Correlation of serum biomarkers with treatment result in women with first trimester threatened miscarriage

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Abstract

Objectives: *To assess the correlation of serum biomarkers with treatment result in women with first trimester threatened miscarriage.*

Materials and methods: *Cross-sectional observative study in 107 patients which were first trimester threatened miscarriage at Department of Obstetrics and Gynecology, Hue Central Hospital and Hospital of Hue Medicine and Pharmacy University from March 2021 to June 2022. Inclusion criteria included women with threatened miscarriage and gestational age ≤ 13 weeks and 6 days. Participants were measured the levels of biomarkers (PAPP - A, CA 125, β-hCG, progesterone) and divided into two groups: group 1 included 90 women with successful treatment (continued pregnancy), and group 2 included 17 women with failed treatment (miscarriage, stillbirth).*

Results: *The level of maternal serum PAPP - A, β-hCG and progesterone for the successful treatment group were higher than the failed treatment group (p < 0.05). The median PAPP - A in group 1 and group 2 were 0.59 (0.12 - 2.04) IU/L and 0.02 (0.01-0.04) IU/L, respectively. The cut-off limit of 0.088 IU/L of PAPP - A level achieved sensitivity of 77.8% and specificity of 94.1%. The cut-off limit of 8004.5 mIU/ml of β-hCG level for ≤ 6 weeks gestational age group achieved sensitivity of 92% and specificity of 84.6%. The cut-off β-hCG value of 29162.5 mIU/ml for over 6 weeks to 13 weeks and 6 days group gave a sensitivity of 98.5% and specificity of 100%. The threshold progesterone value of 14.13 ng/dl gave a sensitivity of 93.3%, specificity of 94.1%. The median CA 125 in the group 2 was 74.01 (32.75 - 103.00) U/ml, that was higher than group 2 which was 40.90 (27.28 - 76.76) U/ml, p > 0.05. The cut-off CA 125 value of 68.55 U/ml for miscarriages gave a sensitivity of 58.8% and specificity of 71.1%.*

Conclusions: *Levels of maternal serum PAPP – A, CA 125, β-hCG and progesterone were early predictors of pregnancy outcome in women with first trimester threatened miscarriage.*

Keywords: *CA 125, Progesteron, PAPP - A, first trimester, threantend miscarriage.*

1. INTRODUCTION

Threatened miscarriage - miscarriage is one of the causes of bleeding in the first trimester of pregnancy. Spontaneous abortion is defined as pregnancy loss at a stage when the embryo or fetus is not capable of independent survival and that is before 20 weeks of gestation, with the exception of hydatidiform mole and ectopic pregnancy [1]. Early pregnancy loss is common, occurring in 10% of all clinically recognized pregnancies. Approximately 80% of all cases of pregnancy loss occur within the first trimester [2].

Causes of threatened miscarriage - miscarriage are diverse, complex and may have many causes together. It is estimated that 60% to 80% of all spontaneous abortions in the first trimester are associated with abnormal chromosomes, of which 95% are due to errors in maternal gametogenesis. In these 95%, autosomal trisomy is the most common chromosomal abnormality. Other factors associated with spontaneous abortions include infections, maternal anatomic defects, immunologic factors, environmental exposures, and endocrine factors. A large number of first trimester abortions have no obvious cause [3]. In a 2016 meta-analysis by Pillai et al of 19 studies in women with threatened miscarriage between 5 and 23 weeks, threatened miscarriage is reported to occur in about one-fifth of pregnancies but an estimated 3 - 16% of these subsequently miscarriage. Women with threatened miscarriage end up with repeated scans in early pregnancy units to allay their anxieties, which in turn adds to the increase in waiting times and costs. In the presence of reliable predictive biomarkers, the above challenges can be mitigated, and potentially new therapeutics can be directed at those identified at an increased risk of miscarriage. Various biochemical markers have been studied to establish if they are able to predict the outcome of threatened miscarriage, however results have been conflicting. Some of the commonly studied biochemical markers are serum hCG, progesterone, estradiol, pregnancy associated plasma protein A (PAPP-A), cancer antigen 125 (CA 125), human placental lactogen (HPL), alpha feto-protein (AFP), inhibin A, follistatin and activin A. Johns et al. (2007) studied inhibin A, activin A, hCG, PAPP-A and follistatin in a threatened miscarriage population. They showed significantly lower

concentrations of inhibin A, PAPP-A and hCG in those who had first trimester miscarriage compared with those who had term pregnancies. Ruge et al (1990) observed that serum levels of PAPP-A were significantly lower in women with vaginal bleeding in early pregnancy than normal pregnant women, however they failed to differentiate between those who either later miscarried or continued with their pregnancy [4].

Studies on the role of biomarkers in assessing treatment outcomes in patients with threatened miscarriage in our country are limited and have not received much attention. From that fact, this study aimed to assess the correlation of serum biomarkers with treatment result in women with first trimester threatened miscarriage.

2. METHODS

This cross-sectional observative study was conducted on 107 patients which were first trimester threatened miscarriage at Department of Obstetrics and Gynecology, Hue Central Hospital and Hospital of Hue Medicine and Pharmacy University between March 2021 and June 2022.

Inclusion criteria:

(1) Pregnancies with live embryo intrauterine, diagnose with threatened miscarriage was based on clinical assessment and evaluation by ultrasonography

(2) Gestational age ≤ 13 weeks and 6 days. Gestational age was calculated from the first day of the last menstrual period or confirmed by first trimester ultrasonography

(3) Participants were measured the levels of biomarkers: PAPP – A, CA 125, β-hCG, progesterone

Exclusion criteria:

(1) Cases of miscarried

(2) Cases of assisted reproduction

(3) Multiple pregnancies

(4) Vaginal bleeding cases such as: stillbirth, ectopic pregnancies, cervical insufficiency, fibroids, cervicitis...

(5) Pathologies that increase the blood levels of CA 125, PAPP – A such as: adenomyosis, asthma, renal impairment...

The recommended protocol was followed in the treatment of women with threatened miscarriage, after that, these patients were assessed the result of treatment upon discharge. Patients with threatened miscarriages were divided into two groups: group 1 included 90 women with successful treatment (continued pregnancy), and group 2 included 17 women with failed treatment (miscarriage, stillbirth).

Data analysis

The results are expressed as mean standard deviation if the data followed a normal distribution, while the nonnormally distributed measurements are reported as median (interquartile range). The differences in Progesterone concentrations among groups were compared using Independent-Samples T Test or One-Way ANOVA Test. The differences in PAPP – A, CA 125 and β-hCG concentrations among groups were compared using Wilcoxon-Mann-Whitney U Test or Kruskal-Wallis H Test. P value less than 0.05 was considered statistically significant. The discrimination attained between the two study groups was evaluated by receiver operating characteristic (ROC) curve analysis. Sensitivity, specificity, and area under the ROC curve (AUROC) were obtained for each model. 95% Confidence Interval (CI) were calculated for each of the estimates.

3. RESULTS

Table 1. Gestational age

Gestational age	n	%
≤ 6 weeks	38	35.5
> 6 - 8 weeks	26	24.3
> 8 - 11 weeks	22	20.6
> 11 - 13 weeks 6 days	21	19.6
Total	107	100.0

Table 1 show that the incidence of threatened miscarriage was higher in pregnant women in less than 6 weeks of gestation age (35.5%) whereas it was decreased gradually in the next gestational age groups.

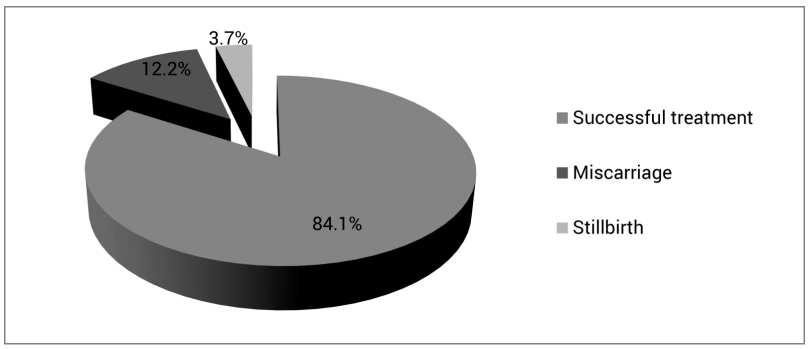

Figure 1. Treatment result

The rate of successful treatment was 84.1%, 17 failed treatment cases accounted for 15.9%, of which 12.2% cases of spontaneous abortion, 3.7% cases progressed to stillbirth (Figure 1).

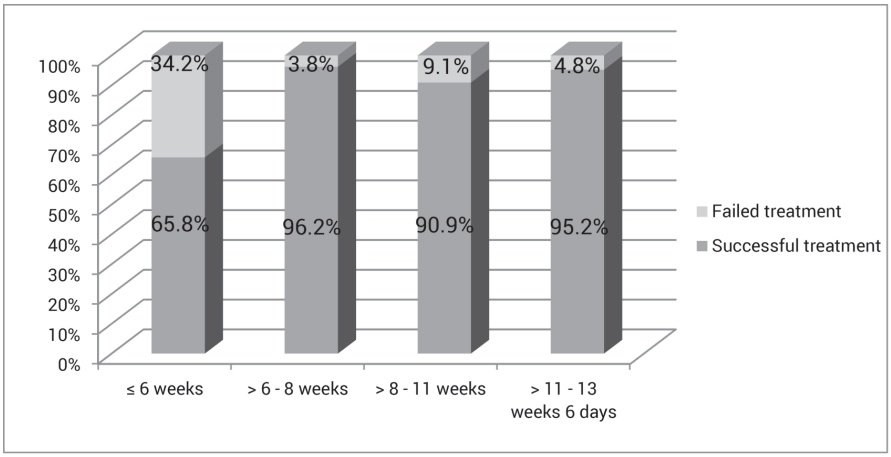

Figure 2. Treatment result according to gestational age

Figure 2 show that the rate of successful treatment in the gestational age groups over 6 weeks is high (> 90%). In the group of gestational age less than 6 weeks, there were 13/38 failed treatment cases accounting for 34.2%.

Table 2. Treatment result according to levels of PAPP - A, CA125, β-hCG and progesterone

Treatment result	n	PAPP-A IU/L	CA 125 U/ml	β-hCG mIU/ml	Progesterone ng/ml
Successful	90	0.59	40.90	87070.50	26.76
Failed	17	0.02	74.01	4333.00	6.60
		p < 0.05	p > 0.05	p < 0.05	p < 0.05

The levels of PAPP - A, β-hCG and progesterone were statistically significantly higher in the successful treatment group than in the failed treatment group. The level of CA 125 in the group of failed treatment was higher than in the group of successful treatment, p > 0.05 (Table 2).

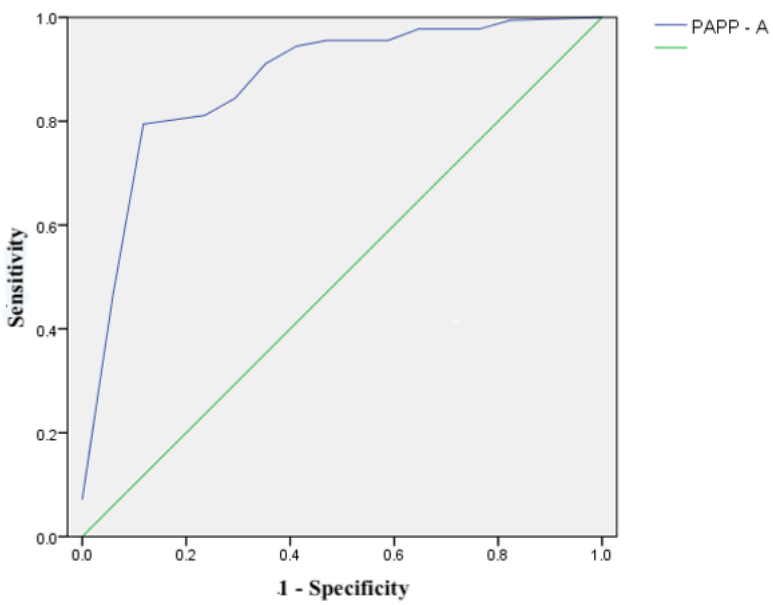

Figure 3. ROC curve of PAPP - A for successful treatment

The area under curve was calculated and was found to be 0.879 with 95% confidence interval (0.779 - 0.980) and with the cut-off PAPP – A level of 0.088 IU/L achieved sensitivity of 77.8% and specificity of 94.1% in predicting successful treatment (Figure 3).

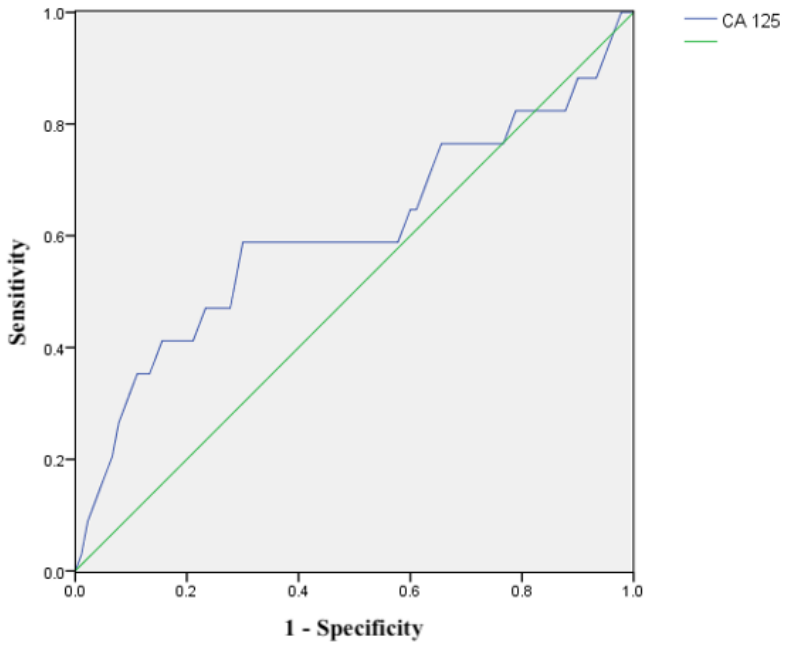

Figure 4. ROC curve of CA 125 for failed treatment

The area under curve was calculated and was found to be 0.606 (p > 0.05) with 95% confidence interval (0.436 - 0.775). The cut-off CA 125 value of 68.55 U/ml for miscarriages gave a sensitivity of 58.8% and specificity of 71.1% (Figure 4).

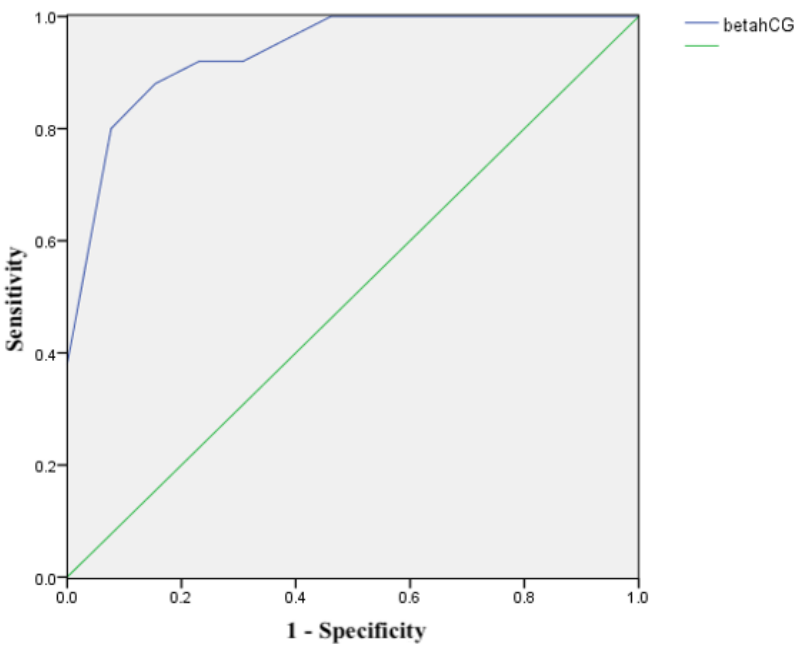

Figure 5. ROC curve of β-hCG in less than 6 weeks of gestational age group for successful

The area under curve was calculated and was found to be 0.951 with 95% confidence interval (0.889 - 1). The cut-off limit of 8004.5 mIU/ml of β-hCG level for ≤ 6 weeks gestational age group achieved sensitivity of 92% and specificity of 84.6% in predicting successful treatment (Figure 5).

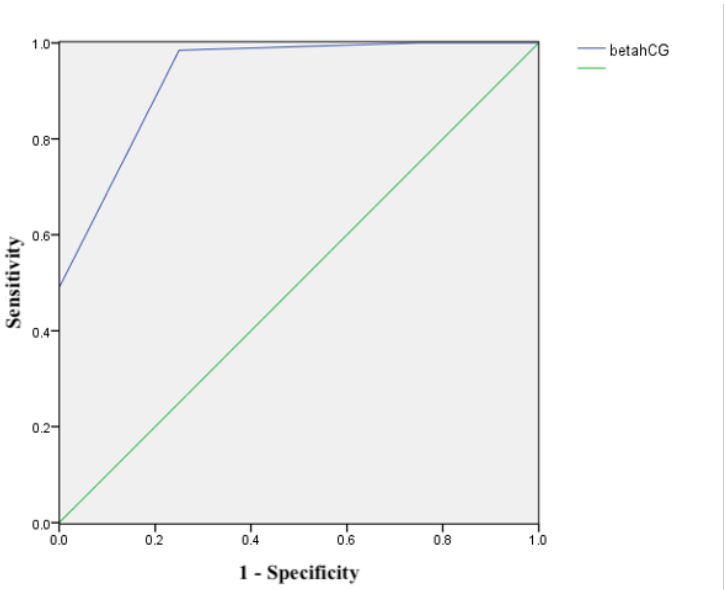

Figure 6. ROC curve of β-hCG in the group over 6 to 13 weeks 6 days of gestational age for successful treatment

The area under curve was calculated and was found to be 0.992 with 95% confidence interval (0.974 - 1). The cut-off β-hCG value of 29162.5 mIU/ml for over 6 weeks to 13 weeks and 6 days group gave a sensitivity of 98.5% and specificity of 100% in predicting successful treatment (Figure 6).

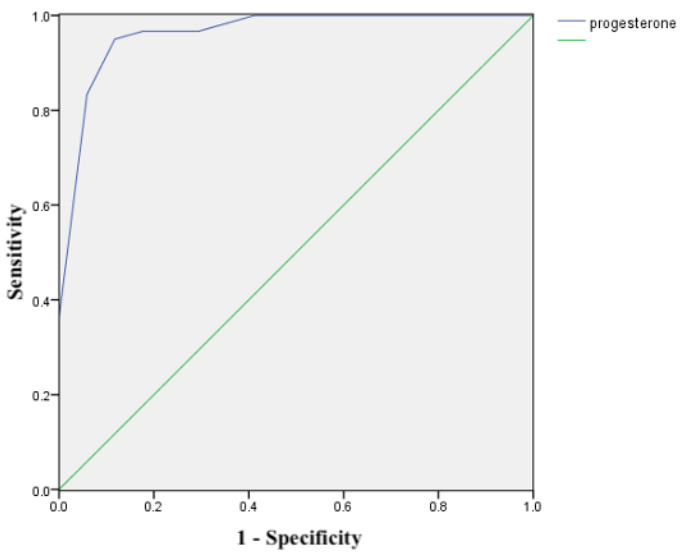

Figure 7. ROC curve of progesterone for successful treatment

The area under curve was calculated and was found to be 0.973 with 95% confidence interval (0.939 - 1). The threshold progesterone value of 14.13 ng/dl gave a sensitivity of 93.3%, specificity of 94.1% in predicting successful treatment (Figure 7).

4. DISCUSSION

Table 1 shows that the group of threatened miscarriage with gestational age ≤ 6 weeks accounted for the highest rate of 35.5%, followed by the group of pregnancies > 6 - 8 weeks accounted for 24.3%. According to Quyen H. Nguyen (2015), the rate of threatened miscarriage in the group ≤ 6 weeks was 41.2% and 6 - 8 weeks was 35.8% [5]. Kamble et al (2017) studied on 1007 women with vaginal bleeding in the first trimester pregnancy. It was seen that 77% patients who presented before 6 weeks aborted whereas only 7% patients who presented after 10 weeks aborted [6].

When the gestational age is smaller, the development of the fetus in the uterus is not stable, so the risk of

threatened miscarriage and miscarriage is high. In very early pregnancy, the corpus luteum produces progesterone. The shift from luteal production to placental production of progesterone occurs by the 7th week of pregnancy and can lead to a temporary reduction in progesterone levels if the placenta is not producing sufficiently. A drop in progesterone levels can cause vaginal bleeding and miscarriage [7].

There were 90/107 cases of threatened miscarriage that treated successfully accounting for 84.1%. This result was similar to that of some studies:

Table 3. Comparison of successful treatment rate with other studies

Author	Year	N	Materials	Successful treatment (%)
We	2022	107	Threatened miscarriage	84.1
Anh B.H. Nguyen [8]	2018	98	Threatened miscarriage	84.7
Quyen H. Nguyen [5]	2015	148	Threatened miscarriage	82.4
Loc T.V. Vo [9]	2014	117	Threatened miscarriage	83.8
Huong T. Le [10]	2013	328	Threatened miscarriage	83.8
Vijay K. Kadam [11]	2019	150	Threatened miscarriage	70.0
Maged Al Mohamady [12]	2016	100	Threatened miscarriage	80.0

The rate of successful treatment of threatened miscarriage in the gestational age groups over 6 weeks is high (> 90%). In the less than 6 weeks of gestation group, there were 13 failed treatment cases accounted for 34.2% and 25 successful treatment cases accounted for 65.8%. These results were quite similar to that of author Anh B.H. Nguyen (2018), the successful rate of treatment in more than 6 weeks group was high. Author observed that the rate of failed treatment in less than 6 weeks group was 26.7% and rate of successful treatment was 73.3% [8]. The rate of failed treatment in less than 6 weeks group in our study was higher than the author's, this was probably due to the small materials and uneven distribution of patients with threatened miscarriage between gestational age groups.

Progesterone, a female sex hormone, is known to induce secretory changes in the lining of the uterus essential for successful implantation of a fertilized egg. It has been suggested that a causative factor in many cases of miscarriage may be inadequate secretion of progesterone. Therefore, clinicians use progestogens, beginning in the first trimester of pregnancy, in an attempt to prevent spontaneous miscarriage [13]. Therefore, luteal insufficiency has a great influence on early fetal development.

In our study, levels of PAPP - A, CA125, β-hCG, progesterone correlated with the treatment results. The median of PAPP - A level in the successful treatment group with 0.59 (0.12 - 2.04) IU/L was higher than that in failed treatment group with 0.02 (0.01 - 0.04) IU/L (Table 2). Goetzl et al (2004) studied to estimate the likelihood of clinical early and late pregnancy loss as a function of first trimester maternal serum analytes and fetal nuchal translucency measurements. The cohort consisted of women who had a live fetus between 10 and 14 weeks of gestation and had no significant vaginal bleeding. Loss rates were only 0.36% at less than 20 weeks after normal PAPP - A levels, conversely, low levels of PAPP - A were individually associated with increased early loss. This association suggests that abnormally low value of PAPP - A may be early marker of abnormal implantation or other trophoblast dysfunction. Ong et al described a significantly lower median maternal serum PAPP - A between 10 and 14 weeks of gestation in women with subsequent pregnancy loss in a large prospective [14]. PAPP - A is produced in the placenta, the values of PAPP - A was significantly higher in the threatened miscarriage group with successful treatment than that in the failed treatment group. Using ROC curve for PAPP - A levels in evaluating the treatment result in threatened miscarriage, the cut-off limit of 0.088 IU/L of PAPP - A level achieved sensitivity of 77.8% and specificity of 94.1% (Figure 3).

The median of CA 125 values in the failed treatment group was 74.04 (32.75 - 103.00) U/ml, was higher than that in successful group with values 40.90 (27.28 - 76.76) U/ml (Table 2). These result were equivalent to that of author Anh B.H. Nguyen (2018), CA 125 levels in the failed group was 125.01 U/ml, compared to that in successful group was 55.05 U/ml [8]. Mohamady et al (2016) studied on 100 pregnant women with threatened miscarriage in Egypt showed that the levels of serum CA 125 for the threatened miscarriage (miscarried) group was 54.28 ± 11.4 IU/ml; while for the threatened miscarriage (continued) group was 18.81 ± 8.02 IU/ml. The difference was statistically significant ($p < 0.001$). The concentrations of CA 125 in the pregnant women who subsequently miscarried were higher than those

who did not, thus suggesting that the serum CA 125 levels are not so important in maintaining successful pregnancy. CA 125 might have a role in the preparation of the endometrium for successful implantation. More trophoblastic damage is associated with higher levels of CA 125 and CA 125 can be used as a prognostic factor to the outcome of pregnancy as it might be related to the extent of trophoblastic destruction [12]. Comparing between the levels of CA 125 and treatment results, we observed that the concentrations of CA 125 in women with failed treatment was higher than that in successful treatment group. Utilizing ROC curve analysis, this study determined that the best discriminatory value of serum CA 125 for the determination of miscarried was 68.55 U/ml with regard to sensitivity and specificity of 58.8 and 71.1% (Figure 4). Anh B.H. Nguyen (2018) studied on 98 patients with threatened miscarriage and used a cut-off value of 52.55 U/ml with a sensitivity of 72.3% and specificity of 60.0% [8]

The concentrations of β-hCG and progesterone in the successful treatment were significantly higher than those in the failed treatment group. Duan et al (2011) studied on 245 intrauterine pregnant women, 175 women with threatened miscarriages who consulted for vaginal bleeding received exogenous progesterone supplements, control group included 70 pregnant women. The mean serum levels of progesterone and β-hCG in patients with inevitable miscarriages (13.76 ± 5.52 ng/ml, 3647.00 ± 2123.00 mIU/ml, respectively), were significantly lower than these levels in ongoing pregnancies (25.47 ± 6.18 ng/ml, 8492.00 ± 2389.00 mIU/ml, respectively) [15].

β-hCG and progesterone are commonly markers that used to assess the fetal viability. In this study, serum β-hCG and progesterone levels showed a significant difference between the group of women that continued and the group of women that failed pregnancies. There was a highly significant increase in serum β-hCG and progesterone levels in women who continued pregnancies compared with failed pregnancies.

Utilizing ROC curves analysis, this study determined the best discriminatory values of serum β-hCG and progesterone for the determination of continued pregnancy. The cut-off limit of 8004.5 mIU/ml of β-hCG level for ≤ 6 weeks gestational age group achieved sensitivity of 92% and specificity of 84.6%. The cut-off β-hCG value of 29162.5 mIU/ml for over 6 weeks to 13 weeks and 6 days group gave a sensitivity of 98.5% and specificity of 100% (Figure 6). The threshold progesterone value of 14.13 ng/dl gave a sensitivity of 93.3%, specificity of 94.1% (Figure 7).

Anh B.H. Nguyen (2018) showed that β-hCG and progesterone were both very valuable in early diagnosis of pregnancy with threatened miscarriage, whether it continues to develop normally or not. Author used a cut-off β-hCG value of 28374 mIU/ml with a sensitivity of 88%, specificity of 86.7% and reported 16.51 ng/ml as a cut-off progesterone value that ongoing pregnancy and reported a sensitivity of 91.6% and specificity of 86.7% for this level [8].

Duan et al (2011) used ROC curves to estimate for β-hCG, progesterone and the combinations of two variables regarding outcome of threatened miscarriage. On the basis of this study, serum β-hCG level had 64.1% sensitivity and 81.4% specificity at a cut-off value of 7236 mIU/ml in diagnosis of inevitable miscarriage. Serum level of progesterone had 76.1% sensitivity and 70.4% specificity at a cut-off value of 16 ng/ml [15]. Kadam et al (2019) researched on 150 patients with early pregnancy threatened miscarriage less than 12 weeks, complaints of vaginal bleeding with or without lower abdominal pain to evaluate the predictive value of single serum progesterone level. The area under curve was calculated, and a cut-off value of 10.08 ng/ml was obtained, 83.8% of viable pregnancies had serum progesterone more than or equal to 10.08 ng/ml which was statistically significant [11].

The cut-off values of β-hCG and progesterone that we found were quite similar to other authors. β-hCG and progesterone are both very valuable in following up the treatment of women with first trimester threatened miscarriage.

5. CONCLUSIONS

Levels of maternal serum PAPP - A, CA 125, β-hCG and progesterone were early predictors of pregnancy outcome in women with first trimester threatened miscarriage.

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