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SERUM TOTAL PROTEIN AND ITS ELECTROPHORETIC PATTERNS IN THREATENED ABORTION WOMEN

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ABSTRACT
The goal of our prospective and controlled study was to dose the serum total protein and its electrophoretic patterns by electrophoresis technique in case of patient in the second gestational trimester with threatened abortion of unknown cause that did or did not evolve towards spontaneous abortion during hospitalization or with recurrent abortion in their priors, or with a normal pregnancy. Our results constantly indicate a significant decrease in serum total protein, globulins, and gamma globulin concentration with respect to those from a normal pregnancy or non-pregnant women. The obtained results suggest that a measurement of the serum total protein and its electrophoretic pattern concentration could be a valuable predictive marker for threatened abortion in the second trimester.

KEY WORDS: total serum protein, electrophoretic pattern, fasting blood glucose, threatened abortion.

INTRODUCTION

Human pregnancy is an efficient process because 70% of conceptions fail to achieve viability, and the specific cellular and/ or molecular mechanisms underlying the maintenance of normal pregnancy and the induction of abortion remain poorly understood [(1)].The precise incidence of early pregnancy loss (EPL) at different periods of gestation has been more clearly defined with the routine use of transvaginal ultra sound and urine tests for human chorionic gonadotrophin (hCG). The incidence of pregnancy loss prior to completion of the first trimester is high, estimated at 50% to 70%. Loss rates drop rapidly with increasing gestational age[(2)]. In medical, the word abortion" refers to any process by which a pregnancy ends with the death and removal or expulsion of the fetus, regardless of whether it is spontaneous or intentionally induced[(3)].The etiology of recurrent spontaneous abortion (RSA) remains unknown in a high proportion of the cases. Unequivocal causes include genetic disorders and uterine malformation. Immunological theories are still a matter of discussion[(4)].Recurrent spontaneous abortion (RSA) is one of the important complications in pregnancy. Half of recurrent miscarriages loss is multifactorial, can be explained by genetic, hormonal, anatomical, metabolic abnormalities, infections or autoimmune mechanisms and can be divided into embryological driven causes (mainly due to abnormal embryonic karyotypes) and maternally driven causes, which affect the endometrium and/ or placental development[(5)]. Known causes of maternal defects include coagulation disorders, autoimmune defects, endocrine disorders and endometrial defects[(6)].The etiology of spontaneous abortion includes chromosomal rearrangements, uterine anomalies, thyroid dysfunction, autoimmune disorders, and infection. However, the etiology of approximately 40% of patients is not well unknown. Tumor necrosis factor (TNF) and its receptor play an obvious role in the inflammatory process. Recently, a number of studies have claimed that TNF alpha expression can be detected in human villi and decidua of early pregnancy[(7)] and locally produced TNF plays one or more roles in the pregnant corpus luteum, as an autocrine and/or paracrine mediator[(8)].Threatened abortion is characterized by uterine bleeding in the first half of the pregnancy, to which, sometimes, a faint sacral or hypo gastric pain can be associated[(9)]. It is assumed that uterine bleeding in late threatened abortion would originate either in the partial detachment of the placenta or in vascular anomalies from the place of implantation or union of the decidua capsularis with the decidua vera[(10)]. The association between low pregnancy –associated plasma protein A (PAPP-A) at 11 to 14 weeks of gestational age has been known for a number of years {Ong et al 2000[(11)], Smith et al 2002[(12)], 2006[(13)], Spencer et al 2008[(14)]}.Early first trimester (PAPP-A) levels can predict early pregnancy failure and the risk of aneuploidy, particularly Down syndrome[(15,16)]. Low maternal serum protein A at 11-14 weeks of gestation is associated with subsequent development of pregnancy complications. There is some evidence that low levels of maternal serum (PAPP-A)in the first trimester may be associated with miscarriage, low birth weight and preterm delivery[(17,18)].As many as 20% of patients have vaginal bleeding during early pregnancy. The high rate of abortion in this group of women causes considerable concern both to patients and their families[(19 20)]. Much has been written about both biophysical and biochemical methods of assessing pregnancy progress in such patients' as the quest continues to find both a rapid and reliable method of predicting pregnancy outcome[(21, 22)]..

MATERIAL AND METHODS

Subjects

Ninety seven women were recruited from the Baghdad City Hospital "between" (April.2010–Jan.2011). 28 women with threatened abortion (their age range (18–40) years, average 29 years, 28 women were control pregnant

(age range 18–40) years, average 29 years, and 23 women were control non pregnant (age range 20–38) years, average 29 years. The characteristic of all women are represented in table 1.

TABLE 1: history of women on admission

	Threatened abortion	Control pregnant	Control non pregnant
Number	28	28	23
Age range/year	18---40	18--40	20—38
Average/year	29	29	29
B.W/kg Range	56—90	45--100	45—110
Average	73	72.5	77.5
Wight/cm	152---168	156--178	100—168
Average /cm	160	167	134
BP mm/Hg	100/60-140/90	100/60—140/90	100/60—130/80

Electrophoresis assayof serum protein and its pattern for studies on early conceptual loss and for pregnancy associated plasma protein were used in a prospective study of 28 threatened abortion women areadmitted to hospital and 28 control pregnant women and 23 non pregnant women come to check. Serum sample at the time of venipuncture was taken for protein and its pattern (albumin, globulins,α1, α2, β and γ) and fasting blood glucose assay and stored at -20^{o}C. Each patient had an ultrasound scan and management was based in accordance with normal unit policy, on clinical and ultrasound findings. We characterized plasma protein by analyzing total protein concentration and its electrophoretic pattern, by RANDOX KIT, of total protein (TP) and electrophoretic method (SHANDON ae 2761) and fasting blood glucose by enzymatic method.

METHOD

Principle of total protein method (Biuret Method):

Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a colored complex, read at (530-570 nm)[23].

Principle of electrophoretic method:

Electrophoresis which separates proteins according to their different electrical charges is usually performed by applying a small amount of serum to a strip of cellulose acetate or agarose and passing acurrent across it for a standard time.

In this way fife main group of proteins, albumin and the α1, α2,β and γ-globulins may be distinguished after staining and may be visually compared with those in a normal control serum[23].

Principle of serum glucose by enzymatic method:

Glucose is oxidized by glucose oxidase (GOD) to gluconate and hydrogen peroxide according to the following equation:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} H_2O_2 + \text{Gluconate}$$

$$2H_2O_2 + \text{phenol} + 4\ \text{Amino–antipyrine} \longrightarrow H_2O + \text{Quinonimine (red color)}^{(23)}.$$

RESULTS

Statistical analysis of data

By ANOVA test and multiple comparisons and Spearman correlation (2-tailed) analysis.[24,25]

By ANOVA test show significant decrease in the serum total protein (STP), globulins, alpha–2 and gamma globulin concentration in threatened abortion than in control pregnant and control non pregnant women (Table 3)(p≥ 0.04, 0.016, 0.092 and 0.001 respectively).

While serum albumin, alpha-1, and β –globulin show no significant change between threatened abortion and control pregnant and control non pregnant women. Fasting blood sugar (FBS) show significant increase concentration in threatened abortion than that control pregnant and control non pregnant women (P≥ 0.001). (Table 3)

The result by Spearman correlation show that no relationship between alpha-1, alpha-2 and FBS in threatened abortion (both spearman correlation coefficient = – 0.348, – 0.134 respectively). (Fig. 1&2)

While the Spearman correlation show significant relationship between β & γ globulin and FBS in threatened abortion women (both Spearman correlation coefficient = 0.59 and 0.265 respectively) (P≥ 0.002, 0.013 respectively). (Fig. 3 & 4).

Correlation control pregnant show significant relationship between FBS and β globulin, and globulin (P ≥ 0.002 & 0.027 respectively) (both Spearman correlation coefficient = 0.472 & 0.591 respectively).

TABLE 2 : Descriptive the association between control pregnant, control non pregnant and threatened abortion women.

	N	Mean	Std.Deviation	Std. Error	Minimum	Maximum
Age Ctrl P	27	28.222	5.220	1.004	18.00	40.00
Age Ctrl non P	22	28.727	6.033	1.28	18.00	38.00
Age Threatened	28	30.57	5.567	1.052	18.00	41.00
Total	77	29.22	5.611	0.6395	18.00	41.00
GAge Ctrl P	28	16.03	5.44	1.029	8.00	24.00
Gage Ctrl no P	0					
Gage threatened	28	11.535	4.686	0.8856	6.00	24.00
FBS Ctrl P	25	82.32	7.824	1.564	60.00	90.00
FBS Ctrl no P	22	79.318	8.493	1.81	60.00	90.00
FBS Threatened	22	89.727	11.418	2.434	75.00	115.00
TSP Ctrl P	24	70.458	5.702	1.164	60.00	82.00
TSP Ctrl no P	23	73.478	7.341	1.530	48.00	84.00
TSP Threatened	28	68.928	6.0731	1.147	56.00	82.
Albumin Ctrl P	25	34.336	4.257	0.851	26.00	43.00
Albumin Ctrl no P	23	38.1739	9.934	2.0715	20.00	73.00
Albumin Threatened	28	36.214	6.22	1.175	20.00	45.00
Globulin Ctrl P	24	35.208	4.510	0.9207	24.00	45.00
Globulin Ctrl no P	23	36.608	5.185	1.081	28.00	46.00
Globulin Threatened	28	32.714	4.665	0.8817	23.00	41.00
Alpha-1 Ctrl P	27	0.563	0.1892	0.0364	0.14	0.83
Alpha-1 Ctrl no P	23	0.6526	0.1551	0.03235	0.46	1.2
Alpha-1 Threatened	28	0.5486	0.15025	0.02839	0.31	0.85
Alpha-2 Ctrl P	27	0.8022	0.11613	0.02235	0.57	1.13
Alpha-2 Ctrl no P	23	0.7662	0.25213	0.05257	0.3	1.46
Alpha-2 Threatened	28	0.6954	0.16329	0.03086	0.46	1.00
Beta Ctrl P	26	0.9768	0.16126	0.03163	0.71	1.26
Beta Ctrl no P	23	0.9261	0.14122	0.02945	0.68	1.15
Beta Threatened	28	0.9261	0.31039	0.05866	0.59	2.23
Gama Ctrl P	27	1.1263	0.20781	0.03999	0.57	1.53
Gama Ctrl no P	23	1.3204	0.23084	0.04813	0.87	1.9
Gama Threatened	28	1.1161	0.15375	0.02906	0.82	1.46

TABLE 3 : levels of fasting blood glucose, total serum protein, albumin, globulin, alpha-1, alpha-2, Beta, and γ- globulin in threatened abortion, control pregnant and control non pregnant women.

Parameters	Control P	Control non P	threatened	P value
FBS	82.32± 1.56	79.31± 1.81	89.72± 2.43	0.001
TSP	70.45± 1.16	73.47± 1.53	68.92± 1.14	0.044
Albumin	34.33± 0.85	38.17± 2.07	36.21± 1.17	0.179
Globulins	35.2± 0.92	36.6± 1.08	32.71± 0.88	0.016
Alpha-1	0.56± 0.03	0.65± 0.032	0.54± 0.028	0.66
Alpha-2	0.802± 0.02	0.76±0.052	0.69±0.03	0.092
B-globulin	0.97±0.03	0.92±0.02	0.92±0.05	0.644
γ- globulin	1.12±0.03	1.32±0.04	1.11±0.02	0.001

DISCUSSION

The endometrium is a dynamic tissue with extensive cell death and tissue remodeling. Macrophages are important as scavengers, in maintaining the equilibrium in this tissue and in reducing inflammation[26]. It is known that a large population of macrophages is found at the site of implantation and remain high throughout pregnancy[27]. Glycodelin A (GdA) formerly known as placental protein 14 (pp 14) is the most abundant secretory glycoprotein of the primate uterine compartment during implantation and early pregnancy[28, 29]. It has been hypothesized to be involved in preparing the endometrium for implantation[30, 31]. The arise in serum total protein and globulins in normal pregnant women, and then decline in missed and threatened abortion is a well-known effect of pregnancy complication. Threatened abortion group showed significant decrease in TSP and globulins than that normal pregnant and non-pregnant group. Pregnancy associated plasma protein- A (PAPP-A) demonstrated in many researches that is the most abundant in the maternal circulation of pregnant women[32]. In women with singleton pregnancies (PAPP-A) was first detected in the maternal blood about 28 days post implantation, and increases exponentially with a doubling time of 3-4 days during the first trimester, then the levels continue in rise throughout pregnancy until delivery[33]. In the women the levels increase by a factor of about 150 as compared to the non –pregnant state[33]. There is in fact an association between low TSP and globulins (alpha-1, alpha-2, beta and gamma) in the first trimester and threatened abortion.

The clinical importance of this finding is that if ultrasound surveillance is instituted in the cortex of low TSP and globulins levels where fetus is thought to be chromosomally normal, it would be most likely to be usefully performed after the first trimester. Alpha-2 band contains haptoglobin may be reduced if there is in vivo hemolysis and split into two if in vitro hemolysis has occurred, an increase in homo–concentration in inflammation lead to stimulate synthesis of so called acute-phase proteins with arise in the alpha-1 & alpha-2 globulin fraction. While in chronic inflammation the usual increase is in immunoglobulin synthesis (γ-globulin).[34]

Our data suggest that where early first trimester TSP and globulin levels are low, they are not predictive of the first trimester growth. However, TSP and globulins are significantly correlated with threatened abortion (Table-3) across the entire range of TSP & globulins. One could speculate that the consequence of combination of late implantation, low TSP and low serum globulins might be associated with abnormal placental implantation and uterine bleeding, manifesting itself in late first trimester when the switch from histotrophic to hemotrophic placentation occurs and is evidenced by maternal and fetal Doppler change .

TABLE 4 : All parameters By ANOVA Test

parameters	Sum of squares	Df.	Mean square	F	Sig.
Age between Groups	83.359	2	41.680	1.335	0.269
Within groups	2309.887	74	31.215		
Gage between groups	283.500	1	283.500	10.983	0.002
Within groups	1393.929	54	25.813		
FBS between groups	1269.192	2	634.596	7.319	0.001
Within groups	5722.576	66	86.706		
TSP between groups	265.832	2	132.916	3.267	
Within groups	2929.555	72	40.688		0.044
Albumin between groups	176.475	2	88.238	1.764	0.179
Within groups	3650.956	73	50.013		
Globulin between groups	200.396	2	100.198	4.380	0.016
Within groups	1647.151	72	22.877		
Alpha-1 between groups	0.155	2	0.078	2.813	0.066
Within groups	2.071	75	0.028		
Alpha-2 Alpha-2 between groups	0.162	2	0.081	2.467	0.092
Within groups	2.469	75	0.033		
Beta between groups	0.044	2	0.022	0.443	0.644
Within groups	3.690	74	0.050		
Gamma between groups	0.646	2	0.323	8.257	0.001
Within groups	2.933	75	0.039		

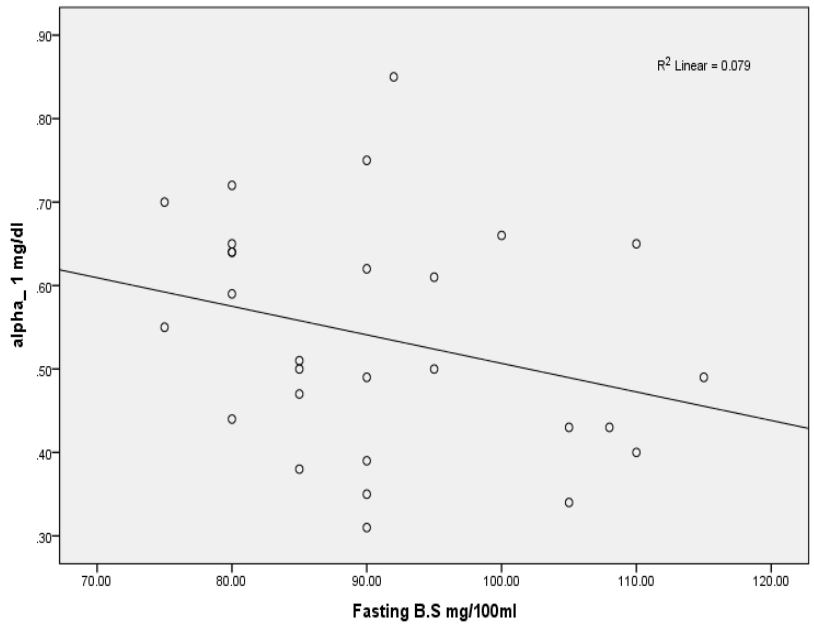

FIGURE 1:the correlation between alpha -1 and FBS in threatened abortion.

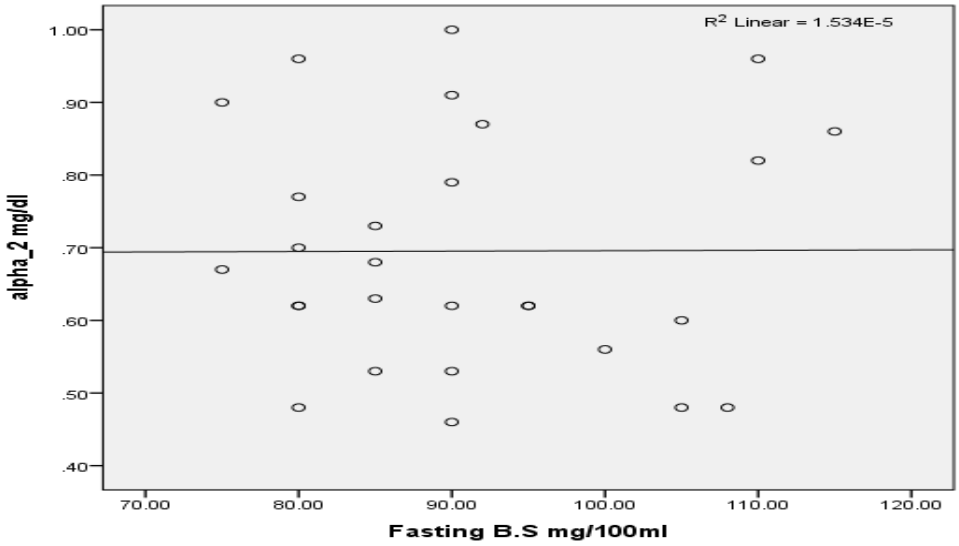

FIGURE 2: the correlation between alpha -2 and FBS in threatened abortion.

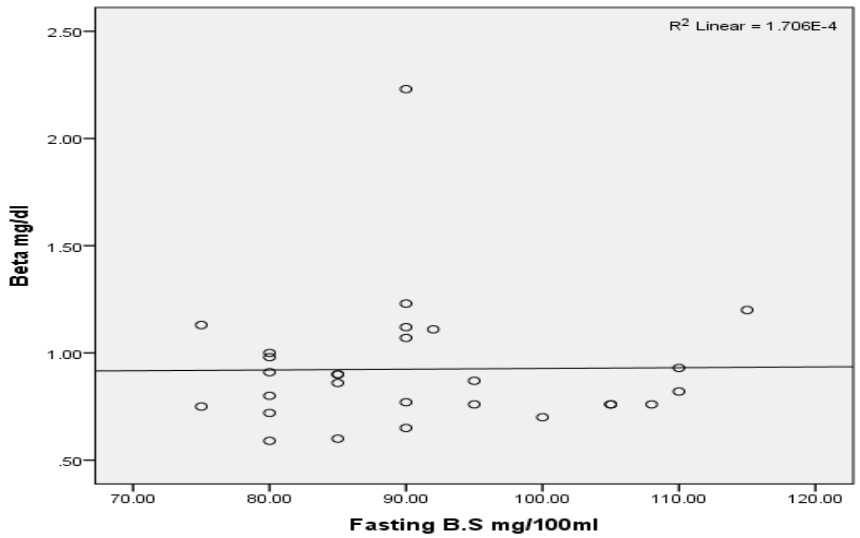

FIGURE 3: the correlation between beta globulin and FBS in threatened abortion.

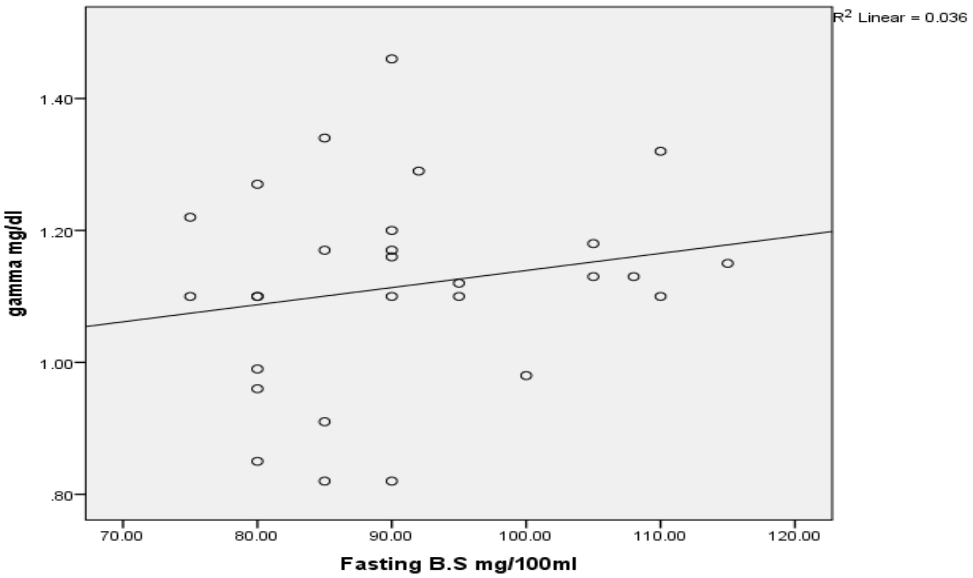

FIGURE 4: the correlation between gamma globulin and FBS in threatened abortion.

CONCLUSION

A recent study demonstrated a significant relationship between TSP and its electrophoretic patterns with threatened abortion.The difficulty in interpreting this relationship is that decreased TSP was not normalized for gestation, and therefore gestation at threatened abortion have been a confounding variable.We have overcome this by relating earlier first trimester TSP and its electrophoretic patterns. This study on this subject relates to normalizing TSP and its patterns levels to gestation related percentiles. It is customary in spontaneous conceptions to date a pregnancy from a first trimester TSP measurement, and then to relate the TSP level to this derived gestation.The only way to overcome this bias would be to date a pregnancy from implantation date, for which there is as yet no clinically robust methodology.

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