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The Reactivity Levels of Progesterone, Nitric Oxide and Nuclear Factor Kappa-B on the Serum of Term and Post-Term Pregnancy, Clinical Study in Padang, West Sumatera, Indonesia

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Abstract

BACKGROUND: A variety of recent evidence exists about the clinical implication of low level of Pregnancy-associated plasma protein A (PAPP-A) in pregnancy. This glycoprotein is a protease, which releases the Insulin-like growth factor from IGFBP 4. Its role is a trophoblastic invasion of decidua, stimulation of cell mitosis and differentiation. It has an immunosuppressive effect in the placenta, inhibition of coagulation and complex role for integration of all these processes in the placenta. Level of PAPP-A (under 0.4 MoM-Multiple of Medians) in first-trimester screening in chromosomally and morphologically normal fetuses, could influence fetal weight, preeclampsia, premature birth and stillbirth. As a result of the complications as mentioned earlier, there is implication on timing, mode of delivery and condition of the newborn.

AIM: The study aims to evaluate the influence of low PAPP-A, measured in the first trimester on the outcome of pregnancy, with accent disorders, which are the result of placental insufficiency. Also, gestational week, mode of delivery and condition of newborn secondary underlying conditions will be evaluated.

MATERIAL AND METHODS: After given information and consultation about the expectation from the screening, pregnant women with a singleton pregnancy were tested for First Trimester Screening to estimate the risk for Trisomy 21, 13, 18- the most frequent chromosomopathies. After exclusion of chromosomopathies and congenital malformations, one hundred and fourteen patients enrolled in the study. The target group (n = 64) with PAPP-A below 0.4 MoM and control group (n = 50) with PAPP-A equal and above 0.4 MoM. An assessment of mode and time of delivery and presence of small for gestational age newborns, preeclampsia, premature birth and newborn condition at delivery was made.

RESULTS: The percentage of the patients delivered in term was similar between the target group (n = 64) and the control group (n = 50), 82.81% vs 82.0% respectively. The rate of cesarean section was 29.7 % in the target group vs 32% in the control group. A significant difference was found about elective vs urgent cesarean section in favour of the target group. The difference was present about the complication in pregnancy before delivery, 56% vs 22%, p = 0.023, which were the main indication for cesarean section. The difference in newborn outcome was not significant.

CONCLUSION: There is a difference in frequency of complications, in the cases with PAPP-A under 0.4 MoM, such as premature birth, preeclampsia compound with SGA fetuses versus the control group. The difference for SGA newborn and premature birth among the groups has statistical significance. The patients delivered with cesarean section were with the main indications SGA or elevated blood pressure, often occurred combined with prematurity. Apgar score and birth weight were similar in target and control group, but the newborns with a birth weight under 2500 g. were more frequent in the target group. Because these results did not show another significance among two groups, probably lower cut-off is needed, combining with another test (Doppler of uterine arteries in the first trimester, biochemical test). Presence of other diseases which could hurt placental function should be emphasised.

Introduction

Term pregnancy generally lasts 37 to 40 weeks or 259 to 280 days counted from the first day of the last menstrual periods. Postdate pregnancy occurs within a period of > 40 weeks to 42 weeks. The post-term pregnancy lasts more or equal to 42 weeks or 294 days, since the menstrual period followed by two weeks later ovulation [1]. The incidence of post-

term pregnancy in the world ranges from 4-19% [2], around 6% of the 4 million babies born in the United States during 2006 were born at 42 weeks of gestation or older [3]. In Indonesia, the incidence of post-term pregnancy is approximately 10% [4].

Post-term pregnancy is often associated with an increased risk of perinatal morbidity and mortality and effected on the development of fetal [5], like perinatal mortality associated with meconium aspiration and asphyxia [6]. Furthermore, labour is one of the triggers of post-term pregnancy caused cephalo-pelvic disproportion and shoulder dystocia [2]. The study in Norway reported that the rate of cerebral palsy in post-term infants is 144 per births [7]. Generally, the post-term pregnancy occurs because of the disruption to the onset of labour, while the onset of labour itself is not yet known clearly [8].

The progesterone decrease causes the release of nitric oxide (NO) in the endometrium and cervix, as well as cytokine activation [9]. Activation of cytokines via the pathway of cyclo-oxygenase (COX) II will lead to an increase in prostaglandin E2 (PGE2) is cause to release of NO and will increase PGE2 to lead the degeneration of cervical collagen and cervical tissue remodelling resulting in cervical ripening [10].

The NF-κB is a protein complex that controls the process of DNA transcription 17, 18. High NF-κB activation decreases insulin and antioxidant capacity and increases endothelial platelet interactions, neutrophil transport and LDL oxidation, which is one of the inflammatory processes [11]. Increased the phosphorylation of A2 will be increasing the arachidonate to convert a prostaglandin by activation of high COX-2. The high COX-2 will lead to the functional withdrawal of progesterone through interaction with progesterone receptors [12]. The NF-κB reported to decrease the insulin and antioxidant and increases endothelial platelet interactions, neutrophil transport and LDL oxidation, which is one of the inflammatory processes [13].

Many theories were to explain the labour of pregnant women, example oxytocin, progesterone theory, fetal cortisol, prostaglandin, uteri structure, nutrition, circulation of blood and nerve, and decreased of the fetal head. All of the theories can be the indicator of expression the progesterone, NO, and NF-κB in term and post-term pregnancy. This study to analyse the level of progesterone, NO, and NF-κB after reactive with the serum of pregnancy.

Material and Method

The model of this study was approached the observational analytic study with cross-sectional study design in July 2017-2018 and was carried out in the maternity clinic, primary health care and Type C Hospital, Padang and reactivity assay were conducted by Laboratory of Biomedical, Faculty of Medicine, Andalas University, Padang-Indonesia. The study population was pregnant women with 37-38 weeks gestation research site. The sample in this study was taken by consecutive sampling in the research period divided into term pregnancy (36 subjects) and post-term pregnancy (36 subjects).

Reactivity Assay

The ELISA Assay was referred to as Gani (2009) [14]. The serum of subjects inserted 10 ml into the vacutainer tubes to measure the levels of progesterone, nitric oxide, and NF-κB was detected by the ELISA method (Biorad, USA). The examination of progesterone, nitric oxide, and NF-κB began with the preparation of each reagent (Progesterone and Nitric Oxide, Colorimetric Assay Kit (R & D Systems, USA) as well as the reagent of Human NF-κB (My Biosource). Then 50 µL, blank and samples were added to 96-well plate also 100 µL HRP conjugate progesterone, nitrate reductase mixture, and NF-κB mixture. In each well added 50 µL of biotinylated antibodies then covered with a plate sealer and incubated for 60 min at 37°C, after that washed with 350 µL of wash buffer solution (three times). Then added 50 µL Substrate A and B and covered with a plate sealer and incubated for 15 min at 37°C. Then add 50 µL stop solution to each well. The colour will change from blue to yellow as the reactivity indicator and read by ELISA reader wavelengths at 450 nm.

Research Ethics

This study was approved the ethical clearance by the Faculty of Medicine, Andalas University, Padang-Indonesia.

Statistical Analysis

The reactivity levels of progesterone, NO, and NF-κB were analysed by bivariate and multivariate with the probability is $p < 0,05$.

Results

The term and post-term pregnancy are not significant ($p > 0.05$) as well as the leukocyte profile ($p > 0.05$) (Table 1). It has no influence on the subjects during the pregnant phase. Based on the leukocyte profile, the second groups have to exhibit the peak conditions or stress during childbirth. The most subjects with first births reached 41.7% within term pregnancy cases and 61% Post-term pregnancy.

Table 1: Distribution of Subjects of Term and Post-term Pregnancy

Characteristics	Term Pregnancy	Posterm Pregnancy	*p-value*
Mother's Age mean ± SD (year)	28.05 ± 4.08	28.69 ± 4.94	0.552
Leukocyte mean ± SD (mm^3)	10776.97 ± 1334.89	10450.75 ± 1319.03	0.301
Parity (%)			
1st parity	15 (41.7)	22 (61.1)	
2nd parity	12 (33.3)	9 (25.0)	
3rd parity	8 (22.2)	3 (8.3)	
4th parity	1 (2.8)	2 (5.6)	
Body Mass Index (%)			
Normal	7 (19.4)	9 (25.0)	
Overweight	23 (63.9)	20 (55.6)	
Obese	6 (16.7)	7 (19.4)	

The profiles of the progesterone, NO, and NF-κB detected in blood serum groups term and post-term pregnancy (Table 2). Our study showed (Table 2) the rate of progesterone in the post-term pregnancy phase is much higher than term pregnancy. This data becomes a reference for controlling post-term pregnancy; also, it can reduce the incidence of post-term pregnancy in the mother and fetus. The NO term pregnancy levels are higher than the post-term pregnancy ($p > 0.05$) as related as the NO levels serum will increase when pregnancy until some postpartum (Table 2).

Table 2: Profile of Progesterone, NO and NF-κBon the serum of Term and Post-term Pregnancy

Variables	Term Pregnancy n = 36	Post-term Pregnancy n = 36	*p-value*
Progesterone (ng/mL) mean ± SD	26.15 ± 13.04	106.73 ± 124.76	0.001
NO (µmol/L) mean ± SD	7.20 ± 5.93	6.24 ± 4.41	0.440
NF-κB (ng/mL) mean ± SD	8.16 ± 2.64	7.97 ± 2.67	0.766

Based on the multivariate analysis show the odd ratio of NO was more dominant than progesterone (Table 3) in the post-term pregnancy is strongly correlated ($p < 0.05$). It assumes the presence of both when pregnancy in the post pregnancy has a connection, so the content of NO levels becomes an indicator of post-term pregnancy.

Table 3: Analysis Results of Multivariate Factors Associated with Post-term Pregnancy

Variables	B	Standard Deviation	Wald	*p-value*	OR
Progesteron Level (ng/ ml)	-0.270	0.078	11.933	0.001	0.763
NO Level (µmol/L)	0.706	0.249	8.075	0.004	2.026

The NF-κB level in term pregnancy is better than a post-term pregnancy ($p > 0.05$).

Discussion

The leukocyte indicators are < 6000/µL (lower limit); 15000/µL (upper limit); and 9000-25000/µL (stress conditions) [15]. These results can be the early detection of that possibility of the post-term pregnancy as well as Body Mass Index (BMI) commonly shows weight gain. Based on recommended of Institute of Medicine BMI < 19.8 kg/m^2 (gain 12.5 -18 kg), BMI = 19.8-26.0 kg/m^2 (gain 11.5-16 kg), BMI > 26.0- 29.0 kg/m^2 (gain 7.0-11.5 kg), and BMI > 29.0 kg/m^2 (gain of 7.0 kg) [16].

In this study, progesterone was higher than NO and NFκB. Verhaegen (2012) reported that the progesterone is an indicator of the development of pregnancy and its production decreases nearing the birth [17].

Kumar (2012) suggested that the progesterone levels also have the variance at the stage of pregnancy 9-47 ng/ml (first trimester), 12-20 ng/ml (first 5-6 weeks). The produce of progesterone in pregnant phase is higher than normal as well as related to the estrogen produced mainly in the first trimester. The second trimester has milk duct development and enlarges of breasts [18]. Bhattarai (2014) reported that progesterone might help to avoid the loss of embryo [19]. Statistically analysed by Slattengren (2013) shown that the cutoff value of progesterone decrease; it has the probability of a survive pregnancy increased to 99.2% [20].

Furthermore, the synthase of NO has progressively during term pregnancy [21]. The increase of NO will cause the cervical ripening as the signs to decrease of progesterone-B receptors in myometrial cells [22]. The NO works to ripen the cervix by increasing vascular permeability, cytokine secretion, and inducing cervical apoptosis [23]. Biochemically, the NO was synthesised by L-Arginine with the help of NO synthase (NOS) and co-factors [24]. Biologically, the NO causes smooth muscle relaxation, inhibits platelet aggregation and adhesion, and inhibits cell proliferation [25] also the significance of NO as the modulator of uterine blood flow by the pathway of cyclic guanosine monophosphate (cGMP) [26].

The NF-κB reported a regulator of cytokine of pro-inflammatory that implicated as substances that trigger the initiation and process of parturition in humans [27]. The results of this study are consistent with previous studies, where the serum NF-κB levels at post-term pregnancy were lower than NF-κB levels in term pregnancy [28]. The Bivariate analyses to explained the progesterone and NO levels were significant ($p < 0.25$) and NF-κB is no significant ($p > 0.25$) it's not included in multivariate analysis. Both progesterone and NF-κB were correlated. Tan (2012) suggested who the progesterone becomes to maintain the chorionic cells from oxidative stress and apoptosis of the cell. Moreover, this hormone possesses anti-inflammatory and inhibits the transcription of NF-κB [29].

This study concluded that The NO would signify used to the variable predictor for detecting of post-term pregnancy (OR 2.026) if linked with the progesterone level in serum of pregnant women ($p < 0.05$).

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References

1. Mandruzzato G, Alfirevic Z, Chervenak F, Gruenebaum A, Heimstad R, Heinonen S, et al. Guidelines for the management of postterm pregnancy. J Perinat Med. 2010; 38(2):111-9. https://doi.org/10.1515/jpm.2010.057 PMid:20156009

2. Galal M, Symonds I, Murray H, Petraglia F, Smith R. Postterm pregnancy Facts Views Vis Obgyn. 2012; 4(3):175-87.

3. Glass HC, Costarino AT, Stayer SA, Brett C, Cladis F, Davis PJ. Outcomes for Extremely Premature Infants. Anesth Analg. 2015; 120(6):1337-51. https://doi.org/10.1213/ANE.0000000000000705 PMid:25988638 PMCid:PMC4438860

4. Research IH. Report of the Indonesia Basic National Health. Annual Review Report. 2010; 1(1):20-45.

5. Mengesha HG, Lerebo WT, Kidanemariam A, Gebrezgiabher G, Berhane Y. Pre-term and post-term births: predictors and implications on neonatal mortality in Northern Ethiopia. BMC Nurs. 2016; 15:48. https://doi.org/10.1186/s12912-016-0170-6 PMid:27499702 PMCid:PMC4974761

6. Aslam HM, Saleem S, Afzal R, Iqbal U, Saleem SM, Shaikh WA. Risk factors of birth asphyxia. Ital J Pediatr. 2014; 40:94. https://doi.org/10.1186/s13052-014-0094-2 PMid:25526846 PMCid:PMC4300075

7. Tollånes MC, Wilcox AJ, Lie RT, Moster D. Familial risk of cerebral palsy: population based cohort study. BMJ .2014; 349:g4294. https://doi.org/10.1136/bmj.g4294 PMid:25028249 PMCid:PMC4099475

8. Caughey AB, Snegovskikh VV, Norwitz ER. Postterm pregnancy: how can we improve outcomes? Obstet Gynecol Surv. 2008; 63(11):715-24. https://doi.org/10.1097/OGX.0b013e318186a9c7 PMid:18928576

9. Schumacher A, Costa SD, Zenclussen AC. Endocrine Factors Modulating Immune Responses in Pregnancy. Front Immunol. 2014; 5:196. https://doi.org/10.3389/fimmu.2014.00196 PMid:24847324 PMCid:PMC4021116

10. Stjernholm-Vladic Y, Stygar D, Mansson C, Masironi B, Akerberg S,Wang H.Factors involved in the inflammatory events of cervical ripening in humans. Reprod Biol Endocrinol. 2004; 2:74. https://doi.org/10.1186/1477-7827-2-74 PMid:15500686 PMCid:PMC534613

11. Liu T, Zhang L, Joo D,Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017; 2:17023. https://doi.org/10.1038/sigtrans.2017.23 PMid:29158945 PMCid:PMC5661633

12. Lawrence T. The Nuclear Factor NF-κB Pathway in Inflammation. Cold Spring Harb Perspect Biol. 2009; 1(6):a001651. https://doi.org/10.1101/cshperspect.a001651 PMid:20457564 PMCid:PMC2882124

13. Zhang C. The role of inflammatory cytokines in endothelial dysfunction. Basic Res Cardiol. 2008; 103(5):398-406. https://doi.org/10.1007/s00395-008-0733-0 PMid:18600364 PMCid:PMC2705866

14. Gani BA, Bachtiar BM, Bachtiar EWB, Wibawan IWT. The ability of IgY to recognize surface proteins of Streptococcus mutans. Dental Journal (Majalah Kedokteran Gigi). 2009; 42(4):189-193. https://doi.org/10.20473/j.djmkg.v42.i4.p189-193

15. Chandra S, Tripathi AK, Mishra S, Amzarul M, Vaish AK. Physiological Changes in Hematological Parameters During Pregnancy Indian J Hematol Blood Transfus. 2012; 28(3):144-46. https://doi.org/10.1007/s12288-012-0175-6 PMid:23997449 PMCid:PMC3422383

16. Carmichael S, Abrams B, Selvin S. The pattern of maternal weight gain in women with good pregnancy outcomes. Am J Public Health. 1997; 87(12):1984-8. https://doi.org/10.2105/AJPH.87.12.1984 PMid:9431288 PMCid:PMC1381241

17. Oyedeji KO. Bolarinwa AF, Adegoke AO. Valuation Of Antifertility And Teratogenic Effects Of Chromatographic Fractions Of Portulaca Leracea In Male And Female Albino Rats. 2013; 5(3);440-4.

18. Kumar P, Magon N. Hormones in pregnancy. Niger Med J. 2012; 53(4):179-83. https://doi.org/10.4103/0300-1652.107549 PMid:23661874 PMCid:PMC3640235

19. Bhattarai T, Chaudhuri P, Bhattacharya K, Sengupta P. Effect of progesterone supplementation on post-coital unilaterally ovariectomized superovulated mice in relation to implantation and pregnancy. Asian J Pharm Clin Res. 2014; 7(1):29-31.

20. Slattengren AH, Prasad S, Oyola S. Is this pregnancy viable? J Fam Pract. 2013; 62(6):305-16.

21. Kho JV, Chua SS, Dallumal RM, Omar SZ. Medications Used By Pregnant Women: Any Safety Concerns? Int J Pharm Pharm Sci. 2017; 9(5):100-6. https://doi.org/10.22159/ijpps.2017v9i5.16057

22. Pang Y, Dong J, Thomas P. Progesterone increases nitric oxide synthesis in human vascular endothelial cells through activation of membrane progesterone receptor-α. Am J Physiol Endocrinol Metab. 2015; 308(10):E899-911. https://doi.org/10.1152/ajpendo.00527.2014 PMid:25805192

23. Korde Choudhari S, Chaudhary M, Bagde S, Gadbail AR, Joshi V. Nitric oxide and cancer: a review. World J Surg Oncol. 2013; 11:118. https://doi.org/10.1186/1477-7819-11-118 PMid:23718886 PMCid:PMC3669621

24. Feng C. Mechanism of nitric oxide synthase regulation: electron transfer and interdomain interactions. Coord Chem Rev. 2012; 256(3-4):393-411. https://doi.org/10.1016/j.ccr.2011.10.011 PMid:22523434 PMCid:PMC3328867

25. Bani D, Failli P, Bello MG, Thiemermann C, Sacchi TB, Bigazzi M, Masini E. Relaxin activates the L-arginine-nitric oxide pathway in vascular smooth muscle cells in culture. Hypertension. 1998; 31(6):1240-7. https://doi.org/10.1161/01.HYP.31.6.1240 PMid:9622136

26. Shahrokh E, Tehraninejad, Khazei N, Ayati E, Movafegh A, Azimaraghi O. Effect of vaginal sildenafil on in vitro fertilization success rates in women with previous failed in vitro fertilization attempts. Asian J Pharm Clin Res. 2018; 11(6):486-88. https://doi.org/10.22159/ajpcr.2018.v11i6.25645

27. Tak PP, Firestein GS. NF-κB: a key role in inflammatory diseases. J Clin Invest. 2001; 107(1):7-11. https://doi.org/10.1172/JCI11830 PMid:11134171 PMCid:PMC198552

28. Vaughan JE, Walsh SW. Activation of NF-κB in placentas of women with preeclampsia. Hypertens Pregnancy. 2012; 31(2):243-51. https://doi.org/10.3109/10641955.2011.642436 PMid:22380486 PMCid:PMC3542769

29. Tan H, Yi L, Rote NS, Hurd WW, Mesiano S. Progesterone receptor-A and-B have opposite effects on proinflammatory gene expression in human myometrial cells: implications for progesterone actions in human pregnancy and parturition. J Clin Endocrinol Metab. 2012; 97(5):E719-30. https://doi.org/10.1210/jc.2011-3251 PMid:22419721 PMCid:PMC3339884