# Serum protein profile in Nigerian women: an analysis by gestation age

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**Abstract:** The effects of pregnancy and advancing gestation on serum proteins pattern of Nigerian women, living in Ikirun, south western Nigeria were examined in sixty-six (66) apparently normal pregnant women. Twenty-five (25) apparently normal non-pregnant (age matched) women were also concurrently studied as controls. The mean total serum proteins and serum albumin concentration of the controls were 70.5 ± 3.3 mg/ml and 40.8 ± 4.0 mg/ml; respectively. Total serum proteins and serum albumin concentrations decreased significantly and progressively as gestation advanced to the lowest level of 58.2 ± 2.7 mg/ml and 29.2 ± 3.7 mg/ml ($p < 0.001$); respectively at 33-36$^{th}$ week of gestation. The total serum globulins concentration remained statistically unchanged throughout the gestation period but for a significant increase to a level of 32.1 ± 1.6 mg/ml ($p = 0.025$) at 21-24 weeks of gestation. Serum protein electrophoresis pattern showed no significant abnormality but for an elevated $\alpha_2$-globulin band at 21-24$^{th}$ week of gestation and did not closely reflect changes observed in the absolute concentrations of serum albumin. The relatively stable total serum globulins (TSG) concentration in the face of normal gestational haemodilution suggested increased TSG mass during normal pregnancy.
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## 1.0 Introduction

Gestation is a period between conception and delivery and has been associated with increased dietary protein requirements in humans. During their period of rapid growth, the foetus and placenta accrue proteins very rapidly (Lewis *et al*., 2010). Protein and vitamin deficiencies are common features in pregnant women belonging to the lower socioeconomic groups, thus affecting serum protein concentrations (Bhatty *et al*., 2001). Protein status is usually assessed by measuring levels of total serum proteins, albumin, or plasma non-essential and essential amino acid ratio (Varma and Yue, 1984). A direct relationship of quality and quantity of dietary proteins with decrease in plasma proteins in cases of protein malnutrition has been reported and maternal malnutrition may be aggravated by pregnancy (Opara *et al*., 2011).

Variations in the level of serum proteins occur between racial groups and under physiological and pathological conditions (Ekeke *et al*., 1983; Cohen *et al*., 1998; Adedeji *et al*., 2004). Changes in serum proteins concentration during pregnancy have been studied by a number of investigators using a variety of techniques. The patterns so far reported are not in complete agreement with each other (Bacq *et al*., 1996; Tantengco *et al*., 1973, Payne *et al*., 1990, Kapsenberg *et al*., 2004 and Ihara *et al*., 2005). We here report the changes in serum protein pattern consequent to advancing gestation in a cross-section of Nigerian women living in Ikirun metropolis, south western Nigeria.

## 2.0 Material and Methods

### 2.1 Selection of Subjects

The population studied consisted of subjects attending the antenatal clinic of the State Hospital, Ikirun, Western Nigeria. The clinic usually refers antenatal subjects for routine blood test, thus providing opportunity for subject selection and sample collection at Bolab Medical Laboratories, Ikirun. Sixty-six (66) apparently normal pregnant women referred for routine laboratory tests were recruited for the study. Subjects were grouped according to gestational age in weeks. The gestational age was estimated from the date of the last menstrual period. Twenty-five (25) normal non-pregnant women (age matched) were selected randomly within the town. Informed consent was obtained from all subjects. Blood samples were drawn by venipuncture and after clotting and retraction at room temperature for 1 hour; serum was separated by centrifugation at 3000 rpm for 10 minutes and immediately stored at -20 $^{O}$C until they were analyzed.

### 2.2 Serum proteins analysis

Total serum proteins (TSP) concentration was determined by the modified Biuret method (Weichselbunm, 1946) and serum albumin (SA) concentration was determined by method of Doumas *et al*. (1971). Total serum globulin concentration was determined by calculating the difference between TSP and SA as reported elsewhere.

### 2.3 Electrophoresis

Cellulose acetate protein electrophoresis was also performed on serum samples using Helena Electrophoresis system, according to manufacturer's instructions (Beaumont, TX; Sebia, Norcross, GA, USA). We used biosystem cellulose acetate membranes pre equilibrated in diethylbarbital buffer pH 8.6. The procedure was carried out on approximately 1µl of each serum using the buffer at 225V (20V/cm) for 25 minutes. The separated fractions were fixed in 5% acetic acid and visualized with Ponceau S stain.

### 2.4 Statistical Analysis

Descriptive analysis, and student t-test were used for the comparisons of data. using Graphpad 5 software (San Diego, CA). p-values $< 0.05$ were considered significant.

### 3.0 Results

The concentrations of total serum protein (TSP), serum albumin (SA) and total serum globulins (TSG) of pregnant women compared with that of apparently normal non-pregnant women is presented in figures 1. TSP and SA concentrations were significantly reduced during normal pregnancy ($p < 0.001$) while concentration of TSG was not significantly different ($p=0.443$). The mean TSP concentration in non-pregnant women was 70.5±3.3 mg/ml. During pregnancy, there was a gradual fall to a level of 62.8±6.5 mg/ml at 17-20 weeks. There was an increase to 63.5±3.6 mg/ml at 21-24 weeks and thereafter, further decline to the lowest level of 58.2±2.9 mg/ml at 33-36 weeks of gestation was discovered (Table 1).

Similarly, the mean serum albumin concentration in non-pregnant women was 40.8 ± 4.0 mg/ml. During pregnancy, a significant fall was first noticed in SA concentration at 9-12$^{th}$ week of gestation and thereafter, there was a progressive fall to the lowest level of 29.2 ± 3.7 mg/ml at 33-36$^{th}$ week of gestation (Table 1). The fall in serum albumin concentration was greater than that of TSP during the same period. This was more apparent when the absolute concentrations were expressed as percentages of the values in non-pregnant control (data not shown). The TSG concentration remained statistically unchanged throughout the gestation period but for a significant rise to a level of 32.1±1.6 mg/ml at 21-24$^{th}$ week of gestation. Albumin: globulin ratio decreased progressively from 1.4 in non-pregnant control to 0.9 at 25-28$^{th}$ week and thereafter to 1.0 till term. Table also highlights an increase in TSP concentration in the face of a progressive disease in SA concentration between 17- 24$^{th}$ week of gestation.

Figure 3 shows the representative serum protein electrophoregram on cellulose acetate membrane during normal pregnancy. Pregnancy was found associated with apparently normal electrophoretic pattern and no apparent effect of advancing gestation age on the electrophoretic pattern of serum proteins during normal pregnancy. However, an elevated $\alpha_2$-globulin band at 21-24$^{th}$ week of gestation was observed. Albumin bands did not closely reflect changes observed in the absolute concentrations of serum albumin.

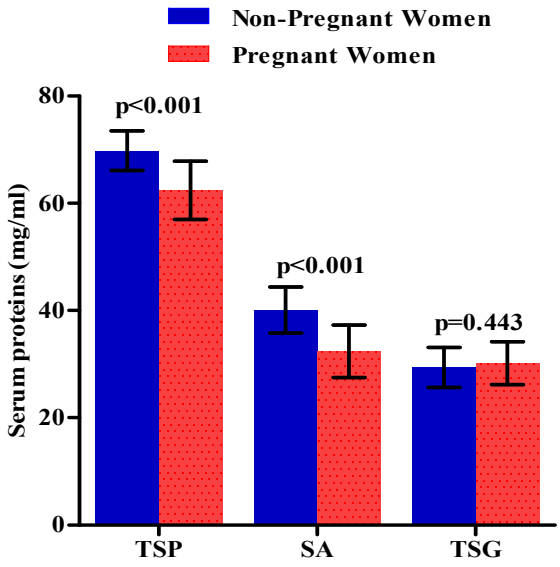


Figure 1: Bar chart comparing the serum proteins in pregnant women (n=66) with non-pregnant women controls (n=25). Each bar and error-bar plot represents the mean and standard deviation of serum proteins concentrations. An unpaired Student't' test was used for the comparison. p-values less than 0.05 was considered significant.

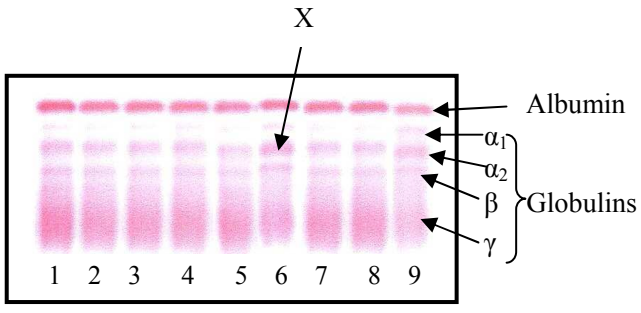


Figure 2: Representative electrophoretic pattern of serum proteins during normal pregnancy. Lane 1: normal non-pregnant control; Lanes 2-9: pregnant women with gestation ages 4-8; 9-12; 13-16; 17-20; 21-24; 25-28; 29-32; and 33-36 weeks; respectively. The arrow 'X' highlights a relative increase in $\alpha_2$-globulin band at 21-24$^{th}$ week of gestation.

Table 1: Serum protein profile consequent to advancing gestation age

| Gestation (Weeks) | N | Total Serum Proteins (mg/ml) | | Serum Albumin (mg/ml) | | Total Serum Globulins (mg/ml) | | A:G Ratio | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean ± SD | P | Mean ± SD | P | Mean ± SD | P | Mean ± SD | P |
| Control | 25 | 69.8±3.7 | ---- | 40.1±4.3 | --- | 29.4±3.7 | --- | 1.4±0.3 | --- |
| 4 - 8 | 7 | 69.4±3.6 | 0.817 | 40.3±2.0 | 0.903 | 29.1±2.5 | 0.845 | 1.4±0.1 | 0.695 |
| 9- 12 | 4 | 64.8±1.3 | 0.014 | 36.8±2.4 | 0.142 | 28.0±1.6 | 0.459 | 1.3±0.2 | 0.837 |
| 13 - 16 | 7 | 62.7±2.5 | 0.001 | 33.6±3.7 | <0.001 | 28.9±3.9 | 0.720 | 1.2±0.2 | 0.108 |
| 17 - 20 | 10 | 61.9±6.5 | <0.001 | 32.9±1.6 | <0.001 | 29.9±5.5 | 0.777 | 1.2±0.2 | 0.057 |
| 21 - 24 | 12 | 63.5±3.6 | <0.001 | 31.4±3.2 | <0.001 | 32.1±1.6 | 0.025 | 1.0±0.1 | 0.025 |
| 25 - 28 | 10 | 61.9±5.1 | <0.001 | 29.9±4.3 | <0.001 | 32.1±3.3 | 0.068 | 0.9±0.2 | <0.001 |
| 29 - 32 | 11 | 58.7±3.8 | <0.001 | 29.4±4.5 | <0.001 | 29.4±6.0 | 0.963 | 1.1±0.3 | <0.001 |
| 33 - 36 | 5 | 58.2±2.9 | <0.001 | 29.2±3.7 | <0.001 | 29.1±1.9 | 0.801 | 1.0±0.2 | 0.015 |

P-values were determined by Student's't' test $p < 0.05$ was considered significantly different.

## 4. 0 Discussions

The results obtained from this study revealed that the mean concentrations of serum proteins exhibited variations with advancing gestational age. In present study, the mean TSP concentration in non-pregnant women is 70.5±3.3 mg/ml. We observed a significant ($p< 0.001$) and progressive fall TSP to a lowest level of 58.2±2.9 mg/ml at 33-36 weeks of gestation. The TSP concentration is known to be of limited value on its own. TSP may be altered by changes in plasma volume without altering the albumin:globulin ratio. Changes in TSP concentration may result from dehydration and over loading with fluid. A short spell of vigorous exercise and stress can result in measurable increase in TSP concentration, as does prolonged venous stasis during phlebotomy (Varley *et al*., 1980 & Van-Hunsel *et al*., 1998).

The decrease in TSP is almost always the result of a fall in that of SA concentration while the TSG concentrations do not always change or increase by small amount (Varley *et al*., 1980). Although the changes in SA and TSG concentrations may cancel each other, and the albumin: globulin ratio may vary widely. The changes are therefore best discussed in terms of individual protein or group of similar proteins. The signifycant decrease ($p< 0.001$) in SA concentration from 40.8±4.0 mg/ml in the non-pregnant women to the lowest level of 29.2±3.7 mg/ml was greater than the fall in TSP during the same period. A significant fall ($p < 0.05$) was first noticed during the first trimester (9-12 weeks of gestation), and thereafter to the lowest level of 29.2±3.7 ($p < 0.001$) at 33-36$^{th}$ week. This bear similarity with the studies of Bacq *et al*. (1996) conducted on pregnant women in France, who observed that serum albumin levels were significantly lower during normal pregnancy. It is also of interest to note that Xydas *et al*. (1996) in the study on certain community in USA found no effects of pregnancy or advancing gestation on maternal serum protein profiles.

In this study, the pattern of changes in serum albumin during normal pregnancy also bear similarity in one way or the other with the findings of previous investigators (Haram *et al*., 1983 & Ekeke *et al*., 1985). The extent of the fall in this study is much higher than the fall in studies carried out on subjects from economically advanced countries. Nutritional deficiencies may be implicated for this phenomenon (Ojofeitimi and Tanimowo, 1980; Butte *et al*., 1981 and Caso *et al*., 2000). The concentration of TSG appear not affected by pregnancy or advancing gestation but for a slight and significant increase at 21-24$^{th}$ week of gestation ($p < 0.05$); and revert to the non-pregnant control levels thereafter. The reasons for this phenomenon and the specific proteins involved are under investigation.

However, Shimokawa *et al*. (1980) demonstrated that certain serum protein fraction of molecular weight of 185,000 Dalton are elevated in relative concentration during the first trimester and decrease to the non-pregnant levels after 25$^{th}$ week of gestation. The serum protein in this range of molecular weight is complement $C_3$ (Burtis & Ashwood, 1994). Later, Richani *et al*. (2005) showed that normal human pregnancy is associated with evidence of complement activation, as determined by increased concentrations of the $C_{3a}$, $C_{4a}$ and $C_{5a}$ in the maternal circulation. Complement $C_3$ is known to migrate between the beta and gamma band. No apparent increase in the region studies was observed (Figure 2). Serum protein electrophoresis pattern showed no significant abnormality but for an elevated $\alpha_2$-globulin band at 21-24$^{th}$ week of gestation and did not closely reflect changes observed in the absolute concentrations of serum albumin. Characterization of specific proteins in

pregnancy migrating in these bands will be required for more insights into this phenomenon.

It is universally known that serum protein anabolism occurs in the liver and plasma cells and catabolism of low molecular weight in the kidneys. Alterations in function of any of these sites will affect the appropriate serum protein fractions. Changes in serum proteins during normal pregnancy can also be attributed to varying factors like increase in plasma volume and wide individual variations at any given stage of pregnancy (Thornburg *et al*., 2000). A considerable amount of albumin passes through the glomerular filtrate daily during pregnancy and most of this is reabsorbed in the renal tubules, being broken down in the process and therefore lost to the body. Moreover, albumin is the major protein frequently found in normal urine (Comper, 2008; Litteboj and Poulik, 1986). On the other hand, Olufemi *et al*., 1991 found that the amount of albumin synthesized in the intravascular compartment was significantly greater at 9.5 g/day in pregnant subjects compared with 6.3 g/day in non-pregnant control subjects.

The increased serum albumin synthesis does not appear to meet the increase protein loss during normal pregnancy. Thus, serum albumin levels fall leading to a relative rise in $\alpha_2$-globulin whose renal loss is least. Theoretically, increased renal albumin loss should only cause a relative rise in TSG; however, there is the possibility that the liver, when attempting to replace excess albumin loss, overproduces serum globulin fractions. If haemodilution constitutes the only variable factor as observed by Thornburg *et al*., 2000, and the amount of albumin remains unchanged, then, for a percentage increase in plasma volume, there would be a proportional decrease in all protein fractions as all the fractions are affected by venous stasis.

The principles of serum protein changes in this study are a lowering of the concentration of albumin fraction probably resulting from renal serum albumin loss, which may have exceeded the reported increase in serum albumin synthesis. Malnutrition, a common feature in rural areas could be a factor, more so that maternal malnutrition could be aggravated by foetal demands. The almost stable TSG in the face of haemodilution suggests an increased TSG content of blood during pregnancy.

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