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Comparative study of serum total protein, albumin and super oxide dismutase in preeclampsia patients with normal pregnancy

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Abstract
Background: Preclampsia is a critical hypertensive pregnancy disorder characterized by new-onset hypertension and often proteinuria with high rate of maternal and fetal morbidity and mortality.
Objective: To determine the levels of serum albumin, total serum protein and serum superoxide dismutase (SOD) in pregnant women with preeclampsia and compare that with normal pregnancy.
Methodology: A case control study involving 50 cases of preeclampsia and 50 matching control of normal pregnancy was carried out in obstetrics department of a tertiary care teaching hospital. Blood samples were collected and tested for serum total protein, serum albumin and SOD in erythrocytes and analysed.
Results: Mean age was 24.13±6.43 vs 27.00±6.59 years in preeclampsia vs control group (p=0.029). The mean gestational age, in weeks, was higher (p<0.001) for patients in the control group 37.17±1.76, than in preeclampsia group 36.30±3.01. The mean systolic and diastolic pressure were significantly higher in preeclampsia group (p<0.05). The mean serum total protein was higher in control group 6.69 ± 0.62 gm/dl compared to 4.75 ± 0.41 gm/dl in preeclampsia group (p<0.05). Mean serum albumin level in preeclampsia was 2.38 ± 0.39 vs 4.35 ± 0.55 in control group(p<0.05). Mean SOD activity of the preeclampsia patients was 280.96 ± 16.8 vs 176.98 ± 14.12 of normal pregnancy(p<0.05).
Conclusion: Patients with preeclampsia had significantly lower levels of mean serum total protein and higher superoxide dismutase activity as compare to normal pregnancy. These biochemical parameters can serve as important markers of risk in such pregnancies for early detection and better management of the patients.
Key words: *Preeclampsia, serum total proteins, serum albumin, serum super oxide dismutase, pregnancy*

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I. Introduction

Preeclampsia is a life-threatening pregnancy disorder characterized by new-onset of hypertension after 20th week of gestation with systolic pressure >140 mm Hg, diastolic pressure >90 mm Hg and in severe cases systolic blood pressure > 160 mm Hg and diastolic blood pressure > 110 mm Hg accompanied by proteinuria and edema. (1) It affects almost 10% of total pregnancy worldwide and have significant impact on maternal and foetal health in immediate and long term. (2, 3) Preeclampsia causes imbalance among various prostaglandins with excessive formation of thromboxane A2 increasing the risk of major cardiovascular disorders and stroke for mother and for foetus it increases the risk of restricted growth, preterm birth, placental abruption and foetal death. Preeclampsia is also characterized by endothelial dysfunction caused by oxidative stress and higher levels of FMS- like tyrosine kinase 1(sFLt- 1), an antagonist of vascular endothelial growth factor and placental growth factor. (4, 5) Women with history of hypertensive disease during previous pregnancy, autoimmune disease, chronic kidney disease, diabetes or body mass index (BMI) ≥ 35 kg/m are at high risk of preeclampsia. (6) Since origin it remains one of the few fatal complications of pregnancy for which no specific cure is present in severe cases. There has been substantial progress in understanding the complex physiology of preeclampsia over the last decade. A defective trophoblastic invasion of spiral arterioles causes hypoxia in the placenta with the release of various factors which causes endothelial dysfunction with increased vascular resistance. Lipid peroxidation which acts as an important source of reactive oxygen species generation and exacerbated

inflammatory response are also important factors that need to be considered in the pathophysiological mechanism of this syndrome. The placental membrane is one of the cellular components which is most affected by lipid peroxidation. Any changes in structure and permeability of cell membrane leads to production of cytotoxic products and free radicals. Involvement of reactive oxygen species (ROS), such as nitric oxide (NO) prevents embryo implantation. (7, 8) ROS leads to vascular damage resulting in an imbalance in concentration of thromboxane and prostacyclin and even it causes release of free radicals, oxidized lipids, cytokines, and vascular endothelial growth factor that damages the endothelium. (9) Superoxide dismutases (SODs), a group of metalloenzymes found in all life forms acts as front line of defence against oxidative stress with higher levels of ROS. It catalyzes the dismutation of superoxide anion free radical (O_2^-) into molecular oxygen and hydrogen peroxide (H_2O_2). During preeclampsia placental SOD gets reduced significantly as it is an important radical for placental lipid per oxidation. (10, 11) Proteinuria is considered to be one of the common manifestations of preeclampsia as hypertension causes renal insufficiency leading to anoxic change in endothelium of the glomerular tuft, resulting in increased capillary permeability and increased leakage of major serum proteins albumin and globulin. (12) The present study aimed to determine and compare the serum superoxide dismutase, serum total protein and serum albumin levels in preeclampsia patients with normal pregnancy.

II. Material and Method

A cross sectional case control study using 50 cases of preeclampsia patients (group 1) and 50 control cases of normal pregnancy (group 2) selected from Civil Hospital and B. J. Medical College, Ahmedabad, Gujarat was performed during the period from February 2017 to September 2017. In group 1 women (age group between 20 to 45 years) with single/multiple pregnancy showing sign and symptoms of preeclampsia were included. In group 2 women (age group between 20 to 45 years) with single/multiple pregnancy without any complications were included. Pregnant woman with pre-existing renal or liver disease, known diabetic or hypertensive, with history of repeated miscarriage, smoking and with severely anemia were excluded. Study was approved by the Ethics committee of the civil hospital and B. J medical college. The work encompasses clinical study of preeclampsia in pregnant women. The practical work was carried out in the Department of Biochemistry and patients and controls were selected from labour room and antenatal wards of Civil Hospital, Ahmedabad. All patients were primary evaluated by clinical examination and then confirmed by investigators for preeclampsia. Blood samples were collected and were tested for serum total protein, serum albumin and activity of superoxide dismutase enzyme in erythrocytes on ERBA XL 640 fully auto analyser, at Hitech Biochemistry Laboratory, Civil Hospital, Ahmadabad.

Sample collection

After aseptic precaution and patient consent blood samples were taken from antecubital vein. From the total blood collected, 5 ml. were distributed in vacuette with EDTA to determine SOD activity and other 5 ml. were placed into a clot activator vacuette. Samples were transported to the laboratory at 2 - 8° C within half an hour. Ensured the complete clot formation has taken place prior to centrifugation in red vacuette.

Sample processing and analysis for SOD

500 microlitre of whole blood was centrifuged at 3000 RPM for 10 minutes and the supernatant was discarded. 0.9 % normal saline was added to the remaining plasma. Again, centrifuged at 3000 RPM for 10 minutes and supernatant was discarded. (This procedure was carried out 3 times) after the last centrifugation the saline was aspirated and discarded. Then cold distilled water was added to wash RBCs and was stored at 4° C for 15 minutes. After 15 minute 100 microlitre of lysate was mixed with 2.5 ml (2500 microlitre) of simple diluents (phosphate buffer) and analysed for the activity of SOD by Randsod Kit Method. (13)

Sample processing and analysis for Serum Total protein and Serum Albumin

For serum total protein and serum albumin 5 ml. of blood collected with clot activator vacuette and sample were transported to the laboratory at 2 - 8° C within half an hour. Ensured the complete clot formation has taken place prior to centrifugation in red vacuette. After serum separated by centrifugation of blood sample, serum total protein and albumin were estimated by Biuret method (14) and Bromo cresol green method (15) respectively. Serum total protein and albumin were measured on ERBA XL 640 fully auto analyser at Hitech Biochemistry Laboratory.

Statistical Analysis: all the data collected were recorded in Microsoft excel and analysed. Data is presented as actual frequencies, mean, SD as appropriate. Unpaired t-test was used for association between the parameters. P value less than 0.05 was considered significant.

III. Results:

The present study was done at Civil Hospital Ahmedabad. Patients of preeclampsia (50 cases) and normal pregnant women as control group (50 controls) where included in this study.

Table–1 Basic characteristics of the control and study group

Variables	Group-I (n=50) Study group	Group-II (n=50) Control group	p Value
Age (Year)	27.00 ± 6.59	24.13± 6.43	0.029
Gestational age	36.30 ± 3.01	39.17±1.76	< 0.001

The mean age of the pregnant women for the control group was 24.13 ± 6.43 and for preeclampsia group the mean age was 27.00 ± 6.59 (p=0.029). The mean gestational age, in weeks, was higher (p<0.001) for patients in the control group 39.17 ± 1.76, than in preeclampsia group 36.30 ±3.01. (Table-1)

Table-2 Serum total protein, albumin, erythrocyte SOD level among study and control group

	Group-I (Study group) Mean ± SD	Group-II (Control Group) Mean ± SD	t Value	p Value
S. Total Protein (gm/dl)	4.75 ± 0.41	6.69 ± 0.62	18.27	< 0.001
S. Albumin (gm/dl)	2.38 ± 0.39	4.35 ± 0.55	20.60	< 0.001
Serum SOD level (U/ml)	280.96 ± 57	176.98 ± 14.12	12.52	< 0.001
Systolic Blood Pressure (mmHg)	158.4 ± 16.8	116.6± 8.6	15.66	< 0.001
Diastolic Blood Pressure (mm Hg)	104.8 ± 12.4	74.2 ± 7.7	14.82	< 0.001

Serum total protein (4.75 ± 0.41) and serum albumin (2.38 ± 0.39) levels were significantly lower (p<0.00) in study group (preeclampsia patient) as compared to control group (6.69 ± 0.62 and 4.35 ± 0.55, respectively). While serum SOD level was significantly higher (p<0.001) in study group (280.96 ± 57) as compared to control group (176.98 ± 14.12). Systolic and diastolic blood pressure were significantly higher (p<0.001) in study group as compared to control group. (Table-2)

IV. Discussion:

Preeclampsia being a hypertensive disorder complicates 7-10% of pregnancies and is responsible for 15-20% of maternal and perinatal mortality. Various cellular level changes occurs during preeclampsia due to endothelial cell dysfunction leading to increased oxidative stress and inflammatory markers due to excess reactive oxygen species. (16) High content of polyunsaturated fatty acid causes mitochondrial lipid peroxidation which leads to higher generation of reactive oxygen species and endothelial cell injury in preclamptic women. (17)

In the present study it was observed that the levels of superoxide dismutase increased in pregnant females with preeclampsia as an adaptive response to counter the effect of increased oxidative stress. This was found to be in consistence to previous studies performed on preclamptic women. (18, 19) Lipid peroxidation has been responsible to be the main contributing factor for oxidative stress in preeclampsia. Free radicals initiate lipid peroxidation by attacking polyunsaturated fatty acids in cell membranes. (20) Various enzymatic or non-enzymatic antioxidants counteract free radicals and prevent oxidative effects on proteins, carbohydrates, lipids and DNA. The most important antioxidant enzyme is superoxide dismutase (SOD), which converts free radicals of both the mitochondria and the cytosol of cells to hydrogen peroxide (H_2O_2). Increased values of activity of superoxide dismutase, that catalysed dismutation of superoxide radical, and removed H2O2 from tissues, indicate antioxidative mechanisms may be an adaptive response to counter the effect of oxidative stress in the cases of preeclampsia. Increase in superoxide dismutase was also found in study by Sharma et al. (21) and Krishna Menon et al (22) from India also

Serum albumin plays an important role as an antioxidant for scavenging free radicals. Human serum albumin helps to recover ATP-sensitive K+ channel causing vasodilation of human omental arteries in damaged endothelium. Also human serum albumin helps to preserve human vascular smooth muscle which have been adversely affected due to increased levels of superoxide. Albumin and tiron has higher tendency to bind with Ca2+- molecules, and thus, they augment the vasodilation by reducing extracellular Ca2+ levels. Human serum albumin also impairs the function of NADPH oxidase in the human vascular smooth muscle cells. Human serum albumin has an inverse relationship with oxidative stress. Lower levels of plasma albumin is associated with various inflammatory disorders and with post-surgical mortality and morbidity. Present study findings suggest that lower levels of plasma albumin is an independent marker in preeclampsia women due to increased capillary permeability and endothelial damage. Similar observations were reported in previous studies on pregnant

women with preeclampsia. (23, 24) Hypoalbuminemia is an early sign in developing preeclampsia and many study recognizes serum albumin level as an important laboratory findings in treatment of hypertensive disorders in pregnancy. Patients with severe preeclampsia were associated with lower levels less than 3.0 gm/dl of serum albumin which was evident in present study also. (25) Result of our study indicate significantly decreased in serum total protein in study group compared to control group, which is in accordance with findings of a study by Howlader M et al (26) and Olooto WE et al (25) also.

Proteinuria has been proposed as well as studied as both an indicator of severity of disease and as a predictor of outcome in preeclampsia. Patient with preeclampsia often develop complication due to multiple organ hypoperfusion, production of albumin is decreased due to reduced hepatic blood flow, thus lead to the gradual development of hypoproteinemia. Proteins are normally not excreted in urine. Protein excretion from in urine is prevented by both the glomeruli and the tubules. During normal pregnancy, an increase in urinary protein excretion is detected because of a combination of increased GFR (glomerular filtration rate) and increased permeability of the glomerular basement membrane. (27) Glomerular proteins of intermediate size, such as albumin, have been identified alone or along with variable concentrations of tubular proteins (such as β2-microglobulin) indicative the tubular damage that can occur in severe preeclampsia. Preeclampsia is accompanying with increased capillary permeability secondary to endothelial damage and partly responsible for observed proteinuria and consequent significantly lowe serum total proteins and albumin levels. These findings supports earlier work by Bhatia et al (28). Hypoalbuminemia in preeclampsia is thus a resultant of urinary protein loss and reduced hepatic blood flow secondary to haemoconcentration created by higher filtration pressure in the capillaries.

This study has highlighted the protein, albumin and SOD levels during preeclampsia verses normal pregnancy. Few limitation of the study are single centre and long term follow up was not possible. In conclusion, the findings of the study showed that estimation of serum albumin, total serum protein and serum superoxide dismutase levels are significant in the detection of preeclampsia in early pregnancy. However several other indicators like oxidative stress, free radical endothelial damage are important in determining the underlying cause of preeclampsia in higher population. Early detection and anti-oxidant therapy helps in prompt management of preeclampsia to avoid serious complications in later stages of pregnancy.

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Conflict of interest: The authors have no competing interests to declare.
Ethics Approval: The study protocol was reviewed and approved by the Institutional Ethics Committee. The study was carried out following the standards of clinical study as laid down in Schedule Y and new drugs and clinical trial act, 2020.
Guarantor: The corresponding author is taking the full responsibility for the manuscript including accuracy and appropriateness of reference list.
Consent to Participate: All the participants were explained clearly about the nature and purpose of the study in the language they understood and written informed consent was obtained from them. All the participants were ensured that their identity will not be revealed at any stage of the study.

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