ORIGINAL ARTICLE

Potential use of single measurement of serum progesterone in detecting early pregnancy failure

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Abstract

Early pregnancy failure is a common pregnancy complication. In clinical practice, the time delay to distinguish viable from nonviable pregnancy is often distressing to patients and doctors. A highly sensitive and specific biomarker that accurately discriminates between viable and nonviable pregnancy would be useful for early intervention. Progesterone has been shown as a biomarker of early pregnancy failure. However the usefulness is still questionable due to the different cut-off values used. A study was conducted to determine the role of progesterone as a marker of early pregnancy failure and to establish the cut-off value in discriminating between viable and nonviable pregnancy. The study was carried out in the Obstetric and Gynecology Patient Admission Centre (OBPAC), Universiti Kebangsaan Malaysia Medical Centre (UKMMC) for a period of twelve months. Ninety-five pregnant women of 13 weeks or less period of amenorrhoea (POA) were recruited. Fourteen normal pregnant women were controls. The patients with early pregnancy failure were classified according to types of abortion. Single measurement of serum progesterone was carried out during admission. The outcome of pregnancy was followed up until 22 weeks of POA to ascertain viability of the fetus. Median progesterone levels were significantly lower in women with nonviable pregnancies compared with viable pregnancy [10.7ng/ml (0.60-49.80) vs. 45.9ng/ml (15.40-127.20) respectively, $p<0.001$]. Progesterone levels were also significantly lower in threatened abortion patients with outcomes of nonviable pregnancy compared with pregnancies that progressed on to the viability period [23.3 ± 12.0 vs. 89.7 ± 33.2 respectively, $p<0.001$]. At cut-off value of 32.7ng/ml, progesterone had 90% sensitivity with 75% negative predictive value and 92% specificity with 97% positive predictive value. The area under curve for progesterone was 0.95 (95% Confidence Interval, 0.903-0.990). In conclusion, these findings indicate that serum progesterone can be used as a marker for early pregnancy failure.

Key words: progesterone, pregnancy, abortion

INTRODUCTION

Early pregnancy failure is a common pregnancy complication whereby 15% to 20% of clinically recognized pregnancies end up as abortions.[1] Historical definitions of early pregnancy failure include threatened abortion, incomplete abortion, complete abortion, inevitable abortion and missed abortion with the outcome of viable or nonviable pregnancy. In clinical practice, the time delay to distinguish viable from nonviable pregnancy is often distressing to patients and doctors.

Ultrasound scanning is probably the best single diagnostic and prognostic test available for diagnosing early pregnancy failure. However, there were certain conditions where both the ultrasonographic evidence and clinical findings were indeterminate.[2] In addition, this technique is dependent upon the skill of the operator and thus the results are not always consistently reproducible. Therefore, a highly sensitive and specific biomarker is required to determine the pregnancy viability for early intervention.

Progesterone is a C-21 steroid hormone secreted by granulosa cells of the ovary. This hormone is important to promote endometrial decidualization by preparing the uterus for

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implantation of the blastocyst and in maintaining the pregnancy.[3] Other important physiological function of progesterone is to inhibit smooth muscle contractility, decrease prostaglandin formation which help maintain myometrial quiescence and prevent the onset of uterine contractions, and inhibit immune responses like those involved in graft rejection.

Progesterone assays are currently available in most immunoassay platforms and have shown excellent performance in terms of assay sensitivity, specificity, accuracy and precision with rapid turn around times. Furthermore, the cost per test for progesterone assay is affordable.

Several studies have shown that progesterone is the most specific biomarker for distinguishing viable from nonviable pregnancies.[4,5] The downfall of progesterone as a biomarker is due to the different cut-off values used by researchers.[4,6-8] The cut-off values were also determined on different study populations.

The aim of our study is to determine the role of progesterone in detecting early pregnancy failure and the cut-off value of progesterone level in discriminating between viable pregnancy from nonviable pregnancy among the Malaysian population attending antenatal check up in the Obstetric and Gynecology Patient Admission Centre (OBPAC), Universiti Kebangsaan Malaysia Medical Centre (UKMMC)

MATERIALS AND METHODS

A cross sectional study was carried out in OBPAC, UKMMC for a period of twelve months. Women who conceived spontaneously and presented with clinical evidence of abortion under 13 weeks of gestation were included in this study. Pregnant women with vaginal bleeding due to local causes (cervical polyp, cervical cancer, local trauma) or who had assisted pregnancies or ectopic pregnancies were excluded from this study.

A control group of normal pregnancies with the same gestational age was selected. Normal pregnancies were categorized as viable intrauterine pregnancies documented by ultrasonography. For all patients, the gestational age was calculated from date of last menstruation. All patients with threatened abortion were followed-up until age of viability of 22 weeks of gestation to determine the outcome of pregnancies (viable or nonviable pregnancy).

Blood samples were collected immediately after admission and before initiation of treatment or surgical intervention. A maximum of 5ml

venous blood was collected in a plain tube, centrifuged and the serum were aliquoted and stored at -70°C until analysis was carried out. Serum progesterone was measured on a fully automated analyzer (ARCHITECT i2000sr) using Chemiluminescent Microparticle Immunoassay (CMIA). The ARCHITECT progesterone assays measure progesterone concentrations between 0.1ng/ml to 36.0ng/ml. The intra-assay coefficient of variation (CV) ranged from 3.4% to 5.5% and 1.6% to 2.2% for low and high progesterone levels respectively. Measurements of progesterone concentrations were done in batches to minimize analytical variation. For each batch, three levels of internal control were performed to validate the assay performance. All clinical and laboratory data were stored and analyzed using the Statistical Package for Social Sciences (SPSS) software version 11.0.

The values of progesterone were expressed as mean values and standard deviation for normally distributed data and as median values and ranges for data that were not normally distributed. The Shapiro-Wilk test was used to test the normality of distribution as the sample size was small.

Comparison of median values of progesterone between viable and nonviable pregnancies groups was performed using the Mann-Whitney U-test (nonparametric test). Student's t-test (parametric test) was used to compare the mean progesterone levels in the outcome of threatened abortion (viable and nonviable pregnancies). Receiver Operator Characteristic (ROC) curve was constructed to evaluate the level of serum progesterone in distinguishing viable pregnancies from nonviable pregnancies. The diagnostic value of progesterone as a prognostic value in pregnancy failure was established by its best sensitivity, specificity and optimal diagnostic cut-off from the Receiver Operator Characteristic (ROC) curve analysis. In all statistical analyses, $p<0.05$ (95% confidence interval) was considered significant.

Ethics
The study was approved by the research and ethical review boards of the Universiti Kebangsaan Malaysia Medical Centre (UKMMC).

RESULTS

A total of 81 patients with clinical evidence of abortion and 14 normal pregnancies were recruited for this study. The patient group comprised 83 Malays (87%), 2 Indians (2%), 9 Chinese (10%) and 1 patient under the

"others" race category (1%). Patients with clinical evidence of abortion were further grouped according to the type of abortion i.e. missed, complete, incomplete and threatened abortion. Of these 81 patients, 57% had missed abortion, 10% had threatened abortion, 28% had incomplete abortion and 5% had complete abortion. The median maternal ages for the group of patients with clinical evidence of abortion and control groups were 30 years (22-42 years) and 29 years (24-39 years) respectively. There were no significant differences in maternal or gestational ages between the patients and normal control group. There were also no significant differences in race, gravidity or parity. No significant difference was observed between the gestational age of women who subsequently went on to have viable pregnancies with the gestational age of those who went on to have nonviable pregnancies.

Six out of eight patients with threatened abortion had pregnancy that reached the viability period (viable pregnancy) while the other two remaining pregnancies failed to progress to the viability period (nonviable pregnancy) making the pregnancy outcome of this study population of 20 patients with viable pregnancies and 75 nonviable pregnancies. Demographic information and clinical characteristics are presented in Table 1.

Serum progesterone values were significantly lower in women with nonviable pregnancies, compared with those of women with viable pregnancy. Progesterone values ranged between 15.40ng/ml and 127.20ng/ml for viable pregnancies and between 0.60ng/ml and 49.80ng/ml in nonviable pregnancies. Median progesterone values were 10.7ng/ml (0.60-49.80) for nonviable pregnancies and 45.9ng/ml (15.40-127.20) for viable pregnancies ($p<0.001$). In the threatened abortion patients, mean serum progesterone levels were significantly lower in patients with nonviable pregnancy outcome (23.3 ± 12.0ng/ml) as compared to those progressed onto viability period (89.7 ± 33.2ng/ml) ($p< 0.05$).

The ROC curve analysis demonstrated a significant ability of serum progesterone to differentiate between viable and nonviable pregnancies. The area under curve (AUC) for progesterone was 0.95 (95% CI, 0.903-0.990) with the parameters calculated from the ROC curve shown in Figure 1.

When using a progesterone concentration of less than 32.7ng/ml as a cut-off value for the diagnosis of nonviable pregnancy, sensitivity was 90% (95% CI), specificity 92% (95% CI), the positive predictive value was 97% (95% CI) and the negative predictive value 75% (95% CI). Taking cut-off values of progesterone as 32.7ng/

TABLE 1: Demographic profile of the study population (n= 95)

	Clinical abortion	Control	p value
Number of patients (n)	81	14	
Race			
Malay	70	13	
Chinese	9	1	
Indian	1	-	
Others	1	-	
Age, years (median)	30 (22-42)	29 (24-39)	0.559*
Gravidity (median)	2 (1-8)	2 (1-4)	0.965*
Parity (median)	1 (0-6)	1 (0-3)	0.581*
Gestational age (weeks)	10 (5-13)	10 (4-12)	0.454*
Types of abortion			
Missed abortion	46 (57%)		
Incomplete abortion	23 (28%)		
Complete abortion	4 (5%)		
Threatened abortion	8 (10%)		
1. Viable	6		
2. Nonviable	2		

Values are expressed as median (range) or number (%)
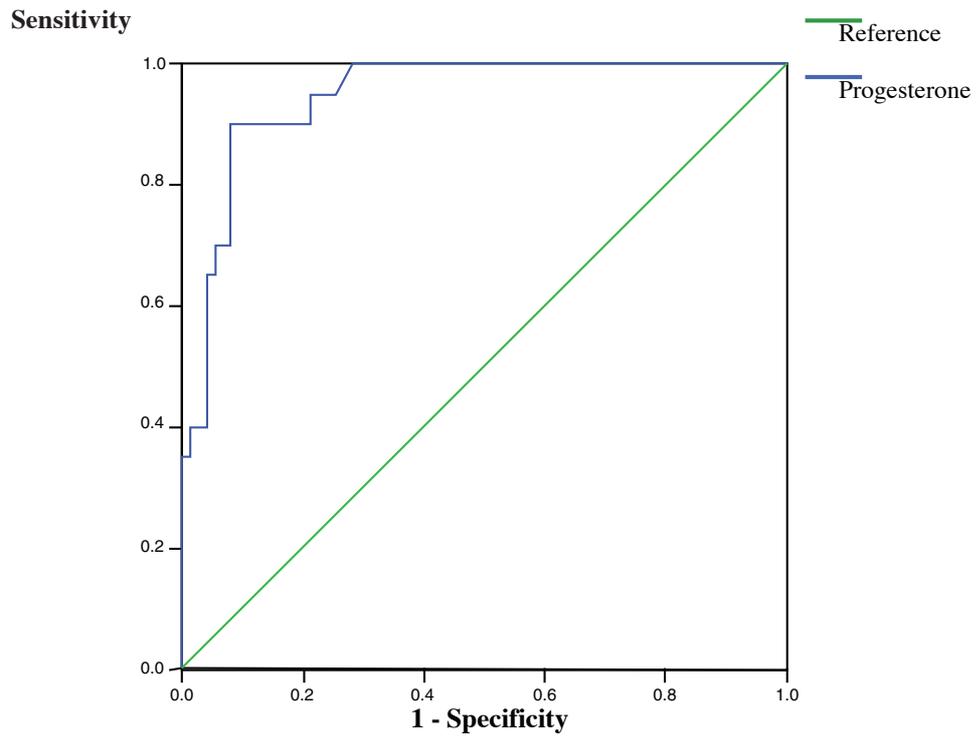By Mann-Whitney U test, statistical significant at $p<0.05$

FIG. 1: Receiver operator characteristic (ROC) curve of progesterone levels as diagnostic test for nonviable
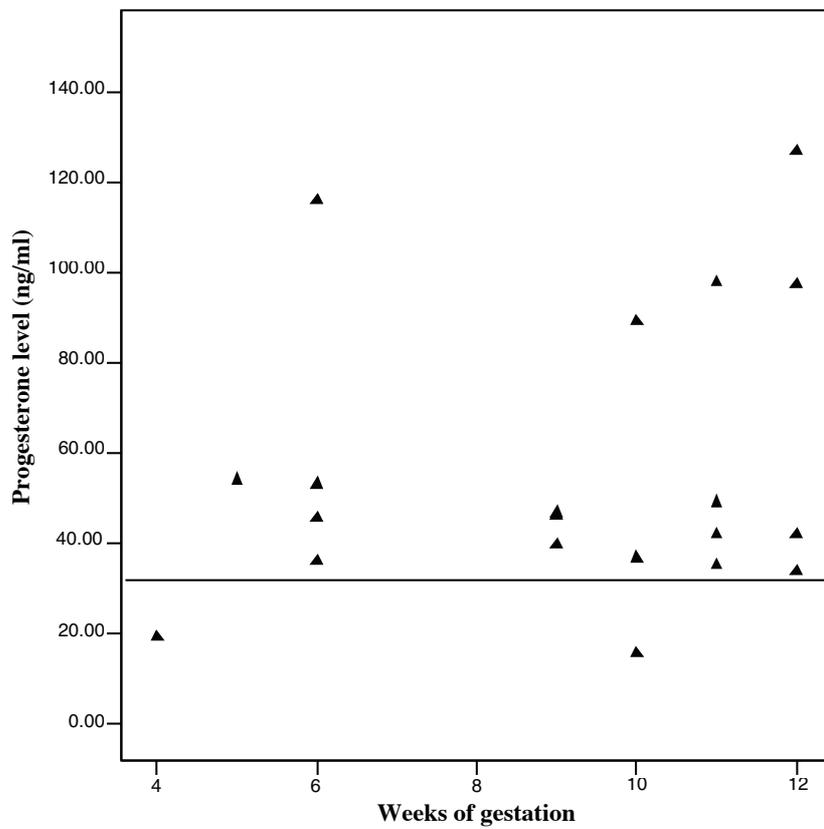pregnancy

FIG. 2: Progesterone level in the viable pregnancies. The line is at the suggested 32.7ng/ml cut off level

ml revealed that 90% of viable pregnancies and 92% of nonviable pregnancies had concentration of progesterone over and less the assigned threshold value of 32.7ng/ml respectively. The details of the distribution of viable and nonviable pregnancies values above and below the suggested cut-off value of 32.7ng/ml respectively are shown in Figure 2 and Figure 3.

DISCUSSION

This study has evaluated the role of a single serum progesterone measurement in the diagnosis of early pregnancy failure in women who presented with signs and symptoms of abortion. From the study, we found that the concentration of serum progesterone is significantly higher in the viable pregnancy compared to nonviable pregnancy. Our findings concur with the majority of researchers on the usefulness of progesterone as a diagnostic tool of nonviable pregnancies.[4,9]

We examined the possibility of using a single serum progesterone measurement in providing immediate prognosis of fetal viability in threatened abortion. From this study, we found that patients with threatened abortion in whom the pregnancy progressed to viability period had significantly higher progesterone values compared to those with nonviable pregnancy. This finding, was similar with the study conducted by Midha *et al*.[8]

From our study, the best combined specificity and sensitivity of progesterone for diagnosis of early pregnancy failure were 92% and 90% respectively. However, when compared to other studies, our cut-off value was higher as most researchers used the cut-off of 10ng/ml to diagnose nonviable pregnancies.[7,10] As studied by Mol *et al*,[11] differences in data collection or study design have a significant impact on the estimated discriminative capacity of serum progesterone measurement. Most of the previous study designs[4,12] were cohort studies which involved larger sample sizes. In addition, some studies have also included ectopic pregnancy as part of nonviable pregnancy, which was an exclusion criteria in our study.[9,13] Differences in progesterone assay measurement, standardization
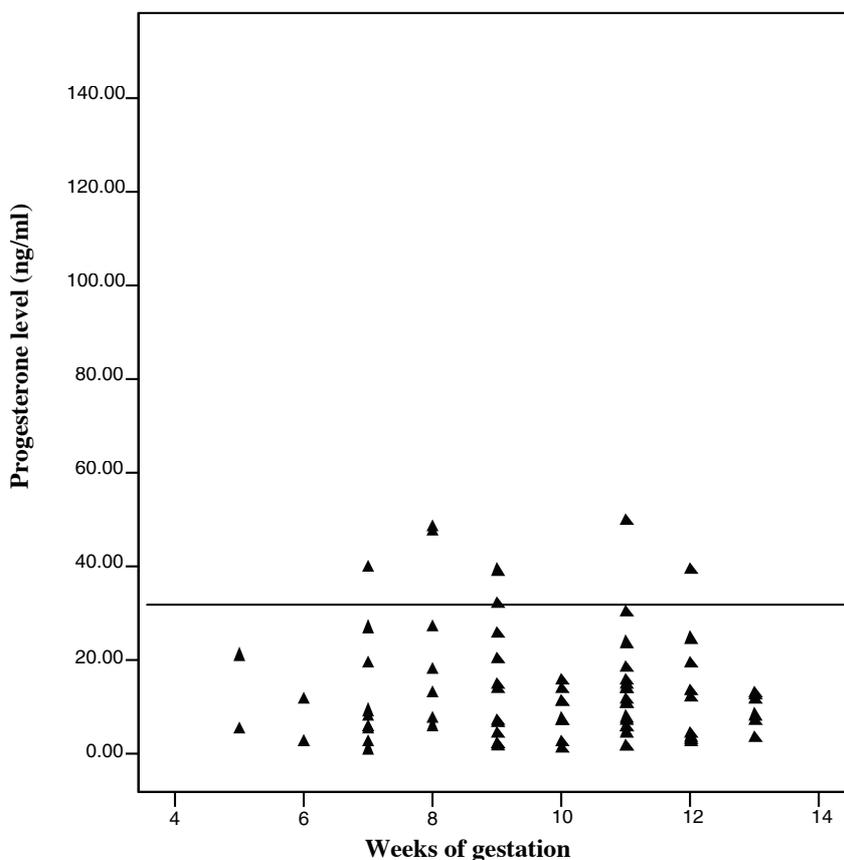

FIG. 3: Progesterone level in the nonviable pregnancies. The line is at the suggested 32.7ng/ml cut off level

and performance may also contribute to the variation of progesterone cut-off value. The other probable explanation of the different in our study compared to other studies may be attributed to variation in progesterone values among women from different population geographic background[14].

From this study, we concluded that a single measurement of serum progesterone has a role in determining pregnancy viability, and helps in the mode treatment and emotional support to the patient. Due to the different cut-off values, caution should be exercised in the interpretation of the progesterone values for determining nonviable pregnancy. The progesterone cut-off value obtained through studies on the local population is useful in the management of early pregnancy failure.

ACKNOWLEDGMENT

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