Asian Journal of
Biological
Sciences

OPEN ACCESS

Assessment of Total Protein, Calcium, Albumin and Glucose in Pregnant Women in Southern Nigeria

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ABSTRACT

Background and Objective: Pregnant women are particularly susceptible to various infections. The evaluation of some micronutrients in pregnant women is of major importance in monitoring the successful outcome of pregnancy. This study aimed to assess some micronutrients (total protein, calcium, albumin and glucose) in pregnant subjects attending Federal Medical Center, Asaba, Delta State. **Materials and Methods:** A total of 160 samples were recruited for this study including 120 pregnant women, 40 for each trimester and 40 non-pregnant women which serve as control. Calcium, total protein, albumin, globulin and glucose were determined. The mean and standard deviation of the results obtained were calculated. Values with $p<0.05$ shall be considered statistically significant in this study. **Results:** Albumin and glucose levels were significantly lower ($p<0.05$) in the pregnant subjects compared to control. Calcium levels were significantly higher ($p<0.05$) in the pregnant subjects compared with the control. Total protein levels were significantly lower ($p<0.05$) in 2nd trimesters compared with control, 1st and 3rd trimesters. Albumin and calcium levels were significantly lower ($p<0.05$) in 3rd trimesters compared with control, 1st and 2nd trimesters. Glucose levels were significantly higher ($p<0.05$) in control compared with 1st, 2nd and 3rd trimesters. **Conclusion:** To reduce the likelihood of micronutrient deficiencies in the mother and the foetus, it is preferable to eat a sufficient amount of these nutrients prior to conception. Additionally, eating it during pregnancy to ensure that the mother and foetus are getting enough vitamins.

KEYWORDS

Maternal health, protein-energy malnutrition, fetal development, biochemical markers, nutritional status, antenatal care, pregnancy complications, reproductive health

ISSN: 1996-3351 (Print)
https://doi.org/10.3923/ajbs.2024.657.665

Received: 22 Jul. 2024
Accepted: 04 Sep. 2024
Published: 31 Dec. 2024

INTRODUCTION

A foetus or embryo is an offspring that develops and becomes fertilized in a woman's uterus during pregnancy[1]. Usually, a pregnancy is broken up into three roughly three-month-long phases, or trimesters. The first trimester of pregnancy is defined as the first 12 weeks of pregnancy. The second trimester of pregnancy is defined as weeks 13 through 28. Pregnancy's 3rd trimester begins at week 28 and ends with delivery. The term refers to the time between 37 and 42 weeks when regular labour, regular uterine contractions and then birth takes place. Normal physiological changes that support the foetus's survival and upbringing are linked to pregnancy[2]. To support these processes and prevent potentially unfavourable maternal and pregnancy outcomes, adequate pregestational nutritional status, as well as appropriate gestational weight gain and dietary intakes, are essential[1,2]. For this reason, it's critical to assess, track and, if necessary, adjust maternal nutritional status before and during pregnancy. Additionally, high and low food intakes have been linked to long-term consequences and noncommunicable diseases in the progeny (Barker Hypothesis, a developmental paradigm for the origins of disease). According to Sibiak *et al.*[3], in particular, foetal growth under obesogenic intrauterine conditions can permanently affect a person's biological and metabolic pathways, resulting in adaptive pathophysiological changes in the child and a higher risk of non-communicable diseases in adulthood.

The majority of organ systems' adaptation changes are reflected in biochemical parameters, which are obviously different from the non-pregnant condition[4]. But in the event of difficulties, these adjustments become crucial. Micronutrients are vitamins and trace elements that come from our food and are necessary for healthy physiological function and the maintenance of life[5]. More than 2 billion individuals suffer from deficiencies, which are mostly brought on by starvation or a poor diet[5]. In addition to being required for the successful elicitation of an immune response against viral infections, certain micronutrients are also used by viruses, including hepatitis B and hepatitis C viruses, to spread their infections[6]. Essential micronutrients have a key role in a variety of hepatic metabolic pathways, including protein synthesis and enzymatic activities, immunological competence, the regulation of the response to interferon therapy and changes in viral genomes[7].

In contrast to iron-folic acid supplementation alone, the World Health Organization (WHO) currently advises giving multiple micronutrient supplements to expectant mothers from populations with a high prevalence of maternal nutritional deficiencies. This will lower the risks of low birth weight (LBW) and small for gestational age (SGA)[8]. On the other hand, contradictory findings cast doubt on the effectiveness of regular multivitamin supplementation among well-nourished women living in wealthy nations[9].

Micronutrient requirements rise higher during pregnancy than macronutrient requirements do and low intakes (and, consequently, a diet lacking in nutritional quality) can have serious repercussions for the developing baby as well as the pregnant mother. Specifically, data supports the physiological function of certain vitamins and minerals[10]. A recent meta-analysis also demonstrated improved survival and outcomes in cases of female newborns and undernourished/anemic pregnant women, demonstrating that foetal gender, pregestational maternal nutritional status and adherence to supplementation represent significant factors influencing the effect of multivitamin supplementation on pregnancy outcomes[11]. Therefore, the purpose of this study is to assess a few micronutrients in pregnant patients who visit the Federal Medical Center in Asaba, Delta State.

MATERIALS AND METHODS

Area of study: Pregnant patients at the Federal Medical Center in Asaba, Delta State, Nigeria, were the subjects of this study. The geographic coordinates of Asaba, the capital of Delta State, Nigeria, are 6°11'52.23°N, 6°43'42.48°E. It is perched above the place where the Anambra River empties into the lower Niger River on a terrace. Secondary forest vegetation thrives on the high plains that stretch much farther than the river basins, beyond the riverbanks. The ancient Niger River connects West Africa to the Atlantic Ocean, forming a trans-African waterway. Through, the Niger River in the North and the Asaba Niger

Bridge, an iconic east-west link, Asaba serves as a link between Western, Eastern and Northern Nigeria. The analysis was done in the Federal Medical Center's Chemical Pathology section in Asaba, Delta State.

Population of the study: Pregnant patients at Federal Medical Center in Asaba, Delta State, are the study's subjects. For this study, total of 160 samples were gathered: 40 for each trimester of pregnancy, 40 non-pregnant women who served as controls and 120 pregnant women. Names, ages and the number of months the subject was pregnant were also collected. Using the formula below[12], the sample size (N) is determined:

$$\text{Samples size (N)} = \frac{Z^2Pq.}{d^2}$$

Where:

N = Desired size
Z = 1.96 (standard score)
P = Prevalence (12%) (0.12)
q. = 1-P (0.88)
d = Sample error tolerated (0.05)

$$N = \frac{1.96^2 \times 0.12 \times 0.88}{0.05^2} = 162.2 \text{ approximately} = 160$$

Research design: In order to evaluate certain micronutrients (total protein, calcium, albumin and glucose) in pregnant patients attending Federal Medical Center, Asaba, Delta State and compare the results with those of the control group, this study was planned as a prospective, cross-sectional study. This study was conducted from May, 2022 to August, 2022. Every participant's full medical history, including name, age and pregnancy duration, was collected. Appropriate statistical techniques were used to compare the overall study outcomes with the control.

Ethical considerations: The Federal Medical Center's Health Research Ethics Committee in Asaba, Delta State, Nigeria, provided ethical approval for this study. Prior to the patients' samples being collected for this investigation, their informed consent was also requested and acquired. The patients were fully informed about the aim of the study and given assurances regarding the privacy of the data collected from them.

Sample collection: The 5 mL of blood were drawn by venepuncture and placed into appropriately labelled lithium heparin vials for both the experimental subjects and the control group. Within 2 hrs after collection, the blood samples were spun in a laboratory centrifuge set at 4000 rpm for 10 min at room temperature. The serum was then divided into sterile, simple containers labelled with the same names as the original blood sample containers. After that, the serum was kept in storage at -20°C until the samples were analyzed.

Sample analysis

Serum calcium estimation principle: In an alkaline solution, calcium ions combine with O-cresophthalene-complexono to generate a complex with a purple color. The complex's absorbance is directly correlated with the sample's calcium content.

Estimation of total protein principle: The biuret reaction creates a violet-colored complex whose absorbance is evaluated colorimetrically by creating a chelate between the peptide bonds of the protein in alkaline solution and the CU^{2+}. The amount of protein present in the sample is reflected in the colour's intensity.

Estimation of albumin principle: This technique is based on the particular binding of the protein at an acid PH to the anion dye bromocresol green (BCG), which causes a shift in the complex's absorption wavelength. The amount of albumin in the sample directly correlates with the color's intensity.

Estimation of globulin:

$$\text{Plasma globulin}\left(g/dL\right)=\frac{\text{Total protein}\left(g/dL\right)}{\text{Plasma albumin}\left(g/dL\right)}$$[13]

Determination of glucose principle: The oxidation of glucose to produce hydrogen peroxide (H_2O_2) and gluconic acid is catalyzed by glucose oxidase (GOD). When peroxidase (POD) is present, hydrogen peroxide is broken down and the oxygen that is released combines with phenol and 4-aminophenazone (4-aminoantipyrine) to produce a pink colour. The colour produced is measured for absorbance.

Statistical analysis: The collected results' mean and standard deviation were computed. The study was conducted using the SPSS software version 21 and an ANOVA (LSD). In this investigation, values having a p-value of less than 0.05 will be deemed statistically significant.

RESULTS

The results of this study are presented and comparisons were made between the control and micronutrients of pregnant subjects according to their trimesters. Socio-demographic profiles and the history of the subjects were also presented.

Table 1 revealed the socio-demographic characteristics of the study population. The subjects were categorized into four age groups; <21-25; 26-30; 31-35 and >35. The result for age showed that the

Table 1: Socio-demographic characteristics of the study population

Variable	Frequency	Percentage
Age (Years)		
<21-25	35	29.2
26-30	38	31.7
31-35	29	24.1
>35	18	15.0
Total	120	100
Religion		
Christian	117	97.5
Muslim	3	2.5
Total	120	100
Tribe		
Igbo	81	67.5
Urhobo	5	4.2
Hausa	2	1.7
Others	32	26.7
Total	120	100
Occupation		
Traders	74	61.7
Teachers	18	15.0
Civil servants	4	3.3
Others	24	20.0
Total	120	100
Trimesters		
1st	40	33.3
2nd	40	33.3
3rd	40	33.3
Total	120	100
Age (Mean±SD)	28.99±5.96	

Table 2: Comparison of parameters between the subjects and the control

Parameter	Control (n=40)	Subjects (n=120)	t-value	p-value
TP (g/dL)	68.95±25.61	63.70±14.29	1.618	0.108
ALB (g/dL)	40.05±14.56	35.00±10.80	2.336	0.021
CAL (mmol/L)	1.82±0.33	3.01±2.05	3.661	0.000
GLU (mg/dL)	6.16±4.25	4.12±1.40	4.597	0.000

n: Sample size, p>0.05: Not significant, p<0.05: Significant, TP: Total protein, ALB: Albumin, CAL: Calcium, GLU: Glucose, values in a row with different superscripts is significantly different at p<0.05

Table 3: Comparison of parameters between control and subjects according to their various trimesters

Parameter	Control (n = 40)	1st trimesters (n = 40)	2nd trimesters (n = 40)	3rd trimesters (n = 40)	F-value	p-value
TP (g/dL)	68.95±25.61[a]	72.43±18.63[a]	57.95±7.95[b]	60.73±9.90[b]	6.388	0.000
ALB (g/dL)	40.05±14.56[a]	45.75±9.54[b]	30.13±7.33[ab]	29.13±5.72[ab]	26.420	0.000
CAL (mmol/L)	1.82±0.33[a]	1.62±0.38[a]	2.87±1.39[b]	4.54±2.52[ab]	33.612	0.000
GLU (mg/dL)	6.16±4.25[a]	4.41±2.03[b]	4.41±1.03[b]	3.77±0.77[b]	7.475	0.000

n: Sample size, p>0.05: Not significant, p<0.05: Significant, TP: Total protein, ALB: Albumin, CAL: Calcium and GLU: Glucose, values in a row with different superscripts is significantly different at p<0.05

majority of the subjects were within the age range of 26-30 years; 38 (31.7%), this was followed by <21-25 years, 35 (29.2%), 31-35 years; 29 (24.1%) and >35 years; 18 (15.0%). Regarding religion, the results also showed that 117 (97.5%) of the subjects were Christian and 3 (2.5%) were Muslim. As regards tribe, the result showed that the majority of the subjects were from Igbo; 81 (67.5%), this was followed by subjects who were from other tribes; 32 (26.7%), Urhobo; 5 (4.2%) and Hausa, 2 (1.7%). As regards occupation, the result showed that the majority of the subjects were traders; 74 (61.7%), this was followed by others; 24(20.0%), Teachers; 18 (15.0%) and Civil servants, 4 (3.3%). As regards trimesters, the frequency of the subjects was equal among all the trimesters; 40 (33.3%). The age (Mean±SD) was (28.99±5.96).

The results in Table 2 showed the comparison of TP, ALB, CAL and GLU levels between the subjects and the control. The results showed that TP levels were not significantly lower (p>0.05) in the subjects (63.70±14.29 g/dL) when compared with the control (68.95±25.61 g/dL). On the contrary, ALB levels were significantly lower (p<0.05) in the subjects (35.00±10.80 g/dL) when compared with the control (40.05±14.56 g/dL). The CAL levels were also significantly higher (p<0.05) in the subjects (3.01±2.05 mmol/L) when compared with the control (1.82±0.33 mmol/L). Furthermore, glucose levels were significantly lower (p<0.05) in the subjects (4.12±1.40 mg/dL) when compared with the control (6.16±4.25 mg/dL).

The results in Table 3 showed the comparison of TP, ALB, CAL and GLU levels between the controls and the various trimesters of the subjects. The results showed that TP levels were significantly lower (p<0.05) in 2nd trimesters (57.95±7.95 g/dL) when compared with 3rd trimesters (60.73±9.90 g/dL), control (68.95±25.61 g/dL) and 1st trimester (72.43±18.63 g/dL). The ALB levels were significantly lower (p<0.05) in 3rd trimesters (29.13±5.72 g/dL) when compared with 2nd trimesters (30.13±7.33 g/dL), control (40.05±14.56 g/dL) and 1st trimester (45.75±9.54 g/dL). Furthermore, CAL levels were significantly higher (p<0.05) in 3rd trimesters (4.54±2.52 mmol/L) when compared with 2nd trimesters (2.87±1.39 mmol/L), control (1.82±0.33 mmol/L) and 1st trimester (1.62±0.38 mmol/L). The GLU levels were significantly higher (p<0.05) in control (6.16±4.25 mg/dL) when compared with 1st trimesters (4.41±2.03 mg/dL), 2nd trimesters (4.41±1.03 mg/dL) and 3rd trimester (3.77±0.77 mg/dL).

The results in Table 4 showed the comparison of TP, ALB, CAL and GLU levels of the subjects according to trimesters. The results showed that TP levels were significantly lower (p<0.05) in subjects in 2nd trimesters (57.95±7.95 g/dL) when compared to subjects in 3rd trimesters (60.73±9.90 g/dL) and 1st trimesters (72.43±18.63 g/dL). The ALB levels were significantly lower (p<0.05) in subjects in 3rd trimesters (29.13±5.72 g/dL) when compared to subjects in 2nd trimesters (30.13±7.33 g/dL) and 1st trimesters (45.75±9.54 g/dL). The CAL levels were significantly higher (p<0.05) in subjects in 3rd trimesters (4.54±2.52 mmol/L) when compared to subjects in 2nd trimesters (2.87±1.39 mmol/L)

Table 4: Comparison of parameters of subjects according to trimesters

Parameter	1st trimesters (n = 40)	2nd trimesters (n = 40)	3rd trimesters (n = 40)	F-value	p-value
TP (g/dL)	72.43±18.63^{a}	57.95±7.95^{b}	60.73±9.90^{b}	14.103	0.000
ALB (g/dL)	45.75±9.54^{a}	30.13±7.33^{b}	29.13±5.72^{b}	58.783	0.000
CAL (mmol/l)	1.62±0.38^{a}	2.87±1.39^{b}	4.54±2.52^{ab}	30.682	0.000
GLU (mg/dL)	4.41±2.03^{a}	4.41±1.03^{a}	3.77±0.77^{ab}	2.123	0.124

n: Sample size, p>0.05: Not significant, p<0.05: Significant, values in a row with different superscripts is significantly different at p<0.05

Table 5: Comparison of TP, ALB, CAL and GLU Levels of the subjects according to age

Parameter	<21-25 years (n = 35)	26-30 years (n = 38)	31-35 years (n = 29)	>35 years (n = 18)	F-value	p-value
TP (g/dL)	65.37±13.64^{a}	64.03±15.32^{a}	63.55±14.78^{a}	60.00±12.90^{a}	0.563	0.640
ALB (g/dL)	33.06±10.85^{a}	36.66±10.53^{a}	35.03±11.32^{a}	35.22±10.69^{a}	0.673	0.570
CAL (mmol/L)	3.37±2.33^{a}	2.59±1.82^{a}	2.99±1.99^{a}	2.23±2.04^{a}	0.955	0.416
GLU (mg/dL)	4.09±1.11^{a}	4.19±1.51^{a}	4.33±1.78^{a}	3.67±0.95^{a}	0.867	0.460

n: Sample size, p>0.05: Not significant, p<0.05: Significant, TP: Total protein, ALB: Albumin, CAL: Calcium and GLU: Glucose, values in a row with different superscripts is significantly different at p<0.05

and 1st trimesters (1.62±0.38 mmol/L). On the contrary, GLU levels were not significantly higher (p>0.05) in subjects in 1st trimesters (4.41±2.03 mg/dL) when compared with subjects in 2nd trimesters (4.41±1.03 mg/dL) and 3rd trimesters (3.77±0.77 mg/dL).

The results in Table 5 showed the comparison of TP, ALB, CAL and GLU of subjects according to age. The results showed that TP levels were not significantly higher (p>0.05) in within the age range of <21-25 years (65.37±13.64 g/dL) when compared subjects within the age range of 26-30 years (64.03±15.32 g/dL), 31-35 years (63.55±14.78 g/dL) and >35 (60.00±12.90 g/dL). The ALB levels were not significantly lower (p>0.05) within the age range of 26-30 years (36.66±10.53 g/dL) when compared with subjects within the age range of >35 years (35.22±10.69 g/dL), 31-35 years (35.03±11.32 g/dL) and <21-25 years (33.06±10.85 g/dL). Furthermore, CAL levels were not significantly higher (p>0.05) in subjects within the age range of <21-25 years (3.37±2.33 mmol/L) when compared with subjects within the age range of 31-35 years (2.99±1.99 mmol/L), 26-30 years (2.59±1.82 mmol/L) and >35 years (2.23±2.04 mmol/L). The GLU levels were not significantly higher (p>0.05) in subjects within the age range of 31-35 years (4.33±1.78 mg/dL) when compared with subjects within the age range of 26-30 years (4.19±1.51 mg/dL), <21-25 years (4.09±1.11 mg/dL) and >35 years (3.67±0.95 mg/dL).

DISCUSSION

Maternal metabolism must undergo significant alterations during pregnancy in order to meet the increasing demands of the intended pregnancy result. All nutrients are metabolized differently as a result of ongoing physiological modifications. The modifications differ based on the mother's lifestyle, genetic predispositions for foetal size and the women's prenatal nutrition. For every vitamin, there are thresholds in the ability to modify metabolism based on the amount supplied[2]. Pregnancy usually causes a drop in many parameters due to increased demands and haemodilution.

The study's findings indicated that, although not statistically substantial, the pregnant subjects' TP levels were lower (p>0.05) than those of the control group. In the second trimester, TP levels were considerably lower (p<0.05) than in the third, control and first trimesters. The findings of other investigations[14-16] are consistent with this one. The hemodilution phenomenon may account for the drop in serum protein profile concentration[17]. According to Wiles *et al.*[15], a possible cause of this decline could be the gradual increase in glomerular permeability to albumin that occurs during pregnancy.

Compared to the control group, the pregnant subjects' ALB levels were considerably lower (p<0.05). When comparing the third trimester to the control, first and second trimesters, the ALB levels were considerably

($p<0.05$) lower in the third trimester. The main protein in plasma, albumin, has been demonstrated to be a favorable target for oxidation in neonatal plasma and is recognized as a significant extracellular antioxidant[18]. Lower albumin levels may result in a decreased antioxidant capacity, which raises the risk of premature births.

A developing foetus needs calcium for development. The participants' calcium levels were significantly greater ($p<0.05$) than the control group's. Third-trimester calcium levels were substantially ($p<0.05$) greater than those of second trimester, control and first trimester. Pre-eclampsia, LBW and preterm birth are all decreased with calcium supplementation[19]. According to Phillips *et al.*[20], gestational hypertensive disorders are linked to a higher risk of preterm birth and fetal development restriction, making them the second leading cause of morbidity and mortality among mothers.

Based on the findings of Zgliczynska and Kosinska-Kaczynska[21], calcium supplementation during pregnancy lowers the risk of gestational hypertension by 35%, pre-eclampsia by 52-55% and preterm births by 24%. Therefore, the World Health Organization (WHO) now advises pregnant women with low dietary calcium intakes to take 1.5 to 2.0 g of elemental calcium per day. Regardless of the mother's age and invariably unchanged from the non-pregnant state, the recommended dietary allowance (RDA) for elemental calcium in pregnant and breastfeeding women is 1000 mg/day[22]. Even in affluent nations, pregnant women frequently consume less calcium than is advised; in the United States, this is estimated to affect 24% of pregnant women, while in some communities in northern Europe, it can account for over 30% of cases. Consuming calcium is crucial for the development of the foetal skeleton, especially during the 3rd trimester. Additionally, supplementation is a potentially effective intervention for preventing pre-eclampsia in females who are high-risk for hypertensive diseases and have poor baseline dietary calcium consumption. This resulted in the WHO recommending that pregnant women who are at risk start taking 1.5-2.0 g of elemental calcium per day starting at 20 weeks of gestation[23].

When comparing the glucose levels of the pregnant women to the control group, there was a significant difference ($p<0.05$). The control group had considerably higher ($p<0.05$) glucose levels than the first, second and third trimesters. Afolayan and Tella[24] achieved a similar outcome. This might be the result of metabolic processes, whereby the foetus's increased demand led to an increase in glucose metabolism. Because the foetus's energy needs are nearly entirely satisfied by the mother's glucose, the serum glucose level drops by roughly 15-20% during pregnancy[25]. Furthermore, as suggested by Riskin-Mashiah *et al.*[26], women with low fasting blood sugar levels may be in the early stages of the first or second trimester. Foetal growth gradually peaks at the start of the third trimester, which causes a corresponding drop in the plasma concentration of the hormones that both the mother and the foetus produce and control glucose mobilization.

CONCLUSION

This study demonstrated the effects of pregnancy on serum glucose, albumin and calcium levels. In particular, level of calcium and glucose was considerably lower in pregnant women's than non-pregnant women. In addition, compared to women who are not pregnant, total protein and albumin levels were lower in pregnant women. Haemodilution and higher requirements were the causes of the differences in these values. It is now established that many women of reproductive age experience micronutrient deficiencies, which are linked to unfavorable outcomes for both mothers and babies. These unfavorable consequences may persist until maturity. To reduce the likelihood of micronutrient deficiencies in the mother and the foetus, it is preferable to eat a sufficient amount of these nutrients prior to conception. Based on the results of this study, it is hereby recommended that regular monitoring of glucose, calcium, albumin and total protein should be done on pregnancy to prevent any underlying onset of neonatal and maternal diseases.

SIGNIFICANCE STATEMENT

The purpose of this study is to assess some micronutrients (total protein, calcium, albumin and glucose) in pregnant patients who visit the Federal Medical Center in Asaba, Delta State. The study aimed to evaluate the levels of some micronutrients in pregnant women (according to trimesters) and the control subjects and to compare the micronutrients of the pregnant women according to age. The regular monitoring of glucose, calcium, albumin and total protein should be done during pregnancy to prevent any underlying onset of neonatal and maternal diseases.

ACKNOWLEDGEMENTS

The Federal Medical Centre's management in Asaba, Delta State, Nigeria, is to be thanked by the authors for establishing a conducive atmosphere for this research. We are grateful to the entire laboratory and technical personnel of St. Kenny Research Consult, located in Ekpoma, Edo State, Nigeria, for their outstanding support and for helping with medical writing and editing in compliance with GPP3 requirements.

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