Original Article

Assessment of Antinuclear Antibodies, Anti-Beta 2-Glycoprotein-1, and Thyroid Peroxidase Autoantibody Levels in Human Immunodeficiency Virus Sero-positive Pregnant Women at Nnamdi Azikiwe University Teaching Hospital, Nnewi Nigeria

John Ekenedirichukwu Okwara[1,2,3], Joseph Eberendu Ahaneku[1,2], Charles Chinedum Onyenekwe[1,2], Gerald Okanandu Udigwe[4,5], Joseph Ifeanyichukwu Ikechebelu[4,5], Emmanuel Chidiebere Okwara[6], Nuratu Adejumoke Okwara[7], Salaam Mujeeb[3], Emeka Callistus Onyeka Izuchukwu[1,8]

Departments of [1]Chemical Pathology and [8]Immunology, Faculty of Basic Clinical Sciences, College of Health Sciences, Nnamdi Azikiwe University, [2]Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University, [5]Department of Obstetrics and Gynecology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, [4]Department of Obstetrics and Gynecology, Faculty of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Awka, [6]Department of Chemical Pathology, College of Health, Imo State University, Orlu, [7]Department of Biochemistry, Faculty of Biological Sciences, Federal University of Technology, Owerri, Nigeria, [3]Department of Pathology, Faculty of Health Sciences, Habib Medical School, Islamic University in Uganda, Kampala, Uganda

Abstract

Background: Pregnancy is associated with biochemical alterations and may be compounded by human immunodeficiency virus (HIV) infection potentially affecting pregnancy outcome. **Aims:** This study evaluated some biochemical parameters that could possibly affect pregnancy outcomes in HIV-infected women. **Patients, Materials and Methods:** The study involved 136 HIV sero-positive on highly active antiretroviral therapy (HAART) and 137 HIV sero-negative pregnant women, recruited from the Antenatal Clinic of Nnamdi Azikiwe University Teaching Hospital, Nnewi. Antinuclear antibodies (ANAs), anti-beta 2-glycoprotein-1 (βGP1), and thyroid peroxidase autoantibody (TPOab) were analysed using the enzyme-linked immunosorbent assay methods. **Results:** TPOab in HIV sero-positive subjects (104.9 ± 51.06 IU/mL) was significantly higher ($P > 0.05$) compared with controls (89.5 ± 33.5 IU/mL). ANA and βGP1 in test group (0.89 ± 0.31; 12.94 ± 8.9, respectively) did not change significantly ($P > 0.05$) compared with the controls (0.84 ± 0.27; 10.37 ± 9.6, respectively). There were no significant changes in measured biochemical parameters between trimesters ($P > 0.05$). Furthermore, there were no significant differences in measured biochemical parameters between subjects with different APGAR scores in all subject groups. **Conclusion:** HIV infection affected TPOab level but had no impact on ANA, βGP1, and APGAR score in HIV pregnancy under HAART.

Keywords: Anti-beta 2-glycoprotein-1, antinuclear antibodies, highly active antiretroviral therapy, human immunodeficiency virus, pregnancy, thyroid peroxidase autoantibody

Introduction

Pregnancy, also known as gravidity or gestation, is the time during which one or more young human life develops in the uterus. The average length of human gestation is 280 days, or 40 weeks, from the first day of the woman's last menstrual period.[1,2] This period is hallmarked with a wide array of metabolic, nutritional, physiological, psychological, and anatomical changes that heralds in a new born.[3-8]

Access this article online

Quick Response Code:

Website: http://journals.lww.com/NJOM

DOI: 10.4103/NJM.NJM_59_23

Address for correspondence: Dr. John Ekenedirichukwu Okwara, Department of Chemical Pathology, Faculty of Basic Clinical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Anambra, Nigeria.
E-mail: je.okwara@unizik.edu.ng

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Okwara JE, Ahaneku JE, Onyenekwe CC, Udigwe GO, Ikechebelu JI, Okwara EC, *et al*. Assessment of antinuclear antibodies, anti-beta 2-glycoprotein-1, and thyroid peroxidase autoantibody levels in human immunodeficiency virus sero-positive pregnant women at Nnamdi Azikiwe University Teaching Hospital, Nnewi Nigeria. Niger J Med 2023;32:302-8.

Submitted: 02-Jun-2023 **Revised:** 31-Jul-2023
Accepted: 08-Aug-2023 **Published:** 22-Sep-2023

It is a period characterised with some signs and symptoms which are often discomforting, and sometimes with complications such as high blood pressure of pregnancy (preeclampsia), gestational diabetes, iron-deficiency anemia, and hyperemesis gravidarum, insufficient or excessive weight gain resulting to microsomia, macrosomia, intra-uterine growth retardation/death, and other untoward pregnancy outcome.[9-14]

Immune system suppression and deregulation is the another feature of pregnancy.[15] This often predisposes the subject to infections such as flu, vaginal yeast infections, uterine infections, Group B *Streptococcus*, bacterial vaginosis, and *Listeria*.[16-18] The changes in immune function may cause this increased risk of infection, and if left untreated, may lead to serious complications. Pregnancy can also increase the risk of contraction of infections such as the human immunodeficiency virus (HIV).[19] Also people living with HIV may suffer increased pregnancy risk.[20-22]

Furthermore, if untreated the risk of transmitting HIV to the baby increases.[23] The use of antiretroviral treatment in pregnancy in a long-course regimen reduces the risk of transmission by as much as two-thirds. Antiretroviral therapy in pregnancy also has been widely reported to improve maternal health and pregnancy outcome.[24,25] Adverse pregnancy outcomes that have been reported in HIV-positive women include increased rates of spontaneous early abortion, low birth weight babies, and stillbirths, preterm labor, preterm rupture of membranes, other sexually transmitted diseases, bacterial pneumonia, urinary tract infections, and other infectious complications.[21,26]

Normal pregnancy in itself is associated with immunological changes.[5] Maternal immune system adapts to maintain tolerance toward the allogenic fetus both to protect the mother and her future baby from pathogens. Some immunologic parameters such as antinuclear antibodies (ANAs), thyroid peroxidase autoantibody (TPOab), and anti-beta-2-glycoprotein-1 (βGP1) were assessed in this study to evaluate their role in pregnancy outcome in women living with HIV. ANAs are serum autoantibodies directed against nuclear cell antigens and bind to proteins, nucleic acids, and protein–nucleic acid complexes.[27,28] They are produced by auto-reactive B lymphocytes and are commonly found in patients with autoimmune diseases. It is believed that abnormal ANA production leads to the formation of antigen-antibody complexes which are implicated in the pathogenesis of autoimmune diseases.[29] There is sufficient evidence of association of HIV infection and autoimmune diseases, thus possibly associating ANA as comorbidity in HIV pregnancy.[30,31]

Thyroid peroxidase (TPO) is essential for physiological function of the thyroid gland. Its key physiological function is the biosynthesis of thyroid hormones which is necessary in pregnancy. The high prevalence of TPOAbs has been implicated in the aetiology of some autoimmune conditions and possibly in HIV pathology.[32] Thyroid autoimmunity can have an adverse impact on the outcome of pregnancy.[33] Although the adverse effects of antithyroid antibodies have been well studied in hypothyroid women, their effects in euthyroid women as well as in HIV pregnancy need to be well evaluated.

Beta-2 glycoprotein antibody is considered one of the primary autoantibodies called antiphospholipid (APL) antibodies that mistakenly target the body's own lipid-proteins (phospholipids) found in the outermost layer of cells (cell membranes) and platelets. βGP1 antibody tests can be utilised along with cardiolipin antibody and lupus anticoagulant testing in the diagnosis of APL syndrome which is implicated in an unexplained blood clot (thrombotic episode), and recurrent miscarriages in women.[34] Immunologic evaluations prior to conception, as well as during pregnancy may be useful in the management of pregnancy, especially in certain groups of individuals such as women living with HIV. Hence, this study aims to evaluate the possible benefits of measurements of these immunological parameters during pregnancy in HIV seropositive individuals.

Patients, Materials and Methods

Two hundred and seventy-three antenatal women attending the antenatal clinic of the Nnamdi Azikiwe University Teaching Hospital Nnewi were recruited for the study. These comprised of 136 HIV sero-positives on highly active antiretroviral therapy (HAART) (test group) and 137 sero-negatives (control group). Every alternate consenting subject was recruited. Questionnaire [Attached in the Appendix] was administered on all participants. Venous blood samples were collected for 4–6 times coinciding with antenatal visit up till delivery, and biochemical parameters such as ANAs, anti-βGP1, and TPOab were analysed using the enzyme-linked immunosorbent assay method. Subjects were monitored till delivery and the delivery outcomes such as appearance, pulse, grimance, activity, and respiration (APGAR) score and IUFD were recorded.

Sample size determination was done using Taro Yamane's formulae, with 95% confidence interval, 0.05 precision.[35] Thus,

$n = N/1 + N\,(e)^2$

n = Sample size

n = The estimated population of study (610)

e = The margin of error (0.05)

$n = 610 = 241$

$1 + 610\ (0.05)^2$

Adjusting the sample size for anticipated 10% attrition, $n = 265$.

Ethical approval

Ethical approval was obtained from the Ethics Committee of the Nnamdi Azikiwe University Teaching Hospital Nnewi. NAUTH/CS/66/VOL. 6/039. Informed consent was obtained from every participant. Questionnaire was administered on the

participants. Participants exercised their free will and could withdraw from the study at any time.

Statistical analysis

Data obtained from the study were presented as mean ± standard deviation. It was analysed using the IBM SPSS (Statistical Package for the Social Sciences) statistics for Windows, version 20.0, Armonk, NY, USA: IBM Corporation. Student's *t*-test, ANOVA, and *post hoc* analysis were done to compare the means among different groups. Level of significance was taken at $P < 0.05$.

Results

Table 1 shows the comparison of biochemical parameters, age, and body mass index between HIV sero-positive and HIV sero-negative pregnant subjects. Anti-TPO antibodies was significantly higher ($P = 0.0012$) in HIV sero-positive subjects compared to control. There were no significant difference ($P > 0.05$) in other parameters compared.

Tables 2 and 3 show the comparison of the biochemical parameters in different APGAR scores. There were no significant differences in the measured biochemical parameters for subjects with different APGAR scores in both test and control groups.

Tables 4 and 5 show the comparison of parameters in different trimesters of the two test groups. There were no significant changes in the tested biochemical parameters in all the different trimesters for the two test groups ($P > 0.05$).

Discussion

Pregnancy is a critical period of the development of life and thus often requires manifold efforts for a good outcome. In the process of antenatal care, additional attention may be required for certain category of subjects, such as subjects with HIV sero-positivity.

Elevation of autoantibodies may be an indication of autoimmunity resulting to aggression on self which is often deleterious. This study showed that serum anti-TPOab was significantly higher among pregnant women living with HIV compared to the sero-negatives. This has the likely implication of resulting to subclinical hypothyroidism, over thyroidism, postpartum throiditis, and autoimmune thyroiditis.[36] Autoimmune thyroid diseases (AITD) are the main causes of thyroid dysfunction and the most common autoimmune diseases in the world and this can be further exacerbated by HIV infection in pregnancy.[31] Further antenatal care toward ameliorating the deleterious effects of the elevated antithyroid

Table 1: Comparison of some biochemical parameters, age, and body mass index between human immunodeficiency virus sero-negative and human immunodeficiency virus sero-positive antenatal subjects

Parameter	HIV negative (*n*=137)	HIV positive (*n*=136)	*t*	*P*
Anti-thyroid peroxidase antibodies (IU/mL)	89.50±33.51	104.90±51.06	3.270	0.0012*
Anti-nuclear antibodies	0.84±0.27	0.89±0.31	1.648	0.1004
Anti-beta 2 glycoprotein 1 IgA (U/mL)	10.37±9.67	12.94±18.90	1.178	0.2404
BMI	26.96±5.05	27.97±6.17	1.190	0.2356
Age (years)	30.78±5.10	31.42±4.63	0.896	0.3714

*Values with $P<0.05$ per row are statistically significant. Values are mean±SD. BMI: Body mass index, HIV: Human immunodeficiency virus, SD: Standard deviation, IgA: Immunoglobulin A

Table 2: The relationship between APGAR score and some biochemical parameters of human immunodeficiency virus sero-positive pregnant subjects

Parameters	Apgar score based groupings				
	0–4 (*n*=06)	5–7 (*n*=06)	8–10 (*n*=35)	*F*	*P*
Anti-thyroid peroxidase antibodies (IU/mL)	96.23±22.65	84.53±17.64	108.40±76.87	0.3554	0.7029
Anti-nuclear antibodies	0.74±0.18	0.87±0.30	0.83±0.27	0.4007	0.6723
Anti-beta 2 glycoprotein 1 IgA (U/mL)	8.43±2.76	8.70±6.25	17.15±26.39	0.6062	0.5499

Values with $P<0.05$ are statistically significant. Values are mean±SD. HIV: Human immunodeficiency virus, SD: Standard deviation, IgA: Immunoglobulin A

Table 3: The relationship between APGAR score and some biochemical parameters of human immunodeficiency virus sero-negative pregnant subjects

Parameters	Apgar score based groupings				
	0–4 (*n*=04)	5–7 (*n*=10)	8–10 (*n*=82)	*F*	*P*
Anti-thyroid peroxidase antibodies (IU/mL)	115.95±52.10	77.77±38.27	91.05±32.67	1.826	0.1668
Anti-nuclear antibodies	1.00±0.27	0.69±0.27	0.83±0.27	2.100	0.1282
Anti-beta 2 glycoprotein 1 IgA (U/mL)	7.33±3.32	7.16±2.07	10.17±6.90	1.242	0.2934

Values with $P<0.05$ are statistically significant. Values are mean±SD. HIV: Human immunodeficiency virus, SD: Standard deviation, IgA: Immunoglobulin A

Table 4: Comparison of some biochemical parameters in different trimester of human immunodeficiency virus sero-positive pregnant subjects

Parameters	Trimester				
	First trimester (n=12)	Second trimester (n=44)	Third trimester (n=49)	F	P
Anti-thyroid peroxidase antibodies (IU/mL)	119.59±27.34	106.79±36.20	99.23±42.18	1.469	0.2351
Anti-nuclear antibodies	0.81±0.19	0.88±0.29	0.89±0.28	0.4101	0.6647
Anti-beta 2 glycoprotein 1 IgA (U/mL)	6.80±2.40	7.04±2.02	7.33±2.83	0.2937	0.7461

Values with P<0.05 are statistically significant. Values are mean±SD. HIV: Human immunodeficiency virus, SD: Standard deviation, IgA: Immunoglobulin A

Table 5: Comparison of some biochemical parameters in different trimester of human immunodeficiency virus sero-negative pregnant subjects

Parameters	Trimester				
	First trimester (n=06)	Second trimester (n=49)	Third trimester (n=90)	F	P
Anti-thyroid peroxidase antibodies (IU/mL)	68.78±19.76	92.98±36.53	86.32±32.32	1.645	0.1968
Anti-nuclear antibodies	1.03±0.30	0.83±0.22	0.81±0.28	1.992	0.1403
Anti-beta 2 glycoprotein 1 IgA (U/mL)	7.82±2.60	9.38±5.17	10.10±9.57	0.3004	0.7410

Values with P<0.05 are statistically significant. Values are mean±SD. HIV: Human immunodeficiency virus, SD: Standard deviation, IgA: Immunoglobulin A

antibodies in women living with HIV will promote good pregnancy outcome. Previous studies have demonstrated an association between HAART and the development of AITD.[37,38] Since abnormal elevations of these antithyroid antibodies may lead to adverse outcomes in both mothers and fetuses, special attention must be paid to the titer of the antibodies during pregnancy. The underlying molecular mechanisms of the variations in those antibodies have yet to be well understood. The determination of the frequency and timing of thyroid antibody evaluation, as well as determining the different reference levels of these parameters, at different stages of gestation, also remain to be elucidated, and would improve antenatal care and outcome.

There was no significant change in serum levels of ANAs and anti-βGP1 in this study. The presence of APL and/or ANA seems to be more frequent in the population of infertile women; serum auto-antibodies are associated with early ovarian failure, and has been implicated in some fertility disorders.[39] There are sufficient evidence implicating their role in certain adverse pregnancy outcomes.[40] However, their mechanism of elevation and prognostic value is not conclusive and need to be further evaluated.[41]

In this study, unlike serum estriol, progesterone, and pregnancy associated plasma protein-A which were elevated at different stages of pregnancy, there were no significant changes in the levels of TPO, ANA, and βGP1 in the three trimesters. Unlike the previously mentioned parameters whose role in progression of pregnancy has been well documented, TPO, ANA, and βGP1 appear not to have any role in pregnancy maintenance and progression.[42-44] It may also imply that sudden elevations of these parameters during pregnancy may portend grave consequences, and may thus serve as index of pregnancy wellbeing evaluation. This may need validation.

The relationship between TPO, ANA, and βGP1 levels and APGAR score was evaluated in this study. Our data showed that the serum levels of these parameters did not significantly affect APGAR score.

Conclusion

HIV infection affected TPOab level, but had no impact on ANA, βGP1 and APGAR score in HIV pregnancy under HAART.

Acknowledgment

The authors acknowledges the contribution of the management and staff of Nnamdi Azikiwe University Teaching Hospital, Nnewi, the Departments of Obstetrics and gynecology, and Chemical Pathology for participant recruitment, sample collection, and sample analysis of all biochemical parameters.

Financial support and sponsorship

This work was funded through grant from Batch 1 2009/2010 Tertiary Education Trust Fund Research project intervention for Nnamdi Azikiwe University.

Conflicts of interest

There are no conflicts of interest.

References

1. The American College of Obstetricians and Gynecologists Committee on Obstetrics Practice Society for Maternal-Fetal Medicine: Definition of Term Pregnancy. Committee Opinion number 579. Obstet Gynecol 2013;122:1139-11.
2. Spong CY. Defining "term" pregnancy: Recommendations from the defining "Term" pregnancy workgroup. JAMA 2013;309:2445-6.
3. Meo SA, Hassain A. Metabolic physiology in pregnancy. J Pak Med Assoc 2016;66:S8-10.
4. Abu-Raya B, Michalski C, Sadarangani M, Lavoie PM. Maternal

immunological adaptation during normal pregnancy. Front Immunol 2020;11:575197.
5. Rodsaward P, Chottawornsak N, Suwanchote S, Rachayon M, Deekajorndech T, Wright HL, *et al.* The clinical significance of antinuclear antibodies and specific autoantibodies in juvenile and adult systemic lupus erythematosus patients. Asian Pac J Allergy Immunol 2021;39. [doi: 10.12932/AP-211218-0465].
6. Troiano NH. Physiologic and hemodynamic changes during pregnancy. AACN Adv Crit Care 2018;29:273-83.
7. Bjelica A, Cetkovic N, Trninic-Pjevic A, Mladenovic-Segedi L. The phenomenon of pregnancy – A psychological view. Ginekol Pol 2018;89:102-6.
8. Kazma JM, van den Anker J, Allegaert K, Dallmann A, Ahmadzia HK. Anatomical and physiological alterations of pregnancy. J Pharmacokinet Pharmacodyn 2020;47:271-85.
9. Wood AM, Livingston EG, Hughes BL, Kuller JA. Intrahepatic cholestasis of pregnancy: A review of diagnosis and management. Obstet Gynecol Surv 2018;73:103-9.
10. Hendriks E, Rosenberg R, Prine L. Ectopic pregnancy: Diagnosis and management. Am Fam Physician 2020;101:599-606.
11. Sapra KJ, Joseph KS, Galea S, Bates LM, Louis GM, Ananth CV. Signs and symptoms of early pregnancy loss. Reprod Sci 2017;24:502-13.
12. Todhunter L, Hogan-Roy M, Pressman EK. Complications of pregnancy in adolescents. Semin Reprod Med 2022;40:98-106.
13. Pontius E, Vieth JT. Complications in early pregnancy. Emerg Med Clin North Am 2019;37:219-37.
14. Antza C, Cifkova R, Kotsis V. Hypertensive complications of pregnancy: A clinical overview. Metabolism 2018;86:102-11.
15. Chen M, Zeng J, Liu X, Sun G, Gao Y, Liao J, *et al.* Changes in physiology and immune system during pregnancy and coronavirus infection: A review. Eur J Obstet Gynecol Reprod Biol 2020;255:124-8.
16. Helmo FR, Alves EA, Moreira RA, Severino VO, Rocha LP, Monteiro ML, *et al.* Intrauterine infection, immune system and premature birth. J Matern Fetal Neonatal Med 2018;31:1227-33.
17. Phillips C, Walsh E. Group A streptococcal infection during pregnancy and the postpartum period. Nurs Womens Health 2020;24:13-23.
18. Bagga R, Arora P. Genital micro-organisms in pregnancy. Front Public Health 2020;8:225.
19. Thomson KA, Hughes J, Baeten JM, John-Stewart G, Celum C, Cohen CR, *et al.* Increased risk of HIV acquisition among women throughout pregnancy and during the postpartum period: A prospective per-coital-act analysis among women with HIV-infected partners. J Infect Dis 2018;218:16-25.
20. Yang M, Wang Y, Chen Y, Zhou Y, Jiang Q. Impact of maternal HIV infection on pregnancy outcomes in southwestern China – A hospital registry based study. Epidemiol Infect 2019;147:e124.
21. Li H, Liu J, Tan D, Huang G, Zheng J, Xiao J, *et al.* Maternal HIV infection and risk of adverse pregnancy outcomes in Hunan province, China: A prospective cohort study. Medicine (Baltimore) 2020;99:e19213.
22. Price JT, Sebastião YV, Vwalika B, Cole SR, Mbewe FM, Phiri WM, *et al.* Risk of adverse birth outcomes in two cohorts of pregnant women with HIV in Zambia. Epidemiology 2022;33:422-30.
23. Hurst SA, Appelgren KE, Kourtis AP. Prevention of mother-to-child transmission of HIV type 1: The role of neonatal and infant prophylaxis. Expert Rev Anti Infect Ther 2015;13:169-81.
24. Moseholm E, Aho I, Mellgren Å, Johansen IS, Storgaard M, Pedersen G, *et al.* The experience of pregnancy among women living with HIV in Nordic countries: A qualitative narrative enquiry. Womens Health (Lond) 2022;18:17455065211068688.
25. Sebitloane HM, Moodley D. The impact of highly active antiretroviral therapy on obstetric conditions: A review. Eur J Obstet Gynecol Reprod Biol 2017;210:126-31.
26. Fentie EA, Yeshita HY, Shewarega ES, Boke MM, Kidie AA, Alemu TG. Adverse birth outcome and associated factors among mothers with HIV who gave birth in Northwest Amhara region referral hospitals, Northwest Ethiopia, 2020. Sci Rep 2022;12:22514.
27. Vlagea A, Falagan S, Gutiérrez-Gutiérrez G, Moreno-Rubio J, Merino M, Zambrana F, *et al.* Antinuclear antibodies and cancer: A literature review. Crit Rev Oncol Hematol 2018;127:42-9.
28. Pisetsky DS. Antinuclear antibodies in rheumatic disease: A proposal for a function-based classification. Scand J Immunol 2012;76:223-8.
29. Aleksandrova EN, Verizhnikova ZG, Novikov AA, Lukina GV. Modern look at the problems of investigation of antinuclear antibodies in systemic lupus erythematosus (literature review). Klin Lab Diagn 2018;63:340-8.
30. Vega LE, Espinoza LR. HIV infection and its effects on the development of autoimmune disorders. Pharmacol Res 2018;129:1-9.
31. Huang YM, Hong XZ, Xu JH, Luo JX, Mo HY, Zhao HL. Autoimmunity and dysmetabolism of human acquired immunodeficiency syndrome. Immunol Res 2016;64:641-52.
32. Kolkhir P, Kovalkova E, Chernov A, Danilycheva I, Krause K, Sauer M, *et al.* Autoimmune chronic spontaneous urticaria detection with IgG anti-TPO and total IgE. J Allergy Clin Immunol Pract 2021;9:4138-46.e8.
33. Dhillon-Smith RK, Coomarasamy A. TPO antibody positivity and adverse pregnancy outcomes. Best Pract Res Clin Endocrinol Metab 2020;34:101433.
34. Walter IJ, Klein Haneveld MJ, Lely AT, Bloemenkamp KW, Limper M, Kooiman J. Pregnancy outcome predictors in antiphospholipid syndrome: A systematic review and meta-analysis. Autoimmun Rev 2021;20:102901.
35. Yamane T. Statistics: An Introductory Analysis. International Edition. Tokyo: Harper; 1970. p. 886-7.
36. Oiwa A, Minemura K, Nishio SI, Yamazaki M, Komatsu M. Implications of thyroid autoimmunity in infertile women with subclinical hypothyroidism in the absence of both goiter and anti-thyroid antibodies: Lessons from three cases. Endocr J 2019;66:193-8.
37. Carvalho LG, Teixeira Pde F, Panico AL, Cohen MV, Pinheiro MF, Barroso PF, *et al.* Evaluation of thyroid function and autoimmunity in HIV-infected women. Arq Bras Endocrinol Metabol 2013;57:450-6.
38. Iordache L, Launay O, Bouchaud O, Jeantils V, Goujard C, Boue F, *et al.* Autoimmune diseases in HIV-infected patients: 52 cases and literature review. Autoimmun Rev 2014;13:850-7.
39. Deroux A, Dumestre-Perard C, Dunand-Faure C, Bouillet L, Hoffmann P. Female infertility and serum auto-antibodies: A systematic review. Clin Rev Allergy Immunol 2017;53:78-86.
40. Ticconi C, Pietropolli A, Borelli B, Bruno V, Piccione E, Bernardini S, *et al.* Antinuclear autoantibodies and pregnancy outcome in women with unexplained recurrent miscarriage. Am J Reprod Immunol 2016;76:396-9.
41. D'Ippolito S, Ticconi C, Tersigni C, Garofalo S, Martino C, Lanzone A, *et al.* The pathogenic role of autoantibodies in recurrent pregnancy loss. Am J Reprod Immunol 2020;83:e13200.
42. Prithviraj P, Anaka M, McKeown SJ, Permezel M, Walkiewicz M, Cebon J, *et al.* Pregnancy associated plasma protein-A links pregnancy and melanoma progression by promoting cellular migration and invasion. Oncotarget 2015;6:15953-65.
43. Nagy B, Szekeres-Barthó J, Kovács GL, Sulyok E, Farkas B, Várnagy Á, *et al.* Key to life: Physiological role and clinical implications of progesterone. Int J Mol Sci 2021;22:11039.
44. Kovács K, Vásárhelyi B, Gyarmati B, Karvaly G. Estrogen metabolism during pregnancy. Orv Hetil 2019;160:1007-14.

APPENDIX

Consent form

Dear Ma,

I am a PhD student of Nnamdi Azikiwe University conducting a study on pregnant women in Nnewi, Anambra State.

My project topic is Measurements of Pregnancy Associated Plasma Protein-A (PAPP-A), Nutritional, Immunological, and some other Biochemical Indices in Different Stages of Pregnancy in Nnewi - Eastern Nigeria.

The essence of the study is to find out the laboratory (biochemical) basis of some problems seen in pregnancy.

Your participation is this study will be voluntary. You are not being compelled. You are also free to withdraw from the study at any time without any repercussion. Participants will not be prone to hazard and will not be paid.

The study will require 8 mL of blood sample collections in the different stages of pregnancy. A questionnaire will also be provided for you to fill.

Please accept my assurance of confidentiality on information obtained in the study.

It is my expectation that the result of this study will contribute positively to pregnancy outcome in this environment.

I thus appeal for your wilful participation in this study.

May you please sign bellow to indicate your approval to participate in this study?

…….

Name of..... Participant Signature/Thumbprint.....Date

Thank you.

OKWARA, JOHN E

Principal investigator (08037109857)

Lecturer 11

Chemical Pathology Department

NAU

For enquiries please contact

Dr G. Udigwe

Consultant, Obstetrics and Gynaecology Department, NAU.

Questionnaire for a study titled: Measurements of pregnancy associated plasma protein-a (PAPP-a), nutritional, immunological, and some other biochemical indices in different stages of regnancy in Nnewi -Eastern Nigeria.

1. Hospital No:……Date…….…….
2. Age:…….
3. Height:…….
4. Weight:……
5. LMP:……
6. Gestational Age…….(WKS)
7. BP:……
8. Race (a) African, (b) White, (c) Others…….
9. Ethinicity (a) IBO, (b) Yoruba, (c) Hausa, (d) Others…….
10. Occupation (a) House wife, (b) Civil servant, (c) Trader, (d) Others…….

11. Presence of systemic disease (a) Diabetes, (b) Hypertension, (c) Renal impairement, (d) Heart disorder, (e) Others…….
12. Presence of some pregnancy related problems (a) Anaemia, (b) GDM, (c) Preenlampsia, (d) Others…….
13. Alcohol intake (a) Yes (b) No
14. Frequency of alcohol intake (a) Occassionaly, (b) Regularly
15. Cigarette smoking (a) Yes (b) No
16. Frequency of cigarette smoking (a) Occassionaly, (B) Regularly
17. Tobacco (Snuff) Usage (a) Yes (b) No
18. Frequency of snuff usage (a) Occassionaly, (b) Regularly
19. How old is your last child?.....
20. How many children have you?.....
21. Did you suffer pregnancy delay? (A) Yes (B) No
22. If Yes, for how long?
23. Did you lose any pregnancy? (a) By miscarriage, (b) Abortion
24. Did you know the cause?
25. At what month of pregnancy?
26. Did you see any doctor? (a) Yes, (b) No
27. Did you use traditional herbs? (A) Yes, (B) No

In your previous pregnancy

28. Did you take routine drugs?
29. Did you notice leg swellings?
30. Did you have protein in urine (a) Yes, (b), No (c) Don't know
31. Did you have sugar in urine (a) Yes, (b) No, (c) Don't know
32. Were you at any time admitted in hospital? (a) Yes, (b) No
33. If Yes, for how long?