Conference Paper

The Accuracy of Single Progesterone, Single β-hCG, and their Combination Measurement in Predicting Early Miscarriage: A Prospective Study in Outpatient Setting

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Abstract

Introduction. Most of miscarriage events occurred during the first trimester of pregnancy. Recent studies found the beneficial effects of maternal serum markers to predict pregnancy outcomes. However, study in Indonesian setting was still limited, especially in outpatient setting. The aim of this study was to evaluate serum progesterone and β-hCG measurement as a beneficial predictor of miscarriage. **Materials and Methods.** This was a prospective study recruiting outpatients pregnant women in Aceh who seek first medical attention for their pregnancy during January 2013 to January 2015. Serum progesterone and β-hCG level were measured beside routine obstetric procedure. The discrimination attained between miscarriage and non-miscarriage groups of pregnant women at the end of first trimester was evaluated using logistic regression and receiver operating curve analysis. **Results.** Among 70 pregnant recruited in this study, nineteen of them (27.1%) experienced miscarriage. Serum progesterone level of women in miscarriage group was lower than non-miscarriage group (17.85 (IQR 13.26-21.15) ng/dl vs 33.67 (IQR 21.83-44.14), $p < 0.001$). Serum β-hCG level was also lower in miscarriage group (10 681 (IQR 5 787.5-26 577.5) mIU/ml vs 48 109 (IQR 17 137-93 915) mIU/ml, $p = 0.001$). Single progesterone measurement gave a good predictor ability for miscarriage with 82.2% accuracy, 86.3% sensitivity and 73.7% specificity if 19.5 ng/dl was used as a cut-off point. **Conclusion.** Maternal serum progesterone level could be a good predictor for miscarriage during the first trimester of pregnancy. Single β-hCG serum in combination with progesterone serum measurement only had little added value for predicting miscarriage.

Keywords: β-hCG, miscarriage, progesterone

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1. Introduction

Miscarriage is one of the stressful experiences faced by pregnant women. Miscarriage is defined as pregnancy loss in the early 20 weeks of gestation, yet most of them occurred during the first trimester. It was reported that up to 20% pregnancy would come into miscarriage [1]. In Indonesia, miscarriage prevalence was varied between

2.5-15%. Miscarriage remains a national problem as its one of the main causes of maternal mortality [2].

Previously, Hasan et al found that early vaginal bleeding could be a predictor for miscarriage. However, there were different risk of miscarriage based on reported duration of bleeding, heaviness of bleeding, and accompanying pain symptoms [3]. Hormonal predictors (progesterone and β-hCG) were raised for potential of miscarriage predictor as their referred as reliable single markers [3]. Progesterone is a steroid hormone secreted by the granulosa cells in the ovary and attributed to the physiology of pregnancy. This hormone play central role for preparing uterus for the blastocyst implantation. The other importances of this hormone were inhibiting contraction of the uterus and suppression of the maternal immune system [4]. Elson et al and Abdelazim et al found progesterone as a potential predictor of successful pregnancy [5,6]. Recent meta-analysis also support a single measurement of progesterone to predict a viable pregnancy outcomes [7]. Besides, combination of serum progesterone with serum β-hCG increased the accuracy for predicting the outcome of threatened miscarriage [8]. Thus, this study aimed to identify potential role of these two hormones in predicting miscariage during the first trimester of pregnancy in outpatient setting of Indonesian population.

2. Experimental Details

2.1. Study Design and Subjects Recruitment

This study was conducted in a private clinic in Banda Aceh, Indonesia, owned by the author over two years from January 2013 to January 2015. Patients were outpatient pregnant women attending for the first visit to obstetric physician. The inclusion criteria were confirmed pregnant women, 20-45 years old, and fulfilled the informed consent form. Patients who had prior history of abnormal vaginal bleeding, currently or recurrent vaginal infections (having symptoms of abnormal *fluor albus* in the current pregnancy), recurrent miscarriage, infertility (defined by failure to pregnant after 12 month or more regular unprotected sexual intercourse), and anatomical abnormality based on general physical examination by physician were excluded. Subjects were recruited consecutively during the study period. All subjects were recruited consecutively and both β-hCG and progesterone measurements were financed by patients themself if they agreed to participated in this study. The study protocol was approved by ethical committee Faculty of Medicine, Syiah Kuala University, Aceh, Indonesia

2.2. Data Collection

Five ml of blood samples were taken by a nurse or midwife from antecubital vein of subjects for serum β-hCG and progesterone assays. A trained laboratory staff seperated serum by centrifugation technique and stored it at 2-8°C. A clot activator container (5 ml Intherma^{TM}: Gel + clot activaor) was used to store blood samples. Progesterone assay was performed using chemiluminescent methods in Prodia® Laboratory,

Variables	Value/n	IQR[a]
Age (years)	30	(27-33)
Gestational week(s)	7	(6-8)
Outcomes of first trimester (n)		
Miscarriage	19	27.1
Non-miscarriage	51	72.9
a. IQR = Interquartile range		

Table 1: Characteristics of subjects.

Banda Aceh, within 48 hours. Meanwhile, β-hCG assay quantitative measurement was performed using Chemiluminescent Microparticle Immunoassay (CMIA) after serum stored in K3-EDTA tube. All subjects were followed during their pregancy course by ultrasonography examination for the viability and the outcome of pregnancy. Outcome at the end of first trimester was recorded.

2.3. Statistical Analysis

All numerical data were presented either as mean $\pm$ standard deviation or median (interquartile range: 25^{th} - 75^{th} percentile) for normally and non-normally distributed data, respectively. Assessment for data normality was based on Kolmogorov-Smirnov test. All subjects were then grouped either as miscarriage or non-miscarriage groups. Comparative analysis of serum progesterone and β-hCG were done using independent t-test for parametric data or Mann-whitney test for the non-parametric ones. P value < 0.05 was considered significant. Statistical analysis was performed using *SPSS (Statistical Package for Social Science, Chicago., Inc) for windows* version 22

3. Results

3.1. Characteristics of Subjects

Seventy patients were recruited for this study. The median age of patients was 30 (interquartile range 27-33) years old. From all subjects, 19 patients confirmed for miscarriage during the first trimester of follow-up (Table 1).

3.2. Progesterone and β-hCG Hormones Level

The median time for serum progesterone and β-hCG being collected was seventh weeks of gestational age. There were significant differences between initial serum progesterone and β-hCG level between groups, as shown in Table 2. These two hormones level were significantly higher in non-miscarriage groups. The area under the curve (AUC) for progesterone and β-hCG testing were 82.2% and 75.0%, respectively (Figure 1).

Hormone	Miscarriage	Non-miscarriage	*P*
Progesterone (ng/ml)	17.85 (13.26 – 21.15)	33.67 (21.83 – 44.14)	<0.001
β-hCG (mUI/ml)	10 681 (5 787.5 - 26 577.5)	48 109 (17 137 – 93 915)	0.001
[a]Mann-Whitney U test; data presented as median (interquartile range)			

Table 2: Progesterone and β-hCG level of subjects.

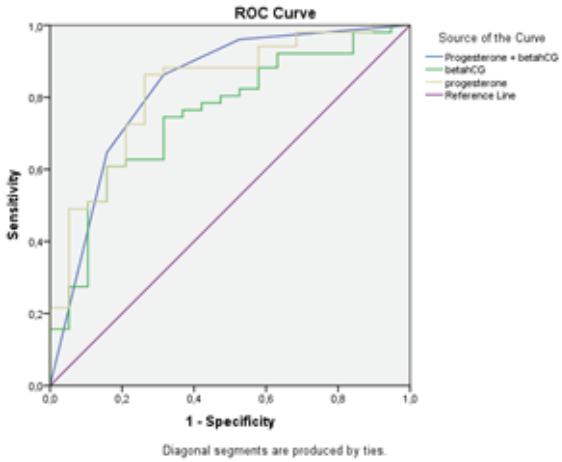

Figure 1: Receiver operating curve (ROC) of progesterone (AUC 0.822), β-hCG (AUC 0.750), and combination of progesterone + β-hCG (AUC 0.827).

We then tried to find the cut-off value for serum progesterone and β-hCG level in order to classify our subjects as at higher or lower risk for miscarriage. We then found the progesterone cut-off point was 19.5 ng/dl (sensitivity= 86.3%, specificity= 73.7%) and β-hCG cut-off point was 18 000 mIU/ml (sensitivity= 74.5% specificity= 68.4%).

Using the logistic regression, we found both progesterone and β-hCG were useful to predict miscarriage during the first trimester of pregnancy. The strength of the association can be seen from the value of odds ratio (ExpB) presented in Table 3. Serum progesterone level had higher strength of association compared to β-hCG. The combination of using two cut-off points from progesterone and β-hCG level had AUC 0.827 (Figure 1).

Variables	B (SE)	Exp(B)	95% CI
Constant	- 2.53 (0.56)	0.08	
Progesterone (< 19.5 ng/dl)	2.46 (0.67)	11.74	3.17 – 43.43
β-hCG (< 18 000 mIU/ml)	1.37 (0.67)	3.95	1.07-14.52
[a]SE = Standard Error			

Table 3: Logistic Regression model for miscarriage using cut-off points of progesterone &β-hCG.

4. Discussion

This study aimed to observe the ability of measuring serum progesterone and β- hCG early in the first visit of pregnancy to predict miscarriage during the first trimester. Around 30% subjects in this study would have miscarriage during the first trimester if their pregnancy, similar with previous report by Abdelazim et al [5]. Previously, Duan et al performed a retrospective study which recruited pregnant women in China population. They found that combination of serum progesterone and β- hCG level had highest sensitivity and specificity compared to single measurement alone, either progesterone or β- hCG level. The combination of these two hormones measurement gave 85.7% accuracy with 88.1% sensitivity and 84.3% specificity. Single progesterone alone only gave 72.5% accuracy with 76.1% sensitivity and 70.4% specificity [8].

Women in early course of pregnancy would seek medical attention especially if they suffered from pain or vaginal bleeding. Although ultrasonography had best diagnostic ability, it may give non conclusive result if gestational age is still too young [7]. A suboptimal rise of serum β- hCG (lower than 66% with two days interval serial measurement) was previously considered having a good prognostic accuracy. However, β- hCG should be carefully interpreted. It is advisable to combine it with the other tests [9]. Current meta-analysis supported the paradigm shift toward benefit of serum progesterone level alone [7]. Al Jufairy also reported that lower level of progesterone could predict not only miscarriage but also ectopic pregnancy [10].

Although our finding found combination progesterone and β- hCG measurements gave highest accuracy, the added value of β- hCG level measurement was not significant compared to single progesterone measurement alone (Figure 1). The explanation of this might be answered by a study by Al-Jeborry which found that serum β- hCG level measurement would predict the prognosis of pregnancy only if it was measured in serial manner during the sixth and eight weeks of gestational age with two to three days apart interval [11]. β- hCG is a glycoprotein produced by trophoblast and being detectable in maternal serum within the few days after implantation. The prognostic significance of serum β- hCG measurement was based on its natural variability level during pregnancy course, with high slope of increase during the first eight weeks of pregnancy and then slowly decline until labor. β- hCG is beneficial to predict complication of pregnancy including pregnancy loss or ectopic pregnancy [1,12]. There were multiple factors being analyzed for their benefits in predicting the outcome of pregnancy, including miscarriage. Gracia et al and de La Rochebrochard et al found that besides serum markers measurement, maternal age including those above 35 years old would predict miscarriage [13,14]. This was contrary to our result that maternal age did not significantly associated with miscarriage. Another potential maternal serum markers currently highlighted was inhibin A. Johns et al found that after controlling for serum β- hCG level, inhibin A was the only predictor for miscarriage [15]. Inhibin A and β- hCG are two substances produced by the growing trophoblast and lower level of these two hormones was associated with miscarriage [16].

Using the cut-off point of progesterone to a level of 19.5 ng/dl gave the best sensitivity and pecificity. This was similar with Abdelazim et al which found a 20 ng/dl as

a cut-off point had 95.1% sensitivity and 98.9% specificity to predict viable and non-viable pregnancy [5]. Progesterone plays important factor for successful pregnancy. It hampered maternal immunological properties and myometrium hypercontractility [17] Thus, based on recent meta-analysis, progesterone administration could prevent preterm birth in high risk maternal group of pregnancy [18]. Whereas, other study still questionned the efficacy of progesterone supplementation due to small sample size of published randomized trials [19]. However, supplementation with β- hCG had no potential advantange to overcome threatened miscarriage [20].

Several limitations encountered in this study. The size of our study population still relatively small compared to previous study in other settings [5,8]. Also, we had limited data regarding other potentials confounders being reported elsewhere (e.g. chromosomal abnormality, quantitative data about maternal complaints of bleeding and pain, paternal age, and maternal consumption of potential related compounds) [7,14,21,22]. However, our study performed in outpatient setting where most of Indonesian women seek medical advice for their pregnancy for the first time based on author's knowledge. Further larger multicenter study was needed involving larger sample size with different ethnicities and locations in Indonesia. Besides, neonatal outcomes were still not reported in this study.

5. Conclusion

Both single measurement of maternal progesterone and β- hCG were helpful to predict miscarriage during the first trimester of pregnancy. However, the combination of these two measurements only added little value compared to single progesterone measurement alone. Single progesterone measurement had accuracy as high as 82.2%. A cut-off value at a level to 20,0 ng/dl had 86.3% sensitivity and 73.7% specificity.

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