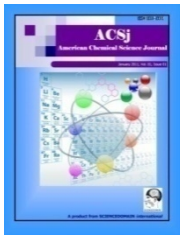

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Assessment of Some Biochemical Parameters in Iraqi Pregnant Women with and Without Complications

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Authors' contributions

This work was carried out in collaboration between all authors. Author IGZ designed the study, managed the the literature searches, wrote the protocol Author ZN managed analyses of the study performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The purpose of this study was to evaluate and compare some biochemical parameters including [ceruloplasmin oxidase(CP), α – fetoprotein (AFP), interleukine-1 (IL-1), α2- macroglobulin (α2 – MG), α1 antitrypsin(α1 – AT)] and β-human gonadotropin(β -HCG)] in Iraqi women with pregnant complications (mole ,ectopic and missed abortion) compared with normal pregnant women .
Study Design: This is a case-control study which included 63 women with pregnant complications (25 with missed abortion having - mean age 26.68 ± 7.54 yrs, 21 with mole having - mean age 26.14± 8.19 yrs and 17 having ectopic - mean age 26.14 ± 6.99 yrs) pregnancy and 25 normal pregnancy women with mean age 25.16 ± 5.97 yrs as control.
Place and Duration of Study: Samples collected from the Baghdad Medical City, AL-Yarmook and Fatema AL-Zahraa hospitals during the period from September 2011 to April 2012.
Methodology: Alpha feto protein, IL- 1, α2 – MG, α1 – AT and β -hCG were measured by immunoassay method. CP activity was measured using the modified Rice method.
Results: AFP and IL-1 were significantly decreased (p≤ 0.05) in all patients groups but Cp, activity was decreased significantly only in EP and MP groups (p≤ 0.05). There was a significantly increased (p≤ 0.05) in β-hCG level in mole or missed abortion and significantly

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decreased (p≤ 0.05) in ectopic pregnancy compared with controls. α2-MG levels were significantly increased in EP , MP compared to control.α1-AT were elevated significantly (p≤ 0.05) in all women with pregnancy complications compared with controls.In mole pregnancy there were positive correlation between α2 – MG with β-hCG (r= 0.51, p= 0.01) or with α1 – AT (r= 0.49, p= 0.02) and negative correlation between AFP with α1 – AT (r= -0.41, p= 0.05), IL- 1 with α2 – MG(r= - 0.44, p= 0.04). CP positively correlated with IL- 1 (r= 0.734, p= 0.00) while in missed abortion there were positive correlations between β-hCG and AFP (r= 0.42, p= 0.034), IL-1α (r = 0.83, p= 0.00) with α2-MG (r = 0.060, p= 0.00) and between AFP with α2-MG (r = 0.45, p= 0.02) and between IL- 1 with α2-MG (r = 0.75, p= 0.00).

Conclusion: This study shows that the above biological parameters could be used for the monitoring the health status of pregnant women with complications.

Keywords: Abortions; alpha fetoprotein; ceruloplasmin; interluekin 1; alpha 2 – microglobin; alpha 1 – antitrypsin; β-human chroniv gonadotropin; Iraq.

1. INTRODUCTION

Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to considerable extent [1]. Normal physiological processes associated with pregnancy may change from onset leading to potentially fatal both maternal and fetal complications if untreated [2]. The most common complications of early pregnancy are missed abortion (MA), mole pregnancy (MP) and ectopic pregnancy (EP) [3] that might be diagnosed by maternal blood biomarkers requiring early diagnosis. But this might be militated against by lack of experimental models. Biochemical changes taking place during pregnancy lead to increases in circulating maternal levels of AFP in a three-domain glycoprotein structurally similar to serum albumin [4]. Alpha-fetoprotein is synthesized during embryonic development primarily by the fetal liver and yolk sac, enters the amniotic fluid through fetal renal excretion, crosses the placenta possibly by diffusion into maternal circulatory system [5]. Although the exact cause for an unexplained elevation in maternal blood is not completely understood, placental study suggested chorionic villitis and placental vascular lesions which allow leakage of the AFP from fetal circulation thereby elevating the maternal serum AFP [6]. Human chorionic gonadotropin (β-HCG) is produced during pregnancy by the developing placenta after conception, and later by the placental syncytiotrophoblast [7]. Human chorionic gonadotropin interacts with the LHCG receptor to promote the maintenance of the corpus luteum from the beginning of pregnancy, causing it to secrete the progesteron. Due to its highly negative charge, β-HCG may repel the immune cells of the mother to protect the fetus during the first trimester. It has also been hypothesized that β-HCG may be a placental link for the development of local maternal immune tolerance [8]. Acute phase proteins (APRs) are plasma proteins which increases in response to an acute-phase reaction may be positive acute- phase proteins like (alpha 1 - antitrypsin, alpha 1-acid glycoprotein, haptoglobin, ceruloplasmin, C3, C4 and C-reactive protein) and negative acute phase proteins (transthyretin, albumin and transferrin) decrease and are known as negative acute-phase proteins [9]. Interleukins-1 (IL-1), IL-6 and IL-8, help to maintain the trophoblast in early pregnancy, that also play major role in intrauterine infection, especially after premature rupture of membranes and in preterm or term labor irrespective of infection [10,11]. Serum proteinase and proteinase inhibitory profile of pregnant women differs from that of non pregnant women [12]. This may be attributed to the production of proteinases and their inhibitors from trophoblastic tissue and also to the production of increased amounts of inhibitors from maternal liver. The invasion of the

trophoblast into the uterine endometrium involves the digestion of the matrix by matrix metallo proteinases [13,14]. Alpha 2-macroglobulin (α2- MG) has a major role in implantation by regulating the bioavailability of proteinases and cytokines involved in implantation. Obstetric complications that occur in the first trimester threaten pregnancy [15]. The continued elevation of α2-MG even after surgical removal of the trophoblastic tissue in EP or evacuation of the MP suggests a long term change in the inhibitory profile of the patients. However, it is certain that α2- MG and not AT or ACT activities reframe in NP, EP and MP [16]. α1-antitrypsin is an acute phase protein; antitrypsin functions to protect tissues from released proteolytic enzymes, a study showed that α1-AT increases by about 100% in the third trimester, increases in α1-AP was hypothesized to be a result of the increased oestrogen concentrations inducing α1-AP synthesis by the liver [17]. This study is evaluated some of biochemical parameters in pregnant women with or without complications and correlated with β-HCG.

2. MATERIALS AND METHODS

2.1 Subjects and Specimens

The study population included women who were admitted to the Gynaecology Units in the Medical City, AL-Yarmook and Fatima AL-Zahraa hospitals during the period from 2011 to 2012. The study populations consisted of 88 pregnant women in the first trimester of gestation with age ranged between (14 – 35) years. These were 17 women with ectopic pregnancy (EP) diagnosed by laparoscopy , 25 women diagnosed (after curettage) as having a missed abortion (MA), 21 women with mole pregnancy (MP) and 25 women with no pregnancy complications . None of the selected women suffered from any other type of diseases. The groups above were matched with gestation age, Hb, BMI, maternal age and number of children.

About 10ml of vinous blood was collected in plane tube using plastic disposable syringes and left for 20 minutes at room temperature (25ºC). After coagulation, sera were separated by centrifugation at 1500 Xg for 10 minutes. Sera were frozen in -20ºC freezer until analysis. Commercial enzyme immunoassay kits as follows: IL-1 from CUSABIO - China catalog no. CSB-E04620h, β-HCG from human – Germany catalog no. 53040, AFP from human – Germany catalog no. 52010, α2-MG from CUSABIO China catalog no. CSB-E08959h and α1-AT from CUSABIO China catalog no. CSB-E11719h. The manufacturer's instructions were followed, The enzymatic assay of ceruloplasmin oxidase activity was carried out using the modified Rice method and p-phenylene diamine-2HCL as a substrate [18]. The CP activity was expressed in term of (U/L).

2.2 Statistical Analysis

Statistical analysis was done using SPSS version 10.0 and values were expressed as mean$\pm$SD and $P \leq 0.05$.The comparison of mean ± SD was performed using Student t - test and correlation analysis was performed using Pearson`s correlation test.Statistical significance was defined as $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Demographic data of the studied groups were summarized in Table 1. There were no significant differences in age, BMI and number of children. However, there were significant

differences (p≤ 0.05) between test groups compared with control group with regards to Hb and, gestation age respectively.

Table 1. Demographic parameters in the studied groups

Parameter	Normal pregnancy	Missed abortion	Ectopic pregnancy	Molar Pregnancy
Maternal Age(years)	25.16+5.97	26.68+7.54	26.41+6.99	26.14+8.19
Hb (g/dL)	10.76+1.47	11.817+1.59*	9.98+0.72*	9.90+1.54*
BMI (Kg/M2)	26.51+4.97	25.61+4.70	26.36+2.37	26.30+4.24
Number of Children	1.72+1.86	2.64+2.41	1.41+1.27	2.00+1.54
Gestation Age(Months)	4.88+0.88	3.04+1.54*	1.94+0.24*	1.62+0.49*

P≤ 0.05 compared with normal pregnancy.

The mean values of all parameters in the studied groups are presented in Table 2. The results indicated that there were statistically significant decreases in AFP and interleukin -1 in missed, ectopic and molar pregnancies when compared to control group, but Cp, activity was decreased significantly only in EP and MP groups (p≤ 0.05).α2-MG levels were significantly increased in EP, MP compared to control.α1-AT were elevated significantly (p≤ 0.05) in all women with pregnancy complications compared with controls.
β-HCG was increased significantly in mole or missed abortion and decreased significantly in ectopic pregnancy compared with control group.

Table 2. Biochemical parameters levels (Mean+SD) in the studied groups

Parameters	β-HCG (IU/L)	AFP (ng/ml)	CP (U/L)	IL-1α (pg/ml)	α2-MG (ng/ml)	α1-AT (nIU/ml)
Normal Pregnancy(control) (N=25)	155.74 +85.39	108.60+ 95.67	47.11+ 13.7	244.40+ 155.02	167.52+1 49.04	255.80+ 120.92
Missed abortion (N=25)	196.26* +73.03	39.45*+ 59.91	42.68+ 8.98	136.56* +97.85	188.96+9 0.79	320.54* +194.09
Ectopic Pregnancy (N=17)	144.93* +76.93	22.90*+ 53.31	36.90+ *7.144	180.41* +7.17	269.12*+1 48.88	435.31* +147.85
Molar Pregnancy (N=25)	234.16* +38.50	7.55*+ 3.05	31.78+ *11.43	175.33* +7.65	435.317*+ 147.858	469.25* +175.28

**P≤ 0.05 compared with normal pregnancy.*

Pearson's product moment correlation analysis was carried out to determine the relationship of all studied parameters with β-HCG. The results indicated that in mole pregnancy there were positive correlation between α2-MG with β-HCG (r = 0.51 , p = 0.01) and with α1-AT (r = 0.49 , p = 0.02) and negative correlation between AFP with α1-AT (r = 0.41 , p = 0.05), positive correlation between IL-1 with α2-MG (r = 0.44, p= 0.04) , Cp positively correlated with IL-1 (r = 0.734 , p= 0.000)in mole pregnancy , while in missed abortion there were positive correlation between β-HCG with AFP (r=0.42 , p= 0.03) , with IL-1 (r=0.83 , p=0.00) , with α2-MG (r = 0.08, p= 0.00) and between AFP with α2-MG (r = 0.45, p= 0.02) and between IL-1 with α2-MG (r = 0.75, p= 0.00).

Pregnancy associated complications are known to often responsible for maternal, fetal and neonatal morbidity and mortality. Early detection followed by preventive therapy may decrease the complications and related feto-maternal risks. This could be possible only if the

pregnant women prone to develop disorders are identified quite early [19]. This study provides additional information using some acute phase proteins, β-hCG and IL-1 in missed abortion, ectopic and mole pregnancies. Other parameters have been tested for their clinical value in the above pregnancy complications in previous studies.

Ceruloplasmin (feroxidase activity) known as oxidant defense enzyme and decreases significantly in pregnant women with complications [20]. Although pregnancy was reported to accelerate the rate of Cp protein synthesis and release with an increase of serum copper [20], the present study showed reduced of Cp activity. The decrease in circulating Cp would be an indicator to the degree of depletion of the mother copper deposits in order to deal with the fetal need, this finding was in agreement with the previous studies by Louro et al. [21] and Savila et al. [22], who studied the activity of Cp enzyme in pregnant women with preeclampsia.

AFP is a negative acute phase protein, which rise in maternal serum AFP until about week 32, because AFP is not metabolized by mother [23]. Krause et al. [24] found that the level of AFP were decreased in pregnancy complications, this is corroborated by the result of present study. Low maternal serum AFP levels had been associated with spontaneous abortion and infant death [25,26,27]. Explained the low maternal serums AFP were associated with chromosomal abnormalities fetal death and hydatidiform molar pregnancy.

There is no single way to characterize the pattern of β-HCG for ectopic pregnancy. The number of women with ectopic pregnancy who experience an increase in β- HCG values is approximately equal to the number of those who experience a decrease. The β-HCG profile in women with ectopic pregnancy can mimic that of an intrauterine pregnancy or a completed spontaneous abortion in approximately 29% of cases [28]. The ability to quantitate the β-HCG level is useful in the monitoring germ cell and trophoblastic tumors follow up care after miscarriage and in diagnosis of and follow up care after treatment ectopic and molar pregnancy. Munim et al. [29] suggested that low maternal serum β- HCG at 10–14 weeks of gestation are associated with subsequent development of pregnancy complications.

In another hand, Cowans et al. [30], describe the first trimester, and conclude that there was an elevation of β- HCG has not been associated with a significant increase in pregnancy complications.

During normal pregnancy, maternal hormones and locally acting cytokines play a key role in regulating the onset of labor, cervical ripening, uterine contraction, and delivery. Maternal infections during pregnancy have been demonstrated to perturb this normal cytokine and hormone-regulated gestation [31].

IL-1α level were decreased in all studied groups this may be contributed to autoimmune susceptibility, inflammatory disorders and pregnancy complications including preeclampsia , preterm delivery, implantation failure and recurrent miscarriage [32]. Resent studies suggest that increased synthesis and secretion of acute phase proteins by heaptocytes is triggered by interleukin 1 produced by mononuclear phagocytes activated directly or indirectly by acute phase stimuli. Thus in pregnancy, mononuclear phagocytes processing to release interleukin 1 to stimulate acute phase protein synthesis in the liver. Gestational hormones could operate at the level of the mononuclear phagocytes or directly on the liver. Alternatively, it must be consider whether in pregnancy the maternal liver is the sole site of synthesis of acute phase protein [33].

Elevation in the levels of both α2- MG and α1- AT in all studied groups in this study compared to control group indicated that the two proteins are involved in inhibits a wide variety of proteases and possesses anti inflammatory activities [34]. Recent reports indicate that α2-MG is capable of binding hydrolases required for enzyme mediated tumor invasion. The body seems to perceive ectopic and molar pregnancy in a similar fashion as a tumor. The nature of enzymes and other molecules interacting with α2-MG in molar pregnancy has not been elucidated as yet although it is reported to bind cytokines and growth factors required for tumor proliferation [35]. An investigation into the plasma proteinase profile of these patients is required. It is a tempting to suggest here, that α2-MG estimation in molar pregnancy may be used as an indicator of trophoblastic disease in conjugation with high β-HCG.Mckay et.al. [36] noted that the trophoblastic becomes thinner as pregnancy progresses and showed that the functional role of α1- AT in coagulation and fibrinolytic system during pregnancy is doubtful.

Maya et al. [37], showed that α1- APand α2- MG increased in molar and ectopic pregnancy, leading to formation of proteinase inhibitor complexes stimulates the synthesis of markedly increased level of α1- AT and α2- MG probably by the maternal liver.

4. CONCLUSION

The study concluded that the above biochemical parameters may play a role in monitoring the pregnancy complications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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