

ORIGINAL ARTICLE

Maternal level of pregnancy-associated plasma protein A as a predictor of pregnancy failure in threatened abortion

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Abstract

Threatened miscarriage is a common complication of pregnancy. Despite initial viability confirmation by ultrasound scan, some of these patients had further spontaneous abortion. A highly sensitive and specific biomarker would be useful to determine the outcome of pregnancy and to prevent emotional impact to these women. A prospective 14-month cohort study was conducted in the Obstetrics and Gynaecology Department of Universiti Kebangsaan Malaysia Medical Centre to determine whether low serum levels of pregnancy-associated plasma protein A (PAPP-A) measured in early pregnancy can predict the outcome of threatened abortion. 42 pregnant women between 6 to 22 weeks of gestation with threatened abortion and 40 controls were enrolled. Serum samples were collected at presentation and PAPP-A was assayed by electrochemiluminescent immunoassay technique. Pregnancies were followed-up until 22 weeks of gestations and the outcome documented. Nine patients (11%) developed spontaneous abortion and 73 patients (89%) had successful pregnancy. The median PAPP-A level was significantly lower in patients with spontaneous abortion compared to those who had successful pregnancies in the threatened abortion group: 0.78 MoM (0.41-1.00 MoM) vs 1.00 MoM (1.00-2.0 MoM) respectively ($p < 0.05$). The best sensitivity of 44% and specificity of 93% were obtained at the cut of value of 0.66 MoM (95% CI, 0.561-0.773). In conclusion, low PAPP-A value in threatened abortion women is associated with pregnancy failure, although the use of PAPP-A as a one-time single marker has limited value.

Key words: pregnancy-associated plasma protein-A, pregnancy, spontaneous abortion, threatened abortion.

INTRODUCTION

Threatened abortion is the most common complication of pregnancy and 15-20% end up with spontaneous abortion.¹ Although abortion is not generally associated with serious morbidity or mortality, very frequently it may cause profound emotional disturbance and significant psychological impact on these women. The physician has an important role in making an accurate diagnosis of pregnancy and to avoid giving false assurance. Ultrasound scanning is probably the best single diagnostic test available, however, more than 30% of these women progress to spontaneous abortion² regardless of viability confirmation initially by ultrasound scan. Therefore, a biomarker with good sensitivity and

specificity to successfully predict the outcome of pregnancy is of value.

Many studies have examined biochemical markers such as serum human chorionic gonadotrophin (hCG), progesterone, activin A and inhibin A to predict the outcome of threatened abortion.³⁻⁵ However, there were discrepancies in cut-off values, sensitivity and specificity among these studies. Recent studies have focused on pregnancy associated plasma protein A (PAPP-A) as a predictive marker of adverse pregnancy outcome including abortion.⁶⁻⁸

Pregnancy-associated plasma protein A (PAPP-A) is a protease for insulin-like growth factor binding protein-4 (IGFBP-4).⁹ Low levels of PAPP-A are associated with increased IGFBP

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and, consequently, low level of IGF which plays a key role in regulating trophoblast invasion of the decidua and thus fetal growth.¹⁰ Many studies have shown that abnormal serum level of PAPP-A may be a sign of abnormal function of the placenta that may manifest in placental-related complications such as spontaneous abortion.¹¹⁻¹²

The concentration of PAPP-A in maternal blood is influenced by gestational age at the time of blood sampling.¹³ For chromosomal testing, for instance, sampling between 9 to 14 weeks of gestation resulted in best detection rates.^{14,15} Since many of previous studies examining the relationship between PAPP-A levels and adverse outcome have obtained PAPP-A values from screening tests for chromosomal abnormalities, PAPP-A values were mostly available between 9 to 14 weeks of gestation. Our present study was designed to look at the earlier gestational age of 6 to 22 weeks. We did not include patients below six weeks of gestation because at the earlier gestational age of less than five weeks, the serum PAPP-A levels is not sufficient to differentiate between normal pregnancies with those that developed adverse outcome.¹³

The aim of study was to determine whether a low level of serum PAPP-A measured in threatened abortion pregnant women was associated with pregnancy failure.

MATERIALS AND METHODS

A prospective cohort study was conducted at the Antenatal Clinic, Patient Admission Centre (PAC) for Obstetrics and Gynaecology of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) from August 2010 to October 2011. Pregnant women between 6 to 22 weeks of gestation, who presented with vaginal bleeding, with or without abdominal pain, and with no cervical dilatation were included in this study. The diagnosis of threatened miscarriage was established by presence of clinical symptoms and ultrasound demonstrated intrauterine sac with cardiac activity.

Pregnant women with missed abortion confirmed by ultrasound scan, those with local cause of per vaginal bleeding like cervical polyp, cervical carcinoma or local trauma, those confirmed to have congenital anomalies and twin pregnancy and pregnant women who smoked were excluded from the study. Age and gestation matched pregnant women were recruited as the control. Pregnant women who were eligible for the study were recruited after full verbal

explanation and signing the consent form. They were also informed that the result of PAPP-A measurement in this study would not be used in the management of any pregnancy associated condition during their current pregnancy.

Ultrasound scan was performed for all patients and the control group. All patients and controls were followed-up until 22 weeks of gestations to determine the outcome which was either spontaneous abortion or pregnancy progress beyond 22 weeks.

PAPP-A measurement

Upon recruitment, 5 mls of venous blood were drawn and collected into plain tubes containing separating gel. The blood samples were centrifuged and the serum separated and stored at -70°C until analysis. Serum PAPP-A was measured on the fully automated Cobas e411 system (Roche Diagnostics) using electrochemiluminescence (ECLIA) technology. The Cobas e411 PAPP-A assay measured PAPP-A concentration from 4 to 10,000 mIU/L. The intra-assay coefficient of variation (CV) and inter-assay were less than 2% for all three levels of controls (144 mIU/ml, 3361 mIU/ml, 6630 mIU/ml). Measurements of serum PAPP-A were done in batches to minimize analytical variation. For each batch, 3 levels of internal control were performed to validate assay performance.

The PAPP-A values in mIU/L were converted into MoM PAPP-A values by dividing PAPP-A levels in mIU/L by median PAPP-A for corresponding gestational age of pregnancy.¹³ The values of MoM PAPP-A were expressed as median and ranges as the data was not normally distributed. Continuous variables were summarized by median and range while categorical variables were summarized using frequency measures.

Statistical analysis

Comparison of median values of PAPP-A between threatened abortion women and the control group, and in the outcome of the threatened abortions were performed using Mann-Whitney U-test (non-parametric). The Kruskal-Wallis test was performed to compare the levels of PAPP-A among different ethnic groups of study population.

A receiver operator characteristic (ROC) curve was constructed to evaluate the PAPP-A level in distinguishing between the two threatened abortion outcomes. The predictive value of PAPP-A as a predictor of pregnancy failure was

TABLE 1: Demographic profile of the study population (n=82)

	Threatened abortion (n=42)	Control group (n=40)	p value
Race			
Malay	33 (78.6)	35 (87.5)	
Indian	7 (16.7)	2 (5.0)	
Chinese	-	3 (7.5)	
Others	2 (4.8)		
Maternal age (years) (median)	29 (26 – 31)	28 (26 – 31)	0.96
Gravidity (median)	2 (1 – 4)	2 (1-3)	0.36
Parity (median)	1 (0 – 2)	1 (0 – 2)	0.35
Gestational age (weeks) (median)	14 (8 -16)	16 (9 -21)	0.01*

Values are expressed as median (range) or number (%)

* By Mann-Whitney test, statistical significance at $p < 0.05$

established by the best sensitivity, specificity and optimal cut-off from the ROC analysis.

All clinical and laboratory data were stored and analyzed using the Statistical Package for Social Science (SPSS) software version 17.0. In all statistical analyses, $p < 0.05$ (95% confidence interval) was considered significant.

Ethics

The study was approved by the institution research and ethical review boards.

RESULTS

Characteristics of study population

A total of 82 pregnant women were enrolled in this study; 42 patients were those with threatened abortion and 40 were normal pregnant women (Table 1). The majority of women recruited were Malays; 68 (83.0%) followed by Indian; 9 (11.0%), Chinese; 3 (3.70%) and others 2

(2.4%).

The median and range of PAPP-A levels of each ethnic group of the study population are shown in Table 2. There was no significant difference of PAPP-A level between different ethnicities (Kruskal-Wallis test, $p = 0.43$).

The median maternal ages for the threatened abortion and control groups were 29 years (26-31) and 28 years (26-31) respectively. There were no significant differences in maternal ages between the two groups. The median gravidity was 2 (range 1-4) in threatened abortion group and 2 (range 1-3) in control group while the median parity of both groups were Para 1 (range 0-2). There were no significant differences in terms of gravidity and parity in these two groups.

The median gestational age of threatened abortion patients was 14 weeks (range: 8-16 weeks), and for the control group the median was 16 weeks (range: 9-21 weeks). Statistically,

TABLE 2: Level of PAPP-A in different ethnic groups

	Race	Number	Median	Range	P
MoM PAPP-A	Malay	68 (82.9%)	1.00	1.00-2.00	0.43
	Chinese	3 (3.7%)	1.00	1.00-2.00	
	Indian	9 (11.0%)	1.00	0.80-1.00	
	Others	2 (2.4%)	1.00	1.00-1.00	
	Total	82 (100%)			

*By Kruskal-Wallis test, statistical significance at $p < 0.05$

there were significant differences in gestational ages between both groups (Mann-Whitney test, $p = 0.01$). Nine from 42 patients with threatened abortion had pregnancy failure while the other 33 patients and all patients in control group had successful pregnancies that progress beyond 22 weeks of gestations.

Pregnancy-associated plasma protein levels

The median and range of PAPP-A levels in threatened abortion and control groups were 1.00 MoM (1.00-2.00) and 1.00 MoM (1.00-1.57) respectively. There was no significant difference in PAPP-A levels between the two groups ($p = 0.83$). The median MoM PAPP-A in pregnancies that progressed in both groups was 1.00 (ranged: 1.00 - 1.90 MoM) while in spontaneous abortion patients, the median was 0.83 MoM (ranged: 0.41-1.5 MoM). Although the median level of serum PAPP-A in patients with pregnancy failure was lower compared to the PAPP-A levels in pregnancies that progressed, this failed to reach statistical significance ($p = 0.68$) (Figure 1).

When we examined the PAPP-A levels in

threatened abortion patients exclusively, the PAPP-A levels of those with pregnancy failure were significantly lower compared to those who had successful pregnancies. The median value of pregnancy-failure and pregnancy-progressed patients were 0.78 (0.41-1.00 MoM) and 1.00 (1.00-2.00 MoM) respectively ($p < 0.05$) (Figure 2).

To differentiate women who had pregnancy failure from those who had progressed pregnancy, ROC curve was constructed based on 42 patients with threatened abortion and showed area under the curve of 0.674 (95% CI, 0.561-0.773) with optimized cut-off value of 0.66 (Figure 3). This cut off value gave 44% sensitivity, specificity of 93%, positive predictive value (PPV) of 80% and negative predictive value (NPV) of 86%.

DISCUSSION

This study has evaluated the use of a single measurement of serum PAPP-A as a predictor of pregnancy failure among threatened abortion women. We found that, among those with threatened abortion, the level of PAPP-A was significantly low in patients who had spontaneous

MoM PAPP-

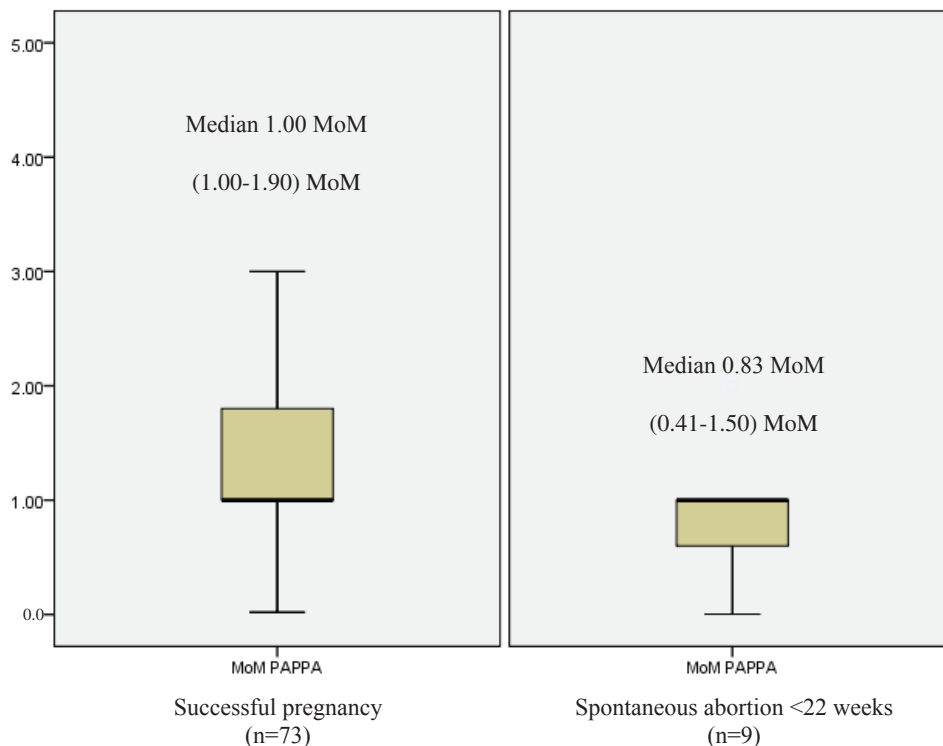


FIG. 1: Box plots showing median levels of PAPP-A in MoM in outcome of pregnancy. Boxes show interquartile ranges and I bars represent highest and lowest value

MoM PAPP-

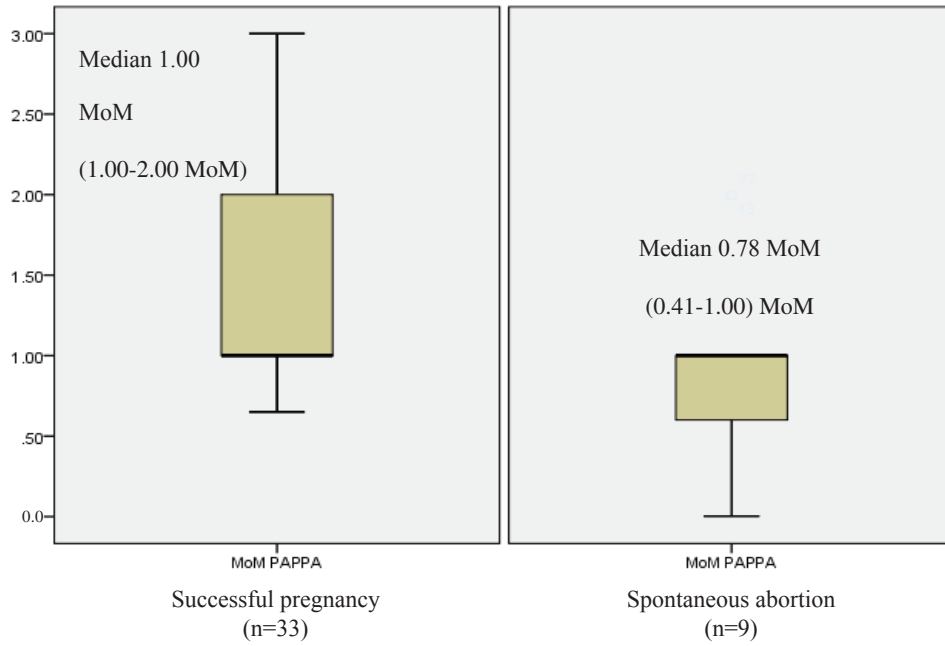


FIG. 2: Box plots showing median levels of MoM PAPP-A in threatened abortion patients with outcome of successful pregnancy as compared to those who had spontaneous abortion. The MoM values in brackets represent 25-75 percentile of the PAPP-A level in each group. Boxes show interquartile ranges and I bars represent highest and lowest value.

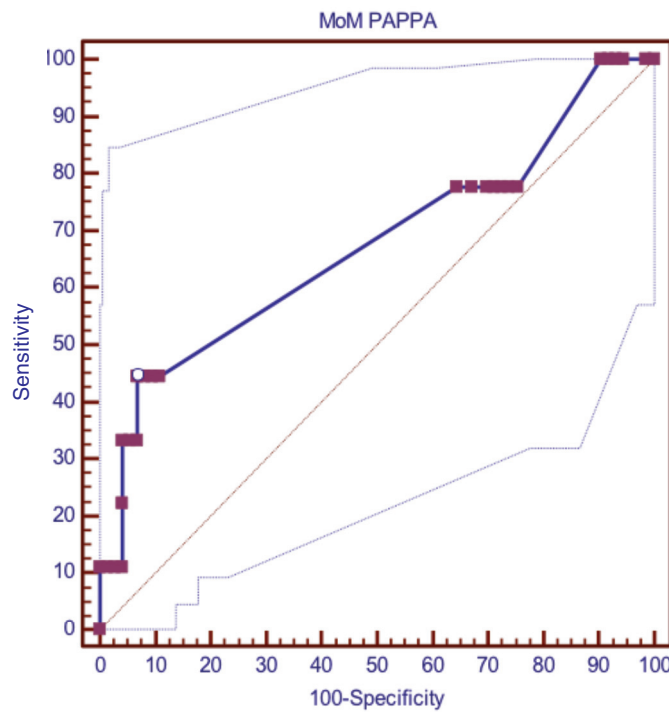


FIG. 3: Receiver operator characteristic (ROC) curve of MoM PAPP-A for the determination of outcome of pregnancy among the threatened abortion patients.

abortion compared to those who had successful pregnancies. This result concurred with the results of other investigators.⁶⁻⁸ However, when we compared the threatened abortion patients and the control group, the levels of PAPP-A in these two groups was not significantly different.

Our study suggests that women with threatened abortion who have a low PAPP-A levels early in their pregnancy, have a higher risk of pregnancy failure. This result supports the notion that low levels of PAPP-A may be an early marker of abnormal implantation or other trophoblast dysfunction that leads to abortion.

Although our findings are similar to previous studies,^{6-8,13} we found that a cut-off value in our study was different. While most other researchers used the cut-off value of between 0.25 to 0.5 MoM,^{6-8,13} we found a higher cut-off value of 0.66 MoM with the best combined sensitivity and specificity of 44% and 93% respectively. Patrick *et al.*⁷ suggest a PAPP-A cut-off of 0.30 MoM with a sensitivity of 64.5% and specificity of 99%, while Evin *et al.*⁶ suggested a cut-off value of 0.45 MoM with a sensitivity of 51.8% and specificity of 86.2%.

The difference in cut-off values could be attributed by many factors like different methodologies in PAPP-A measurement. Most recent studies used automated immunoassay platform. Our study used ECLIA technology, which has good accuracy and precision. The unresolved issue of standardization of this assay may also contribute to the differences in the cut-off value used.

The definition of "low" PAPP-A varies among studies and influences both the statistical and clinical significance of results. Low level has been defined as a range or cut-offs value including in percentiles or arbitrary of MoM ranging from <0.25 to <0.50 MoM. It is conventional in prenatal medicine to report maternal screen markers as gestational age-adjusted multiples of median (MoM). Our study reported the PAPP-A value in MoM. To convert to MoM value, it was required to divide the PAPP-A level in mIU/L to median PAPP-A of corresponding gestational age. To date, there has been no data on median PAPP-A of corresponding gestational age established among normal pregnant women in the South East Asian region, hence we used the median level from a Canadian study by Anne *et al.*¹³ who determined the median level from 407 normal pregnant women with the gestational age of four to 20 weeks. This could have contributed to the difference in cut-off values found in this study.

Studies have shown that different ethnicity has different values of serum PAPP-A. Spencer K. *et al.*¹⁶ found that Afro-Caribbean women had significantly higher PAPP-A levels of 48% compared to Caucasian women and Asian women have 35% higher PAPP-A levels compared to Caucasian women. Other studies showed that Chinese women have a significantly higher serum level of PAPP-A in the first trimester compared to Caucasian.¹⁷ In our study, there is no significant difference between PAPP-A levels among the different ethnic groups, which could be attributed to the small sample size of the study population.

This study examined the PAPP-A level at a wider range of gestational age. However, the small sample size had limited our ability to adjust to possible confounding factors like nulliparity and to adequately assess interaction. The median PAPP-A values for each gestational week were taken from populations which may have no similarity with our study population.

Our population had a higher cut-off PAPP-A value of 0.66 MoM with the best combined sensitivity and specificity of 44% and 93% respectively. This study also found that a low PAPP-A value in threatened miscarriage women is associated with pregnancy failure, although the use of PAPP-A as a one-time single marker has limited value.

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REFERENCES

1. Regan L, Rai R. Epidemiology and the medical causes of miscarriage. *Baillieres Best Pract Res Clin Obstet Gynaecol.* 2000; 14(5): 839-54.
2. Elson J, Salim R, Tailor A, Banerjee S, Zosmer N, Jurkovic D. Prediction of early pregnancy viability in the absence of an ultrasonically detectable embryo. *Ultrasound Obstet Gynecol.* 2003; 21(1): 57-61.
3. la Marca A, Morgante G, De Leo V. Human chorionic gonadotropin, thyroid function, and immunological indices in threatened abortion. *Obstet Gynecol.* 1998; 92(2): 206-11.
4. Phipps MG, Hogan JW, Peipert JF, Lambert-Messerlian GM, Canick JA, Seifer DB. Progesterone, inhibin, and hCG multiple marker strategy to differentiate viable from nonviable pregnancies. *Obstet Gynecol.* 2000; 95(2): 227-31.
5. Hanita O, Hanisah AH. Potential use of single measurement of serum progesterone in detecting early pregnancy failure. *Malays J Pathol.* 2012; 34(1): 41-6.

6. Ugurlu EN, Ozaksit G, Karaer A, Zulfikaroglu E, Atalay A, Ugur M. The value of vascular endothelial growth factor, pregnancy-associated plasma protein-A, and progesterone for early differentiation of ectopic pregnancies, normal intrauterine pregnancies, and spontaneous miscarriages. *Fertil Steril.* 2009; 91(5): 1657-61.
7. Dumps P, Meisser A, Pons D, *et al.* Accuracy of single measurements of pregnancy-associated plasma protein-A, human chorionic gonadotropin and progesterone in the diagnosis of early pregnancy failure. *Eur J Obstet Gynecol Reprod Biol.* 2002; 100(2): 174-80.
8. Tong S, Marjono B, Mulvey S, Wallace EM. Low levels of pregnancy-associated plasma protein-A in asymptomatic women destined for miscarriage. *Fertil Steril.* 2004; 82(5): 1468-70.
9. Lawrence JB, Oxvig C, Overgaard MT, *et al.* The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc Natl Acad Sci U S A.* 1999; 96(6): 3149-53.
10. Conover CA, Bale LK, Overgaard MT, *et al.* Metalloproteinase pregnancy-associated plasma protein A is a critical growth regulatory factor during fetal development. *Development.* 2004; 131(5): 1187-94.
11. Fialova L, Malbohan IM. Pregnancy-associated plasma protein A (PAPP-A): theoretical and clinical aspects. *Bratisl Lek Listy.* 2002; 103(6): 194-205.
12. Santolaya-Forgas J, De Leon JA, Cullen Hopkins R, Castracane VD, Kauffman RP, Sifuentes GA. Low pregnancy-associated plasma protein-a at 10(+1) to 14(+6) weeks of gestation and a possible mechanism leading to miscarriage. *Fetal Diagn Ther.* 2004; 19(5): 456-61.
13. Spencer CA. PAPP-A as an early marker for adverse pregnancy outcomes. Nova Scotia: Halifax; 2007.
14. Cuckle HS, van Lith JM. Appropriate biochemical parameters in first-trimester screening for Down syndrome. *Prenat Diagn.* 1999; 19(6): 505-12.
15. Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10-14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol.* 1999; 13(4): 231-7.
16. Spencer K, Ong CY, Liao AW, Nicolaides KH. The influence of ethnic origin on first trimester biochemical markers of chromosomal abnormalities. *Prenat Diagn.* 2000; 20(6): 491-4.
17. Leung TY, Spencer K, Leung TN, Fung TY, Lau TK. Higher median levels of free beta-hCG and PAPP-A in the first trimester of pregnancy in a Chinese ethnic group. Implication for first trimester combined screening for Down's syndrome in the Chinese population. *Fetal Diagn Ther.* 2006; 21(1): 140-3.