Original Article

Pregnancy-associated Plasma Protein-A, Progesterone, and Oestriol Levels and Some Birth Outcomes in HIV-seropositive Pregnant Women at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria

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Abstract

Background: Pregnancy is associated with biochemical alterations and may be compounded by human immunodeficiency virus (HIV) infection potentially affecting pregnancy outcome such as birth weight, Apgar score, and foetal viability (stillbirth or intrauterine foetal death [IUFD]). **Aims:** This prospective case–control study evaluated some biochemical parameters and their possible effects on pregnancy outcome in HIV-seropositive subjects. **Patients, Materials and Methods:** The study involved 136 HIV seropositives on highly active antiretroviral therapy and 137 HIV-seronegative pregnant women, recruited from the Antenatal Clinic of Nnamdi Azikiwe University Teaching Hospital, Nnewi. Pregnancy-associated plasma protein-A (PAPP-A), oestriol (E3), and progesterone were analysed using an enzyme-linked immunosorbent assay method, and the delivery outcomes were reported. **Results:** Progesterone was significantly higher ($P = 0.002$) in HIV-seropositive individuals (59.3 ± 17.84 ng/mL) compared to controls (54.89 ± 8.24 ng/mL). There were no significant differences in the levels of E3 and PAPP-A between the two groups. In HIV seronegatives, there were no significant changes in measured biochemical parameters between trimesters ($P > 0.05$). There were no significant differences in measured biochemical parameters between subjects with IUFD and subjects with live births ($P > 0.05$) for both the test and control groups. Subjects with significantly lower PAPP-A in HIV seropositives had babies with higher Apgar score. The incidence of IUFD was 7.31% among HIV seropositives and 7.47% among HIV seronegatives. **Conclusion:** HIV infection affects some biochemical indices such as progesterone and PAPP-A but does not adversely affect pregnancy outcomes in HIV seropositives under antiretroviral therapy.

Keywords: Oestriol, human immunodeficiency virus, intrauterine foetal death, pregnancy-associated plasma protein-A, pregnancy outcome, progesterone

Introduction

The first case of acquired immunodeficiency syndrome (AIDS) was reported in 1981 in the United States.[1] Since then, the human immunodeficiency virus (HIV) epidemic expanded worldwide with varying mortality, morbidity, and prevalence in different regions of the world.[2] Although there is a global decline in its prevalence, its impact on quality of life, socioeconomic indices, neonatal health and maternal health

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are still considerable in regions of the world, especially the less developed and developing nations in Africa and Asia.[3-6] Its impact on birth outcomes has been extensively studied with various interventions aimed at improving antenatal, prenatal, and neonatal outcomes such as the Prevention of Mother-to-Child Transmission (PMTCT) initiative of the Nigerian government.[7-12] There have been various global interventions such as the introduction of highly active antiretroviral therapy (HAART) which became a standard of care in the late nineties.[13-15] The use of HAART in pregnancy has considerably improved pregnancy outcomes.[8,16]

HIV infection in pregnancy can be very challenging. The prevalence rate of HIV infection among pregnant women in Nigeria is 7.2%, ranging from 3% to 8% in different regions of the country.[17-19] Although HIV infection is reported to have adverse pregnancy outcomes both in the developed and less developed countries, it is reported more frequently in a number of African studies, and this could be associated with sociodemographic and economic indices.[20-22]

Adverse birth outcome occurs at various degrees at various levels of health-care services in Nigeria, but there could be a lot of unreported cases and inadequacies in the investigations, leaving an information gap that needs to be explored.[23-25] The determination of a consistent biochemical index will elucidate potential threats to normal pregnancy and will suggest precautionary obstetric measures in Nigeria. Pregnancy-associated plasma protein-A (PAPP-A) promotes steroidogenesis, glucose transport, and trophoblast invasion of the uterine endometrium and could be a useful index of pregnancy assessment.[26] In addition, variations in the levels of hormones in pregnancy may correlate with certain clinical conditions. Progesterone, prolactin, oestriol (E3), and human chorionic gonadotrophin are elevated in pregnancy and are often affected in foetal growth retardation, preeclampsia, and other pregnancy-specific pathophysiological clinical conditions resulting in foetal distress and possibly intrauterine death.[27-32]

Poor utilisation of biochemical testing in pregnancy may contribute to poor pregnancy outcome. Biochemical tests have long been established as a reliable index of health assessment.[33,34] Already poor laboratory utilisation has been reported in this region.[35] Furthermore, there is a paucity of record on pregnancy outcomes in relation to the mother's biochemical status during pregnancy. Continued biochemical evaluation of pregnancy is expected to substantially improve pregnancy outcomes in our environment.

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 seropositives (test group) and 137 seronegatives (control group). Every alternate consenting subject was recruited. Questionnaire (attached in the appendix) was administered to all subject. Venous blood samples were collected 4–6 times coinciding with antenatal visit up till delivery, and biochemical parameters such as PAPP-A, E3, and progesterone were analysed using an enzyme-linked immunosorbent assay method. Subjects were monitored till delivery and the delivery outcomes such as Apgar score, birth weight, mode of delivery and intrauterine foetal death (IUFD) were recorded.

Sample size determination was done using Cochran's formula, with 95% confidence interval, 0.05 precision, and 5.3% prevalence rate.[36] In this study, a birth rate of 53 births per 1000 Nigerian population was used in place of estimated prevalence.[36] Thus $P = 0.053$.

$n = Z^2PQ/d^2$

n = sample size

Z = standard normal deviation at 95% confidence interval which is1.96

d = degree of precision (taken as 0.05)

P = proportion of the target population (estimated at 5.3% which is 5.3/100 = 0.053)

Q = alternate proportion (1 − P) which is 1 − 0.053 = 0.947

$$n = \frac{(1.96)^2\ (0.053)\ (0.947)}{(0.05)^2} = 77$$

Adjusting the sample size for anticipated 10% attrition, $n = 87$.

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. A questionnaire was administered to 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:IBM Corp. using appropriate statistical tools. The 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-seropositive and HIV-seronegative pregnant subjects. Progesterone was significantly higher in HIV seropositives (59.43 ± 17.84) compared to HIV seronegatives (54.89 ± 8.24) ($P < 0.05$). There were no significant difference ($P > 0.05$) in other parameters compared.

Table 2 shows the relationship between Apgar score and some biochemical parameters of HIV-seropositive pregnant subjects.

Post hoc analysis in Table 3 shows that the PAPP-A level in subjects with babies with good Apgar score

(8–10) (23.58 ± 13.92) was significantly lower than the level in subjects with babies with poor Apgar score (5–7) (45.13 ± 14.66) ($P < 0.05$).

Table 4 shows the relationship between Apgar score and some biochemical parameters of HIV-seronegative pregnant subjects. There were no significant differences in all parameters compared ($P > 0.05$).

Table 5 shows the comparison of biochemical parameters in different trimesters of pregnancy among HIV-seropositive pregnant subjects. *Post hoc* analysis, Table 6 shows that E3

Table 1: Comparison of some biochemical parameters between human immunodeficiency virus-seronegative and human immunodeficiency virus-seropositive antenatal subjects

Parameter	HIV negative ($n=137$)	HIV positive ($n=136$)	*t*	*P*
Estriol (ng/mL)	4.78±3.28	4.63±3.72	0.372	0.7099
Progesterone (ng/mL)	54.89±8.24	59.43±17.84	3.062	0.0024*
Pregnancy-associated plasma protein-A (μg/mL)	24.85±9.47	27.60±19.56	1.674	0.0951
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. HIV: Human immunodeficiency virus, SD: Standard deviation, BMI: Body mass index

Table 2: The relationship between Apgar score and some biochemical parameters of human immunodeficiency virus-seropositive pregnant subjects

Parameters	Apgar score-based groupings			*F*	*P*
	0–4 ($n=15$)	5–7 ($n=15$)	8–10 ($n=87$)		
Oestriol (ng/mL)	2.50±1.53	4.63±2.15	3.65±3.89	0.5477	0.5822
Progesterone (ng/mL)	55.50±10.32	65.85±11.58	53.44±15.94	1.764	0.1833
Pregnancy-associated plasma protein-A (μg/mL)	35.23±8.77	45.13±14.66	23.58±13.92	7.536	0.0015*

*Values with $P<0.05$ are statistically significant. Values are mean±SD. SD: Standard deviation

Table 3: *Post hoc* analysis of the relationship between Apgar score and some biochemical parameters of human immunodeficiency virus-seropositive pregnant subjects

Parameter	Apgar score		
	0–4 versus 5–7	0–4 versus 8–10	5–7 versus 8–10
Pregnancy-associated plasma protein-A	NS	NS	*

*Values with $P<0.05$ are statistically significant. Apgar score 0–4: $n=6$, 5–7: $n=6$, 8–10: $n=35$. NS ($P>0.05$). NS: Not significant

Table 4: The relationship between Apgar score and some biochemical parameters of human immunodeficiency virus-seronegative pregnant subjects

Parameters	Apgar score-based groupings			*F*	*P*
	0–4 ($n=15$)	5–7 ($n=15$)	8–10 ($n=82$)		
Oestriol (ng/mL)	5.73±2.53	5.99±3.69	4.70±3.03	0.7234	0.4878
Progesterone (ng/mL)	54.68±5.86	59.61±5.52	55.42±8.04	1.342	0.2664
Pregnancy-associated plasma protein-A (μg/mL)	28.85±20.38	27.36±6.70	23.19±8.00	1.182	0.3114

Table 5: Comparison of some biochemical parameters in different trimesters of human immunodeficiency virus-seropositive pregnant subjects

Parameters	Trimester			*F*	*P*
	First trimester ($n=12$)	Second trimester ($n=44$)	Third trimester ($n=49$)		
Oestradiol (ng/mL)	0.35±0.49	3.63±2.88	6.51±3.71	21.85	<0.0001*
Progesterone (ng/mL)	37.34±15.33	61.01±17.82	65.61±17.10	13.02	<0.0001*
Pregnancy-associated plasma protein-A (μg/mL)	12.36±12.84	29.15±21.76	29.32±20.12	3.713	0.0278*

*Values with $P<0.05$ are statistically significant. Values are mean±SD. SD: Standard deviation

showed significant ($P < 0.05$) progressive increases from the first trimester to the third trimester (0.35 ± 0.49, 3.63 ± 2.88, and 6.51 ± 3.71, respectively). Progesterone and PAPP-A were significantly lower in the first trimester (37.34 ± 15.33 and 12.36 ± 12.84, respectively) compared with the second trimester (61.01 ± 17.82 and 29.15 ± 21.76, respectively). There were no significant differences in progesterone and PAPP-A levels between the second and third trimesters. There were no significant differences in all other parameters compared ($P > 0.05$).

Table 7 shows the comparison of biochemical parameters in different trimesters of pregnancy among HIV-seronegative pregnant subjects. *Post hoc* analysis, Table 8 shows that progesterone showed significant ($P < 0.05$) progressive increases from the first trimester to the third trimester (37.82 ± 4.95, 52.11 ± 7.20, and 56.54 ± 7.11, respectively). E3 was significantly higher in the third trimester (6.12 ± 3.10) compared with the first (0.35 ± 0.33) and second trimesters (2.73 ± 1.62). There was no significant difference in E3 level between the first and second trimesters.

The incidence rate of IUFD among HIV-seropositive pregnant subjects was 7.31%, while the incidence rate among HIV-seronegative pregnant subjects was 7.47%. The overall incidence rate among pregnant women was 7.42%.

Antenatal attendance by HIV-seropositive pregnant women constituted 25.39% while attendance by HIV-seronegative pregnant subjects constituted 74.61%.

Antenatal attendance in the first and second trimesters by HIV-seropositive pregnant women (11.5% and 41.9%, respectively) was greater than the attendance by HIV-seronegative pregnant subjects (4.14% and 33.79%, respectively). The trend changed in the third trimester with a decline in the attendance by HIV-seropositive pregnant women (6.86%) with respect to the attendance by HIV-seronegative pregnant subjects (62.07%).

Discussion

Biochemical investigation of pregnancy and antenatal care (ANC) is a useful practice toward improved pregnancy outcome, while additional attention is needed in subjects with HIV seropositivity.

Maternal serum progesterone level in this study was significantly higher ($P < 0.05$) in HIV-seropositive subjects (59.43 ± 17.84 ng/mL) compared to controls (54.89 ± 8.24 ng/mL). Progesterone is an essential hormone in the maintenance of pregnancy. Studies have demonstrated its beneficial roles in prevention of recurrent miscarriages, improved birth weight, and minimised delivery complications. Progesterone, cortisol, and prolactin have strong immunomodulatory effects leading to immunotolerance during pregnancy.[37] Progesterone has been shown to be efficacious when continuation of pregnancy is hampered

Table 6: *Post hoc* analysis of the comparison of some biochemical parameters in different trimesters of human immunodeficiency virus-seropositive pregnant subjects

Parameters	First trimester versus second trimester	First trimester versus third trimester	Second trimester versus third trimester
Pregnancy-associated plasma protein-A	*	*	NS
Oestriol	*	*	*
Progesterone	*	*	NS

*Values with $P<0.05$ are statistically significant. First trimester: $n=12$, second trimester: $n=44$, third trimester: $n=49$. NS ($P>0.05$). NS: Not significant

Table 7: Comparison of some biochemical parameters in different trimesters of human immunodeficiency virus-seronegative pregnant subjects

Parameters	Trimester			*F*	*P*
	First trimester ($n=6$)	Second trimester ($n=49$)	Third trimester ($n=90$)		
Oestriol (ng/mL)	0.35±0.33	2.73±1.62	6.12±3.10	35.07	<0.0001*
Progesterone (ng/mL)	37.82±4.95	52.11±7.20	56.54±7.11	23.13	<0.0001*
Pregnancy-associated plasma protein-A (µg/mL)	19.32±16.22	23.65±8.67	26.46±9.39	2.602	0.0777

*Values with $P<0.05$ are statistically significant. Values are mean±SD. SD: Standard deviation

Table 8: *Post hoc* analysis of the comparison of some biochemical parameters in different trimesters of human immunodeficiency virus-seronegative pregnant subjects

Parameters	First trimester versus second trimester	First trimester versus third trimester	Second trimester versus third trimester
Progesterone	*	*	*
Oestriol	NS	*	*

*Values with $P<0.05$ are statistically significant. First trimester: $n=6$, Second trimester: $n=49$, Third trimester: $n=90$. NS ($P>0.05$). NS: Not significant

by immunological factors, luteinic and neuroendocrine deficiencies, and myometrial hypercontractility.[38] These suggest that elevation in serum progesterone level in pregnancy is protective. Thus, the HIV-seropositive subjects receiving HAART may enjoy further protection from their elevated serum progesterone level. This may explain the similarity of pregnancy outcome such as incidence rate of IUFD observed in the two studied groups. Progesterone and PAPP-A were significantly lower in the first trimester (37.34 ± 15.33 and 12.36 ± 12.84, respectively) compared with second trimester (61.01 ± 17.82 and 29.15 ± 21.76, respectively). Progesterone increased significantly throughout pregnancy in HIV seronegatives, but there was no significant increase between its value in the second and third trimesters among HIV-seropositive individuals. The decline could possible increase the risk of bad pregnancy outcomes in this group, because a sustained progesterone level is required for proper maintenance of pregnancy.[38]

There was a significant positive correlation between PAPP-A and gestational age. HIV-seropositive subjects did not have a significant difference in their plasma level of PAPP-A compared to controls. This could possibly be the result of good ANC coupled with proper antiretroviral therapy. Protease inhibitors used in the treatment of HIV infection do not affect plasma levels of PAPP-A in HIV-seropositive pregnant women.[39] Thus, HIV-seropositive subjects receiving HAART and who do not have other complications of pregnancy will most likely have normal PAPP-A level. Sudden drop in plasma level of this parameter in such subject could be an indication of foetal abnormality. It was observed to be lower in HIV subjects with good Apgar score at delivery. PAPP-A plays an essential role in releasing insulin-like growth factor, thereby functioning as growth-promoting enzyme necessary in pregnancy. Inappropriate levels in certain stages of pregnancy could be deleterious.[40,41] PAPP-A usually rises through pregnancy. Consistent low levels, sudden rise, or decreases especially toward delivery may entail foetal distress. Higher PAPP-A levels observed in HIV-seropositive pregnancies with poor Apgar score in this study possibly suggest a higher risk of IUFD. Its evaluation near term may be beneficial in addition to plasma E3 and progesterone determination. It is also important to exclude other pathogenic causes of PAPP-A elevation. Elevation in PAPP-A could be in response to intrinsic foetal abnormality. PAPP-A elevation may be associated with pregnancy disorders. In pregnancy, melanoma migration evasiveness and progression are promoted by PAPP-A. PAPP-A is widely expressed by metastatic melanoma tumors and is elevated in melanoma cells exhibiting mesenchymal, invasive, and label-retaining phenotypes.[42]

There was no significant difference in level of E3 between HIV-seropositive subjects and HIV-seronegative subjects. An earlier study among HIV-seropositive women in their first and second trimesters did not show any statistical difference in serum E3 level compared to normal. Although serum E3 along with beta-human chorionic gonadotropin, alpha-fetoprotein, and PAPP-A can be utilised in the diagnosis of foetal abnormality in HIV-seropositive women, it has been shown that protease inhibitors used in the treatment of HIV infection do not affect plasma levels of E3 in HIV-seropositive pregnant women.[39,43] The findings on E3 level in this present study further support proper antiretroviral therapy in pregnancy as a way of assuring good pregnancy outcome. In this study, estradiol, like progesterone, showed significant progressive increases throughout the trimesters.

E3 levels were significantly lower in subjects with IUFD compared to subjects that had live birth. Decreased second trimester E3 has been shown to be a marker for Down's and trisomy-18 syndromes. It is also low in cases of gross neural tube defects such as anencephaly. Based on this observation, low levels of E3 have been associated with pregnancy loss. High levels of E3, or sudden increases in maternal E3 levels, are markers of pending labor. Sudden variation may be predictive of pregnancy outcome.

E3 increased steadily from the early weeks of gestation till term in good pregnancy outcome (live birth). The level of increases was, however, higher throughout the gestational period in HIV-seronegative subjects than in HIV seropositives. Toward term (at 32–34 weeks) and at 40 weeks, there were drops in E3 levels in HIV-seropositive subjects relative to HIV-seronegative pregnant subjects. Although this late variation was not sufficient to cause significant difference in pregnancy outcome, it could imply that HIV-seropositive subjects may have a higher risk of adverse pregnancy outcome toward term than their seronegative counterparts.

In this study, the pattern of antenatal attendance increased steadily all through the different trimesters for both HIV-seropositive and HIV-seronegative subjects. The antenatal attendance in the first trimester by HIV-seropositive subjects (11.5%) was, however, higher compared to antenatal attendance in the first trimester by HIV-seronegative subjects (4.14%). Gill *et al.* reported similar earlier antenatal visits by HIV-infected subjects.[44] This could imply a perceived awareness of the benefits of early antenatal booking by the known HIV subjects who took advantage of the PMTCT scheme. This should be further encouraged. Although an earlier study showed that the timing of a woman's ANC visit may not be an important determinant of incidence of stillbirths in isolation, this study further suggest the possible role of effective biomedical evaluations and interventions,such as the PMTCT scheme, as important factors influencing outcomes in this setting.[45]

The comparison of pattern of antenatal attendance in different trimesters of HIV-seropositive and HIV-seronegative pregnant subjects at NAUTH, Nnewi, between June 2010 and December 2013 shows disparity in antenatal attendance. Antenatal attendance in the first and second trimesters by HIV-seropositive pregnant women (11.5% and 41.9%, respectively) was greater than the attendance by HIV-seronegative pregnant subjects (4.14% and 33.79%, respectively). The trend changed

in the third trimester with a decline in the attendance by HIV-seropositive pregnant women (6.86%) with respect to the attendance by HIV-seronegative pregnant subjects (62.07%). This may imply that the HIV-seropositive subjects tend to withdraw from ANC in tertiary health centres toward term, with the likelihood of patronising local birth attendants for deliveries. This practice may negatively affect pregnancy outcomes, as well as increase HIV transmission rate from mother to child and to the care provider.

Late antenatal booking has been widely reported in Nigeria and other African countries.[46,47] This relates directly with bad pregnancy outcome. Some suggest that most women book late because of a belief that there are no advantages in booking for ANC in the first three months of pregnancy. This seems to be because ANC is viewed primarily as curative rather than preventive in the studied population.[48] Research is needed to determine the best approaches for health education programs to correct the misconceptions about ANC. However, efforts toward maternal education, public health enlightenment campaigns, poverty reduction, and use of focused ANC model should be sustained as measures to encourage early initiation of ANC. Research has indicated that antenatal education for expectant mothers results in sustained improvement in knowledge of newborn care.[49] Another study done in Enugu, Nigeria, showed that the prevalence of anemia in pregnancy at booking was high (40.4%) and recommended that early antenatal booking and improved ANC are necessary for early diagnosis and treatment of the condition.[50] These studies emphasised the importance of early ANC in ensuring good pregnancy outcome.

This study showed that the incidence rate of IUFD among HIV-seropositive pregnant subjects was 7.31%, the rate among HIV-seronegative pregnant subjects was 7.47%, while the overall incidence rate among pregnant women at the centre was 7.42%. Higher rates have been reported in different centres in Nigeria. These findings still put Nigeria among the leading countries with high rates of IUFD worldwide. Intrauterine foetal death occurs in 2% of the pregnancies worldwide and in about 0.5% of pregnancies in France.[51]

A study identified history of previous pregnancy loss, consanguinity, and viral infections as risk factors for IUFD.[52] Maternal haemoglobin concentration, serum creatinine levels, and blood sugar were also found to be important factors in IUFD, while as these factors were not considered in this study, their monitoring during ANC in developing countries like Nigeria may enhance pregnancy outcome.

There is no significant difference in the incidence rate of IUFD between HIV-infected pregnant women and the noninfected. This underscores the relevance of quality health-care delivery, especially to patients who may have disorders such as HIV infection. The HIV-infected subjects recruited for this study were undergoing the PMTCT of HIV infection scheme of the hospital. The PMTCT scheme is an intervention to ensure that no child is born with HIV and it is an essential step to ensuring an AIDS-free generation. The PMTCT initiative provides drugs, counselling, and psychological support to help mothers safeguard their infants against the virus. Pregnant women infected with HIV are at high risk of transmitting HIV to their infants during pregnancy, during birth, or through breastfeeding. Over 90% of new HIV infections among infants and young children occur through mother-to-child transmission. Without any intervention, the risk of transmission of infection from the mother to the baby is 20%–45%. With a comprehensive intervention, this transmission rate can be reduced to <2%. Intervention applied in PMTCT invariably enhances the general health of the mother, as is evident in this study.

Conclusion

This study shows variations in the biochemical parameters of pregnant women. Progesterone was significantly higher in HIV-seropositive subjects than in HIV-seronegative subjects. E3 increased progressively all through pregnancy. Lower levels were observed toward term in IUFD. Drop in level of E3 could be an early indicator of abnormality. There were no significant differences in the other biochemical parameters tested between these two groups. This could indicate the efficiency of the PMTCT scheme. Pregnancy outcome assessed by the incidence rate of IUFD between the HIV-seropositive pregnant women receiving HAART compared to HIV-seronegative pregnant women showed no significant difference. The incidence rate of IUFD in the studied population is high compared to findings in other countries. Biochemical monitoring of pregnancy will help reduce the incidence of bad pregnancy outcome. Regular serum progesterone, E3, and PAPP-A determinations could be good indices of assessment of foetal distress. However, studies involving more biochemical parameters covering a longer duration may be more beneficial. There is a need to establish modern prenatal and postnatal screening centres as these are unavailable in the country at present. Appropriate screening is useful in prevention and early diagnosis of congenital abnormalities. Record keeping is poor in our centre. The improvement of this will make prospective and retrospective studies easier and more meaningful.

Author contribution

OJE, AJE, and OCC conceived and designed the research proposal. UGO and IJI reviewed participants. OJA and OJM reviewed methodology. OJE, OEC, and ONA performed sample collection, experiments, and data analysis. All authors contributed to the final version of the manuscript. All authors have read and approved the final manuscript.

Data availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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Appendix

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 pregnancy in Nnewi – Eastern Nigeria.

1. Hospital No:……Date……..
2. Age:……..
3. Height:……..
4. Weight:……..
5. LMP:……
6. Gestational Age……..(weeks)
7. BP:……
8. Race (A) African (B) White (C) Others……..
9. Ethnicity (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 Impairment (D) Heart Disorder (E) Others……..
12. Presence of some pregnancy-related problems (A) Anemia (B) GDM (C) Preeclampsia (D) Others……..
13. Alcohol intake (A) Yes (B) No
14. Frequency of alcohol intake (A) Occasionally (B) Regularly
15. Cigarette smoking (A) Yes (B) No
16. Frequency of cigarette smoking (a) Occasionally (b) Regularly
17. Tobacco (Snuff) Usage (A) Yes (B) No
18. Frequency of snuff usage (A) Occasionally (B) Regularly
19. How old is your last child?.
20. How many children have you?.
21. Did you suffer from 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 to hospital? (a) yes (b) no
33. If yes, for how long?