degree of Doctorate in Physiology 2008.

14. Smallridge RC, Glinoer D, Hollowell JG, Brent G. Thyroid function inside and outside of pregnancy: what do we know and what don't we know? *Thyroid.* 2005; 15: 54-9.

15. Kaprara A., Krassas G. E. Thyroid autoimmunity and miscarriage. *Hormones* 2008; 7: 294-302.

16. Basal AS., Hayman GR. Graves's disease associated with chronic idiopathic urticaria: 2 case reports, *J Investig Allergol Clin Immunol;* 2009; 19: 54-6.

17. Zigman Jeffrey M., Shmuel E. Cohen, Jeffrey R. Garber. Impact of Thyroxine-Binding Globulin on Thyroid Hormone Economy during Pregnancy, *Thyroid* 2003; 13: 1169-1175.

18. Glinoer D. The regulation of thyroid function during normal pregnancy: importance of the iodine nutrition status. Best Practice & Research *Clinical Endocrinology & Metabolism* 2004; 18: 133–152

19. Gosling J. P., Basso L. V. Immunoassay: Laboratory analysis and clinical applications. Isted. Butterworth-Heinemann, 1994;133-50.

20. Kasper D. L., Braunwald E., Fauci A. S., Hauser S. L., Longo D. L., Jameson J. L. Harrison's principles of internal medicine. 16th edition, McGraw-Hill companies USA. Chapter 2005; 320: 2104-2126.

21. Stagnaro-Green A.Maternal thyroid disease and preterm delivery. *J Clin Endocrinol Metab* 2009 ; 94:21-5.

22. Smyth PPA., Wijeyaratne C.N., Kaluarachi W. N. et al. Sequential studies on thyroid antibodies during pregnancy. *Thyroid* 2005; 15: 474–7.

23. Bagis, T., Gokcel, A. , Saygili, E.S. Autoimmune thyroid disease in pregnancy and the postpartum period: relationship to spontaneous abortion. *Thyroid* 2001; 11: 1049-1053.