THE ANTIMICROSOMAL AUTOANTIBODIES LEVELS WITH HORMONAL INDICES OF TESTOSTERONE AND PROGESTERONE CONCENTRATION AS MARKER OF PRIMARY INFERTILITY IN (NIGERIAN) WOMEN

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Summary
Objective: This study was carried out to determine the anti-microsomal antibodies level, being investigated as one of the marker, as cause of primary infertility in women, along with the serum concentration of the female hormones, such as progesterone and testosterone, to serve as indices.
Design: A total of one hundred and twenty (120) Euthyroid female were selected from the volunteers that were recruited, having satisfied the exclusion factor in the designed questionnaire to obtain their informed consents. Thereafter, following the Ethics group recommendations, blood samples were collected from each of the test and control groups. The grouping was designed to contain thirty (30) women each, a week after their menses.
Anti-microsomal antibodies (i.e. anti-thyroid peroxidase (anti-TPO)) were determined, using meridian Bioscience Europe anti-TPO assay. Hormonal assay of progesterone, testosterones were determined, using individual hormonal diagnostic ELISA (enzyme linked immuno-sorbent assay) kits.
Result / Outcome: The mean concentration of progesterone determined in the pregnant women group among the group that were positive to anti microsomal antibodies was 24.10 $\pm$ 8.68 ng/ml. This was significantly higher at $p<0.05$ when compared with the primary infertile women having the concentration of 0.029 $\pm$ 0.05 ng/ml, whereas the testosterone titer value of the same pregnant women group that were positive to anti-TPO was 5.37 $\pm$ 4.92 ng / ml, which was significantly lower at $P < 0.05$, when compared with the testosterone concentration in the primary infertile women group positive to anti-TPO which was 294 $\pm$ 11.96 ng / ml.

Keywords: Progesterone, testosterone, pregnancy, primary infertility and Auto anti-microsomal antibodies

Introduction

Antigenic thyroid peroxidase (TPO) enzyme is responsible for the ionisation of tyrosine residues and the coupling of iodinated residues to form thyroid hormones. Anti-thyroid antibodies have been reported in apparently healthy populations and are observed more frequently in women during their reproductive years, (Matt and MacDonald, (1984), Smith et al., (2012)). It has been suggested that immunological factors may play an important role in reproductive process of fertilisation, implantation and placental development, (Yayima, et al., (2002), Abramson and Stagnaro-Green, (2001)). Women have a high degree of immunological responsiveness which is reflected by their increased susceptibility to non-organ specific autoimmune. Such increased susceptibility is supported by the fact that thyroid auto-antibodies have been associated with increased risk for pregnancy loss, (O'Connor and Davis, (1990), Stagnaro-green et al., (1990) and Mecacci et al., (2000), Lee et al., (2001), De Carolis et al., (2004) Lee and Chiang, (2012)). Other studies have suggested an association between autoimmune factor and pregnancy wastage. It has been suggested that anti-thyroid auto-antibodies may serve as peripheral markers for abnormal T-cell function that may be responsible for pregnancy loss (Gleicher et al., (1989), Pratt et al., (1993), Nielsen and Christiansen, (2005)). Furthermore Pratt et al., (1993), demonstrated that detection of thyroid auto-antibodies before conception carried an increased risk of pregnancy loss.

Infertility in women is the inability of the women of childbearing age to get pregnant after about 12 months of regular unprotected sexual intercourse. Primary infertility is the term used to describe women that has never been able to conceive after a minimum of 1 year of attempting to do so, (Fraser and Loso, (1999)).

Progesterone is one of the two major sex hormones responsible for the physical and physiological differences that exist between males and females, apart from the Estrogen,(Kaider and Coulam, (2000)). Progesterone is first produced by the luteum of the ovary and production from this site is necessary for the first eight (8) weeks of pregnancy. There is always progesterone build up throughout pregnancy phase, (Beer, (2003)). Progesterone is the principal factor responsible for the conversion of a proliferating to a secretary endometrium rendering the endometrium receptive for embryo implantation, (Rodwansea et al., (1978), Luborsky et al., (2002)). It gets to the highest during the ovulation period (i.e. day 13 – 15) of the menstrual cycle and if fertilization does not take place, the secretion of progesterone decreases and menstruation occurs. If fertilization does occur, progesterone is secreted during pregnancy by the placenta and acts to prevent spontaneous abortion, (Fraser and Loso, (1999), Rushworth et al., (2000)). Testosterone, which is the most important of the male sex

hormone, is produced by the testes and in very small amount by the ovaries. Over production of testosterone in females which have been reported to be majorly caused by ovarian and adrenal tumour also result in masculinisation, the symptoms of which include cessation of the menstrual cycle (amenorrhea) and excessive growth of body hair (hairsutism), (Fraser and Loso, (1999). Like progesterone, testosterone can stimulate new bone retention, increase bone density and lack of it causes osteoporosis.

Hormones have been reported to influence the expression of certain autoimmune diseases. They are reported to affect the homeostasis of the lymphoid system and responses to antigens by yet to be characterised mechanisms, (Nielsen and Christiansen, (2005)). The predisposing factors in these instances are the sex hormone and it has been reported that testosterone is an immuno-enhancer, but how these hormones are associated to the diseases state has not been elucidated, (Mecacci et al., (2000)).

This study was carried out to evaluate the in women experiencing, primary infertility and who were positive to anti-thyroid auto-antibodies (anti-TPO) and the progesterone and testosterone concentration so as to establish the connection between these interacting molecules of immunological and hormonal homeostasis.

Materials and Methods

Subjects Recruited

One hundred and twenty (120) women volunteers took part in this study and of these thirty (30) patients of the obstetric and gynaecology unit of Ayinke house at the Lagos State University Teaching Hospital diagnosed of experiencing Primary infertility were recruited as the test subjects. The control groups included thirty (30) pregnant women that undergone antenatal care at the antenatal ward of Ayinke House of the Lagos State University Teaching Hospital., Ikeja. 30 Non-pregnant Nulligravida women volunteers and thirty (30) multiparous non-pregnant women volunteers were also recruited. Those that are hypertensive, experiencing thyroid diseases or on hormonal balancing drugs were ruled out. The control groups were pegged to thirty (30) volunteers each to tally with the recruited test group. The study was conducted according to the ethical standard of the research and ethics committee of the Lagos State University Teaching Hospital.

Biological Samples

Venous blood sample was collected in triplicate into plain tubes of 10 ml each from the one hundred and twenty volunteers. The serums obtained after samples were allowed to stand for an hour and then centrifuged at 400prm for 10 minutes were stored at -4^0C until needed for analysis.

Experimental Procedure

The frozen serum samples were brought to room temperature and tested for positivity to anti-microsomal auto-antibodies (anti TPO) in both the test group and the control groups using a commercially available Enzyme linked immuno-absorbent assay kit (ELISA). Testosterone and progesterone concentrations were determined separately in both groups by using testosterone ELISA Kit and progesterone ELISA Kit using ELISA plate reader at 405nm (Fraser and Loso (1999), Matt and Maccdonals (1984)). A Positive result of antiTPO antibody concentration is defined as titers greater than or equal to 10ui/ml according to the manufacturers instruction (Meridan Bioscience Europe) Testosterone range for adult female is 0.2 – 0.8ng/ml as given by the manufacturer of the kit (Diagnostic Automation Inc) and the normal progesterone range for adult female was given as 10.3 – 229ng/ml by the kit manufacturer (Diagnostic Automation Inc).

Statistical analysis was carried out using the ANOVA Package and the mean $\pm$ standard error analysis on the SPSS v.11 electronic statistical tools of windows www.spss.com.

Results

The Result in Table 1 showed that, of the thirty (30) pregnant women (control 2), only eight (8) women representing 27%, were tested positive to anti-microsomal antibody with titer value 11.86 $\pm$ 1.52 units/ml. And their mean serum testosterone and progesterone concentration, were 5.37 $\pm$ 4.92ng/ml and 24.1 $\pm$ 8.68 ng/ml respectively. Eighteen (18) women which represent 60% of the non-pregnant multiparous women (control 1) tested positive to anti TPO with the titer value of 17.57 $\pm$ 8.85 units/ml and their mean testosterone and progesterone concentration were 0.14 $\pm$ 0.13 ng/ml and 0.54 $\pm$ 0.51 ng/ml respectively. Seven (7) women representing 23% of the non-pregnant nulligravida women (control 3) tested positive to anti-TPO antibodies with titer value of 28.83 $\pm$ 3.06units/ml, having mean testosterone concentration of 1.60 $\pm$ 0.75ng/ml and mean progesterone concentration of 14.85 $\pm$ 8.26ng/ml.

Twenty two (22) out of the thirty primary infertile women representing 73% tested positive to anti-TPO antibodies with titer value of antibodies of 130.11 $\pm$ 11.80units/ml with the mean testosterone concentration of 294.70 $\pm$ 11.96 ng/ml and mean progesterone concentration of 0.029 $\pm$ 0.005ng/ml.

The results obtained for testosterone and progesterone concentration presented in the table 1 showed that in the control group of the pregnant women 22, (73%) tested negative to anti TPO antibodies with the titer value of 9.37 $\pm$ 8.60units/ml had mean concentration of testosterone and progesterone concentration of 0.93 $\pm$ 0.14ng/ml and 39.37 $\pm$ 6.30ng/ml

respectively. 8 (27%) of the primary infertile women had negative anti-TPO antibodies of 5.90 ± 3.99units/ml which had serum testosterone and progesterone concentration of 92.98 ± 13.69ng/ml and 0.026 ± 0.018ng/ml respectively. However, 23 (77%) of the nulligravida non pregnant women were negative to anti-TPO antibodies with 4.61 ± 3.02units/ml titer, with the mean testosterone and progesterone concentrations of 0.94 ± 0.07ng/ml and 37.9 ± 8.60ng/ml respectively.

Discussion and Conclusion

The findings generated in this study, support the findings that progesterone was necessary for effective pregnancy onset, (Fairweather et al., (2012)) however, that elevated testosterone levels in the presence of anti-TPO are indeed significantly responsible for infertility in women as observed in 73% of the primary infertile subjects with high anti-microsomal antibodies titer.

Previous study has demonstrated that the concentration of progesterone in the hormone homeostasis increases dramatically during pregnancy. Further studies on immunological investigation, showed that significant levels of anti-microsomal antibodies have been associated with increased risk for pregnancy lost, (Stagnaro-green, et al., (1990) and Bustos et al., (2006)). This current work is therefore bringing to light among other things the crucial implication of the level of testosterone in women in association with auto-antibodies levels in female reproduction in achieving pregnancy.

The progesterone and testosterone concentration obtained in the non-pregnant control nulligravida women and those in the multiparous women compared with the values in the pregnant women support the view that progesterone increases steadily during pregnancy as against the low level of testosterone obtained, with the concomitant concentration of auto antibodies levels.

The progesterone in the control groups were all significantly higher, when compared to the primary infertile women the in test group with positive anti-microsomal antibodies titer level. Conversely, the serum testosterone was significantly higher in the primary infertile women than the control group. However, the progesterone and testosterone concentration in women without anti-microsomal antibodies in the control group compared with primary infertile women followed similar pattern with the anti-TPO positive.

There was a significant association in the serum progesterone and serum testosterone concentration of the primary infertile women with positive anti-microsomal antibody when compared with non-pregnant multiparous women control. This shows that the elevation of testosterone

and a decrease in progesterone concentration is a significant indicative factor (biochemical indices) underlying cause of the infertility marked by the presence of anti TPO, (Taoff (1978),Nussinovitch et al., (2012)). The results obtained in this study are consistent with earlier findings by Bussen et al., (2000) who stated that an- ovulatory state in a group of thirty five (35) female infertile subjects was due to androgen excess. Gleicher et al., (1989) suggested that the intelligence quotient of children born to mothers with detectable TPOAb, and increased TSH during pregnancy may be compromised, (Bizzaro et al., (2005)).

Since testosterone elevations seem to have direct severe effect on female infertility, it is suggested that, investigation of primary infertile women should embrace determination of antibodies levels alongside the assessment of testosterone in hormonal profile that should be done.

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Table 1 : Comparative Analysis of testosterone and progesterone levels in Euthyroid Nigeria women with and without Anti-microsomal antibody (Anti TPO).

	Infertile Women (Test) N = 30		**Multiparous Women (Control – 1) N = 30**		**Pregnant Women (Control - 2) N = 30**		**Nulligravida Women (Control - 3) N = 30**	
Parameters N = 30	Anti TPO antibody (+) N = 22 22(73%)	Anti TPO antibody (-) N = 8 8 (27%)	Anti TPO antibody (+) N = 18 18 (60%)	Anti TPO antibody (-) N = 12 12(40%)	Anti TPO antibody (+) N = 8 8 (27%)	Anti TPO antibody (-) N = 22 22(73%)	Anti TPO antibody (+) N = 7 7(23%)	Anti TPO antibody (-) N = 23 23(77%)
Antimicrosomal Antibody unit/ml	130.11 ± 11.80	5.90 ± 3.99	17.57 ± 8.85	6.99 ± 1.12	11.86 ± 1.52	9.37 ± 8.60	28.83 ± 3.06	4.61 ± 3.02
Testosterone ng/ml	294.70 ± 11.96	92.98 ± 13.69	0.14 ± 0.13	0.12 ± 0.10	5.37 ± 4.92	0.93 ± 0.14	1.60 ± 0.75	0.94 ± 0.07
Progesterone ng/ml	0.029 ± 0.005	0.026 ± 0.018	0.54 ± 0.51	0.675 ± 0.41	24.10 ± 8.68	39.37 ± 6.30	14.85 ± 8.26	37.90 ± 8.60

Data are expressed as mean + SD and analysed by ANOVA (Two way test) P < 0.05 – Significant
^{a}P < 0.05 compared to control ^{b}P < 0.05 infertile women vs. Multiparous