

**Research Article**

## **SERUM PROTEIN RATIO IN NORMAL AND PRE-ECLAMPTIC WOMEN OF PRIMIPAROUS AND MULTIPAROUS IN RELATION TO AGE**

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### **ABSTRACT**

The objective behind the study was to find out the serum protein ratio in normal and pre-eclamptic women of primiparous and multiparous in relation to age. It was a prospective study. 10 ml venous blood from intracubital vein was collected and was allowed to coagulate for 60 minutes in incubator at 37°C. Serum was separated by centrifugation and 2 ml of serum was frozen at -20°C. The fresh serum was used for estimation of serum total proteins by Biuret method, albumin by BCG method. The total protein in normal pregnancy and pre eclampsia were  $6.705 \pm 0.60$  and  $5.6 \pm 0.57$  respectively. This decrease in total protein was highly significant ( $P < 0.001$ ). Albumin decreased to  $3.95 \pm 0.49$  in normal pregnancy to  $2.9 \pm 0.36$  in pre eclampsia was highly significant ( $P < 0.001$ ). The A : G ratio decreases significantly from  $1.43 \pm 0.24$  in normal pregnancy to  $1.11 \pm 0.17$  in pre eclampsia ( $P < 0.001$ ). There was no significant change in the level of total protein, albumin and globulin in parity and A:G ratio in two age groups respectively. The total proteins, albumin and A:G ratio were found to be decreased, while no significant change in globins with pre-eclampsia as compared to normal pregnancy and not influenced by parity and maternal age.

**Abbreviations:** *Bacillus Calmette–Guérin, BCG; Albumin to globulin ratio, A:G ratio; Pre-eclamptic toxemia, (PET)*

**Keywords:** *Eclampsia, Pre-eclampsia, Primiparous, Multiparous, Albumin, Globulin*

### **INTRODUCTION**

Normal pregnancy is characterized by increased level of circulating steroids, numerous metabolic alterations and presence of rapidly developing and apparently well tolerated allografts i.e. the foetus.

These changes are expected to produce alterations in serum protein pattern of mother. Many investigations studying a single protein or several proteins have detected concentrations in pregnancy which varies significantly from expected average values observed in non-pregnant female (Mack, 1960). Mendenhall (1970) had determined the serum protein concentrations in pregnancy by employing the technique of single radio immune-diffusion. The serum concentrations of proteins were measured in maternal serum during course of pregnancy, at parturition and past partum. Gestation is associated with a decrease in albumin concentration and with no significant variations in concentration of three major classes of immunoglobulins but with an increase in concentration of transferrin, ceruloplasmin and  $\alpha_1$ -antitrypsin.

Tandon *et al.*, (1985) made an extensive quest on serum protein profile in normal pregnancy and in relation to parity. Paper electrophoresis method revealed significant rise in mean levels of serum  $\alpha_1$ ,  $\alpha_2$  and B- globulin in pregnant cases as compared to non-pregnant controls. The rise may be due to increase in pregnancy associated proteins.

Clarke *et al.*, (1971) determined serum proteins in normal pregnancy and mild pre-eclampsia and found that there was no significant variation in  $\alpha_1$ - lipo proteins, hepato globulins and haemo pexin from normal. The only protein which showed a significant difference between normal pregnancy and pre –

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eclampsia was B-1 micro-globulin, the level of which in pre-eclampsia was much higher than normal pregnancy.

Hadded *et al.*, (2000) formed that serum B2 microglobulin levels were significantly higher in women with pre-eclampsia than in control.

## MATERIAL AND METHODS

In the present study 30 normal pregnant and equal number of pre-eclamptic women, in 3<sup>rd</sup> trimester of pregnancy were selected from inpatients and out patients department of Gynecology and obstetrics, Jawaharlal Nehru Medical college, Aligarh Muslim University, Aligarh, Uttar Pradesh, India and enrolled during the period of one year i.e. March 2002 to February 2003 after the research and ethical committee clearance from medical college.

The exclusion criteria were associated with renal, hepatic, cardiac, metabolic disorders, concomitant severe complications of pregnancy, Pregnancy less than 28 weeks or more than 41 weeks and any medication during pregnancy except vitamin, iron, and calcium (Khaliq *et al.*, 1993). Previously healthy normotensive women were considered to have pre- eclampsia if their blood pressure after 20 weeks of gestation was raised to 140/90 mm Hg or more or had mean BP (Diastolic +1/3 of pulse pressure) of more than 110 mm Hg. The increase in BP had to be present on at least two occasion 6 hours apart along with proteinuria of more than 300 mg/day or 100 mg/dl. The written inform consent was taken from all the cases and controls prior to include in study.

10 ml venous blood from intracubital vein was collected. The blood was allowed to coagulate for 60 minutes in incubator at 37<sup>0</sup>C. Serum was obtained by centrifugation and 2 ml of serum was frozen at - 20<sup>0</sup>C. The fresh serum was used for estimation of serum total proteins by Biuret method, albumin by BCG method.

Estimation of Total protein by Biuret Method: (Daumas *et al.*, 1971; Gustafson 1976; Vatzidis, 1977)

Test principle:

In alkaline medium, peptide bonds of proteins react with cupric ions in Biuret reagent to form a violet coloured complex with an absorption maximum at 546 nm (530-570). Intensity of colour formed is directly proportional to concentration of total protein in sample.

$$\text{Total proteins concentration (gm/ml)} = \frac{\text{Absorbance of test} - \text{Absorbance of blank}}{\text{Absorbance of Standard} - \text{absorbance of blank}} \times 6$$

Estimation of serum Albumin by BCG method: (Gustafson 1976; Kaplan & Szabo 1983)

Test principle:

Under acidic conditions, albumin present in serum sample binds to bromocresol green to form a green coloured albumin – BCG complex, which is photometrically measured at 628 nm. Intensity of colour formed is directly proportional to albumin concentration in sample.

$$\text{Albumin concentration (gm/ml)} = \frac{\text{Absorbance of test} - \text{Absorbance of blank}}{\text{Absorbance of Standard} - \text{absorbance of blank}} \times 3.48$$

## Statistical Analysis:

Statistical analysis is done by students “t” test. Once “t” value was estimated “P” value was obtained from standard table of clinical “t” value at appropriate degree of freedom p<0.05 was considered significant

## RESULTS

The table 1 depicts the total protein in normal pregnancy was 6.705 ± 0.60 while in pre-eclampsia it was 5.6 ± 0.57. This decrease in total protein was highly significant ( P<0.001). Albumin decreased from 3.95 ± 0.49 in normal pregnancy to 2.9 ± 0.36 in pre-eclampsia, which was also highly significant (P<0.001). Globulin decreased from 2.75 ± 0.32 in normal pregnancy to 2.6 ± 0.36 in pre- eclampsia. The decrease in

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serum globulin was no significant ( $P > 0.05$ ). The A : G ratio decreased significantly from from  $1.43 \pm 0.24$  in normal pregnancy to  $1.11 \pm 0.17$  in pre-eclampsia ( $P < 0.001$ ).

Table 2 shows that subject were then divided into two group parity. In study group 50% (n=15) women were primiparous and 50% (n=15) were multiparous. Central group was also having equal subjects (n=10) in the two groups. There was no significant change ( $P > 0.05$ ) in the level of total of total proteins, albumin and globulin in two parity group.

Table 3 depicts that the subjects were grouped into two according to age (i) below 25 years and (ii) above 25 years. In Pre-eclampsia 43.33% (n=13) patients were in below 25 years of age group and 56.66% (n=17) were in above 25 years of age group. While in controls there were equal subjects (n=10) in each group. The protein levels were compared in two groups. There was no significant change ( $P > 0.05$ ) in levels of total proteins, albumin, globulin and A : G ratio in two age groups.

**Table 1: Comparison of proteins in normal and pre-eclamptic pregnancies**

	Normal pregnancy	Pre-eclamptic pregnancy
Total proteins (gm/dl)	$6.705 \pm 0.60$	$5.6 \pm 0.57$
Albumin (gm/dl)	$3.95 \pm 0.49$	$2.9 \pm 0.36$
Globulin (gm/dl)	$2.75 \pm 0.32$	$2.6 \pm 0.38$
A:G ratio	$1.43 \pm 0.24$	$1.11 \pm 0.17$

**Table 2: Comparison of proteins at normal and PET in relation to parity**

	Primiparous	Multiparous
Total proteins (gm/dl)		
Normal	$6.56 \pm 0.8$	$6.85 \pm 0.21$
PET	$5.69 \pm 0.63$	$5.6 \pm 0.54$
Albumin (gm/dl)		
Normal	$3.87 \pm 0.68$	$4.03 \pm 0.27$
PET	$2.98 \pm 0.39$	$2.83 \pm 0.34$
Globulin (gm/dl)		
Normal	$2.69 \pm 0.34$	$2.82 \pm 0.33$
PET	$2.71 \pm 0.34$	$2.64 \pm 0.42$
A:G ratio		
Normal	$1.42 \pm 0.18$	$1.45 \pm 0.27$
PET	$1.10 \pm 0.13$	$1.10 \pm 0.20$

**Table 3: Comparison of proteins at normal and PET in two age groups**

	Age < 25 years	Age > 25 years
Total proteins (gm/dl)		
Normal	$6.49 \pm 0.64$	$6.92 \pm 0.46$
PET	$5.7 \pm 0.6$	$5.5 \pm 0.5$
Albumin (gm/dl)		
Normal	$3.76 \pm 0.58$	$4.14 \pm 0.35$
PET	$3.0 \pm 0.35$	$2.82 \pm 0.36$
Globulin (gm/dl)		
Normal	$2.73 \pm 0.39$	$2.78 \pm 0.28$
PET	$2.75 \pm 0.43$	$2.61 \pm 0.33$
A:G ratio		
Normal	$1.38 \pm 0.27$	$1.49 \pm 0.18$
PET	$1.10 \pm 0.15$	$1.10 \pm 0.15$

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### **DISCUSSION**

The present study showed that serum concentration of total protein, albumin, and A:G ratio was significantly reduced in pre-eclampsia, while the change in level of globulin was not significant.

The observation by Studd *et al.*, (1970) showed difference in serum protein level in severe pre-eclampsia compared to normal pregnancy and these changes are to some degree dependent upon the molecular weight of serum proteins (Studd *et al.*, 1970).

The decrease in levels of serum albumin during pregnancy and pre-eclampsia is well known (Hofmeyr *et al.*, 1991; Studd *et al.*, 1970) but Hanger (1967) had shown that the intravascular mass of albumin is unchanged in normal pregnancy and that the sustained hypoalbuminemia is due to hemodilution (Hanger, 1967). On the other hand Mendenhall demonstrated that gestation is associated with a decrease in albumin concentration with no variation in serum immunoglobulins (Mendenhall, 1970).

Honger (1968) had shown that albumin synthesis is significantly greater in pre-eclampsia than in normal pregnancy, probably because of diminished oestrogen production by foetoplacental unit or simply because the more profound hypoalbuminemia of pre eclampsia is a greater stimulus to albumin synthesis by liver. This lower concentration of serum albumin is claimed to be result of proteinuria and hyper catabolism of albumin with no detectable loss of albumin in interstitial fluid or gut.

Mukherjee and Goven (1950) showed that the mean protein content of 0.24 gm/100 ml of oedema fluid of pre-eclampsia was no different from the concentration in the tissue fluid of normal pregnancy. They also found that A:G ratio was much higher in oedema fluid than in plasma.

Studd and Blainey (1969) had suggested that pre-eclamptic toxemia is the most common cause of nephrotic syndrome but De Wardener (1967) did not agree that pre eclampsia fits the definition of nephrotic syndrome, because he claimed that plasma proteins are relatively normal and proteinuria is never more than 5 mg/day. Present study demonstrates that the considerable change in serum proteins that occur in pre-eclampsia can be a result of heavy proteinuria.

These observations indicated that total proteins, albumin, and A:G ratio was found to be decreased, while there was no change in levels of globins with pre-eclampsia as compared to normal pregnancy. There was no influence of maternal age and parity on serum protein levels, but it was influence by urine albumin with which it was inversely related.

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