The Role of Fetal Hemoglobin in Predicting Preeclampsia in Early Pregnancy

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Abstract: Background: Hypertensive disorders are among the most serious complications of pregnancy and diagnosing them early is an important part of prenatal care. Using the hemoglobin test results along with any risk factors seen in the patient's history and physical examinations, including blood pressure measured early in pregnancy, may be effective in forming a predictive model for women likely to develop preeclampsia (PE). This study aimed to investigate the association between fetal Hemoglobin (HbF) and PE in the first and early second trimester of pregnancy. Subjects and Methods: This control case study, carried out at the Department of Obstetrics and Gynecology at King Abdulaziz University Hospital, Jeddah, Saudi Arabia, between March and October 2015, included 80 pregnant women at 11-16 weeks of gestation (40 women with normal pregnancies and 40 women who subsequently developed preeclampsia). Patient data including age, blood pressure, and HbF concentration were recorded. Results: Analysis of venous maternal blood found elevated serum HbF levels in a significant number of women with PE compared to the control group. There was a significant difference in blood pressure measurements (diastolic) between the groups and a significant positive association between HbF and blood pressure parameters (both systolic and diastolic) existed in the case group only. Conclusion: As expected, this study found a significant difference in gestational length at the time of delivery, with shorter gestation in the preeclamptic women than in the women with normal pregnancies. Antenatal care clinics can carry out important risk assessment for the prediction and early diagnosis of preeclampsia by including fetal Hb in their screening tests.
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1, Introduction

Preeclampsia (PE) is a disorder specific to pregnancy characterized by hypertension and proteinuria arising after the 20th week of gestation in a previously non-hypertensive woman. [1,2]. Edema and headache are also associated with the condition, and in severe cases, seizures (eclampsia), clotting abnormalities, hepatic and renal dysfunction, acute respiratory distress syndrome and restricted fetal growth can occur [3,4]. Preeclampsia is one of the major contributors to maternal death and morbidity worldwide, with the World Health Organization (WHO) estimating the incidence of PE in developing countries to be seven times higher than it is in developed countries [5,6].

PE is a condition of unknown etiology, and delivery of the fetus and placenta is the only known cure. Therefore, preventing PE is a significant obstetrical goal. The challenge is to identify women at a higher risk of PE early on in their pregnancies so that they can be monitored more closely.

Several studies have focused on using various clinical and biochemical tests to predict or detect PE early in pregnancy [7,8,9]. For most developing countries, however, these methods are impractical for general use. Currently, no screening test for PE exists which is reliable and cost effective for use in the developing world [7].

The existing clinical and biochemical tests for diagnosing PE in women at high risk are expensive and have been shown to have little value in early diagnosis of the condition [10-12]. Numerous studies have looked at maternal hemoglobin (Hb) concentration and fetal hemoglobin (HbF) concentration as an early PE predictor [13-18]. Measuring hemoglobin isroutinelydoneas part of antenatal care, with PE prediction in mind. Using the hemoglobin test results along with any risk factors seen in the patient's history and physical examinations, including blood pressure measured early in pregnancy, may be effective in forming a predictive model for women likely to develop PE. Therefore, this study aimed to assess the effectiveness of routine measurements of maternal Hb and HbFin the first trimester and early second trimester in predicting the risk of PE.

2. Subjects and Methods

The current case-control study was carried out from March to October 2015 and involved80 pregnant women in their 11-16th weeks of pregnancy. Subjects were selected from women attending the Obstetrics and Gynecology Department at King Abdulaziz

University Hospital (KAUH), Jeddah, Saudi Arabia for a routine antenatal care visit. Based on the last menstrual period, gestational age (GA) was calculated and verified by ultrasound measurement of crown-rump length. After undergoing tests to measure their Hb and HbF levels, all subjects were followed until they delivered, with the presence of preeclampsia being the main outcome studied. The Biomedical Committee at the Faculty of Medicine, KAUH approved the protocol for the study, and all participants gave their written informed consent after receiving comprehensive explanation of the procedure involved.

All subjects involved in this study met the following inclusion and exclusion criteria:

Inclusion criteria

Primigravida, singleton pregnancy, certain last menstrual period, confirmation of pregnancy by serum BHCG and first trimester crown rump length measurements and gestational age (GA) between 11-16 weeks.

Exclusion criteria:

Diabetes, pre-pregnancy hypertension, cardiovascular disease, chronic renal disease, and multiple pregnancies.

The 80 pregnant women of the study group were subdivided into two groups:

1st group (control group): 40 women with normal uncomplicated pregnancies.

2nd group (case group): 40 women who subsequently developed PE.

Following the guidelines of the International Society for the Study of Hypertension in Pregnancy, we defined PE thus: 2 separate measurements of BP greater than 140/90 mmHg taken at least 4 hours apart, and proteinuria concentration≥ 300mg in 24-hour period or, if 24-hour urine collection is unavailable, two readings with a value of at least 2+ on dipstick analysis of urine specimens taken midstream or through a catheter [15,19]. For this study, we classified as normal any pregnancy in which the woman had normal blood pressure and delivered after GA of 37 + 0 weeks. The control samples were chosen as consecutive cases from the same time period as the cases who met the selection criteria.

All the women were informed about the nature of the study and anyone who did not agree to participate was excluded. Demographic data and medical information was recorded for each participant as well as vital signs including blood pressure, pulse, temperature, weight, general condition and chest cardiac examination. Additionally, each subject underwent a physical examination including abdominal obstetric ultrasound to confirm gestational age and normality of pregnancy and to rule out any complications which may result in hypertension. The following investigations were also carried out on each participant: complete blood count; complete urine analysis, serum BHCG, and random blood sugar.

Sample collection

Five ml of maternal venous blood samples were collected at a GA of 11-16 weeks under complete aseptic technique for analysis of total Hb and HbF. The venous blood was in Vacutainer tubes, without additives to allow clotting and centrifuged at 2000 g at room temperature for 10 minutes. The serum was stored at -20c after the careful labeling till the time of analysis. HbF was measured with a sandwich enzyme linked immunosorbent assay (ELISA) using kits from Glory Science Co., Ltd with cat number MBS702332. The sensitivity was 5 ng/mL, with an inter-assay coefficient of variation (CV) of <7.99% and an intra-assay CV of <2.22%. The total Hb concentration in serum was measured by a competitive enzyme linked immunosorbent assay using antibodies against adult Hb. The sensitivity was 40 ng/mL, with an inter-assay CV of <5.5% and an intra-assay CV of <2.04%.

Statistical Analysis

Data were analyzed using SPSS for Windows version 17.0 (SPSS, Chicago, IL. USA). Continuous data were presented as mean values ± standard deviation (SD) or median (range) as appropriate. For categorical variables, percentages were calculated. Characteristics of the control group and the PE group were compared using the unpaired T-test. Results were considered statistically significant if two-sided P values were less than 0.05.

3. Results

Eighty women were included in this study, 40 of whom were preeclamptic (study group) and 40 of whom were normotensive (control). Out of the 40 preeclamptic women, 36 women (40%) and 4 women (10%) developed mild and severe preeclampsia, respectively.

Table1: Percentage of control and preeclampsia cases:

	N (%)
Control	40 (50)
Preeclamptic	40 (50)
Mild preeclamsia	36 (90)
Severe preeclamsia	4 (10)

As shown in Table 2, women in the study group had a mean age of 25.4±4.313 years, whereas those in the control group had a mean age of 29.28±6.552 years (P<0.05). The two groups differed significantly in terms of maternal age, with the preeclamptic women being significantly younger than those in the control group.

As illustrated in Table 3, the mean GA at sampling of the control group was 14.365±0.6562 weeks (wks), while the preeclamptic group was 14.533±0.6036 wks (P>0.05). At the time of sampling, the two groups did not differ significantly with respect to GA. However, GA at delivery did show a statistically significant difference between the study and control groups. At the time of delivery, the mean GA of the control group was 38.9±0.7107 wks, whereas the mean GA of the preeclamptic group was 37.895±0.4145wks (P<0.05).

Table 2: Characteristic of maternal age in the two groups

	Control (n=40) Mean ±SD	**Study (n=40)** Mean ±SD	***p value***
Age (years)	29.3 ± 6.552	25.4± 4.313	0.003*

** Significant difference at p<0.05*

Table 3: Characteristics of GA at sampling and delivery in the two groups

		Control (n=40)	**Study (n=40)**	**Independent test**		**Sig.**
				T	**P**	
GA sampling (week)	Mean ±SD	14.365 ± 0.6562	14.533 ± 0.6036	-1.188	0.23	NS
GA delivery (week)	Mean ±SD	38.9 ± 0.7107	37.895 ± 0.4145	7.726	0.012*	S

** Significant p<0.05*

The mean SBP at sampling in the control and preeclamptic group was 104.25± 6.25833mmHg and 107±7.90975mmHg; respectively. This difference in sampling SBP did not reach statistical significant difference. The mean DBP at sampling in the control and preeclamptic group was 68.5±6.62164mmHg and 72±4.64095mmHg; respectively. Significant differences existed between the two groups with respect to sampling DBP (P<0.05).

Table 4: Characteristics of SBP and DBP at sampling time of the two groups

		Control (n=40)	**Study (n=40)**	**Independent test**		**Sig.**
				T	**P**	
SBP sampling (mmHg)	Mean ±SD	104.25 ± 6.25833	107 ± 7.90975	–1.724	0.089	NS
DBP sampling (mmHg)	Mean ±SD	68.5 ±6.62164	72 ± 4.64095	–2.738	0.008*	S

** Significant p<0.05*

The mean SBP at diagnosing time in the control and preeclamptic group was 107.75± 6.975mmHg and 148.125±6.951mmHg ; respectively. The mean DBP at diagnosing in the control and preeclamptic group was 70.75±5.256mmHg and 94.25 ± 6.751mmHg ; respectively. Significant differences existed between the two groups with respect to SBP and DBP at the diagnosing time in the two groups (P<0.05).

Table 5: Characteristics of SBP and DBP at diagnosing time of the two groups

		Control (n=40)	**Study (n=40)**	**Independent test**		**Sig.**
				T	**P**	
SBP diagnosis (mmHg)	Mean ±SD	107.75 ± 6.97523	148.125 ± 6.9512	–25.931	0.001*	S
DBP diagnosis (mmHg)	Mean ±SD	70.75 ± 5.25625	94.25 ± 6.75107	–17.371	0.006*	S

** Significant p<0.05*

The concentration of total Hb and HbF were analyzed in all plasma samples from women with the controls and the study (Table 6). No significant difference existed between the two groups with respect to Hb-T (P>0.05), while a significant increase of the HbF concentration was seen in the study group as compared to the controls (P<0.05).

On performing a Spearman correlation test for existing correlation between HbF and the studied variables (maternal age, GA at time of sampling or delivery, SBP and DBP at time of sampling and diagnosing with PE), no significant correlation between HbF and any of the studied variables was found in the control (normotensive) group (P>0.05).

Table 6: Characteristics of Hb-T and HbF of the two groups

		Control (n=40)	Study (n=40)	Independent test		Sig.
				t	p	
Hb-T (gm/dL)	Mean ±SD	10.383 ± 0.7327	10.34 ± 0.8205	0.244	0.808	NS
HbF (ng/ml)	Mean ±SD	83.887 ± 31.625	387.763 ± 146.035	–12.862	0.001*	S

** Significant p<0.05*

When subdividing the PE group into mild and severe PE, a statistically significant increase in HbF concentration was observed in women with severe PE as compared to mild PE (P<0.05) (Table 7).

Table 7: Comparison between mild and severe PE regarding HbF

HbF	Mild preeclampsia	Severe preeclampsia	Independent t-test	
	No. = 36	No. = 4	t	P-value
Mean ± SD	346.55 ± 74.02	758.67 ± 97.50	10.270	0.001*
Range	220.76 – 572.88	694.44– 902.97		

**Significant p <0.05*

Table 8: Association between HbF and all the studied variables among the control group

	r	p	Sig.
Age (year)	– 0.001	0.996	NS
GA sampling (week)	0.061	0.707	NS
GA delivery (week)	– 0.131	.421	NS
SBP sampling (mmHg)	0.009	0.955	NS
DBP sampling (mmHg)	0.235	0.144	NS
SBP Diag (mmHg)	0.106	0.516	NS
DBP Diag (mmHg)	– 0.174	0.282	NS
HB (g/dL)	0.188	0.246	NS

**Significant p <0.05*

On the other hand, a statistically significant direct correlation between HbF and BP (both systolic and diastolic) at diagnosing of PE was observed (P<0.05).

Table 9: Association between HbF and all studied variables among the preeclamptic group.

	r	p	Sig.
Age (year)	0.075	0.644	NS
GA sampling (week)	0.277	0.084	NS
GA delivery (week)	0.048	0.767	NS
SBP sampling (mmHg)	– 0.019	0.909	NS
DBP sampling (mmHg)	0.092	0.573	NS
SBP Diag (mmHg)	0.639	0.001*	S
DBP Diag (mmHg)	0.692	0.008*	S
HB (g/dL)	0.173	0.284	NS

**Significant p <0.05.*

4. Discussion

Preeclampsia is the third most common cause of mortality in pregnant women and detecting it early is important in diagnostic medical research. To that end, the current study investigated the effectiveness of measuring maternal Hb and HbF in the first and early second trimester of pregnancy as a predictor of PE. Research has shown the PE risk assessment carried out at antenatal care clinics can play a valuable role in predicting the condition [17]. To be effective, PE screening should be simple, safe, inexpensive, fast, and reproducible. Screening tests should also allow the possibility of intervention, ideally to avoid the development of PE completely, or at least reduce the risk of serious morbidity and mortality [14].

Since PE is such a serious condition affecting pregnant women, a great deal of research has focused on investigating the contributing factors for PE, which include advanced maternal age, multiple pregnancies and chronic hypertension [20,21]. In the present study,

a significant difference in maternal age between normal pregnant and preeclamptic women existed. The mean maternal age of the preeclamptic group was significantly lower than that of the control group. Previous studies found that the incidence of PE was high in women aged 20-29 years [21,22, 23]. In their study of pregnant women in Riyadh, Saudi Arabia, Siddiqui et al. reported an average age of preeclamptic women of 29.0± 6.1 years [23]. Similarly, Hassan et al. reported an average age of 28.6 ± 7.4 years inpreeclamptic women in Abha, Saudi Arabia [21].

In the present study, the investigations for maternal Hb and HbF concentration levels fulfilled the criteria for effective screening tests for PE, as mentioned above [14]. The difference between maternal Hb levels of normal pregnant women and those of preeclamptic women was not statistically significant. There is research to suggest preeclamptic women have higher Hb concentrations than their normal counterparts [24]. Some studies investigating the measurement of maternal Hb as a screening tool for PE reported a significant difference between mean Hb levels in preeclamptic and non-PE women in the first trimester [18,25,26]. However, several other studies found no significant differences between Hb concentrations in preeclamptic women and those with normal pregnancies [21, 27,28]. In terms of maternal Hb levels, the findings of our study are in line with results reported by Makuyana et al. [27], who measured hematological parameters including Hb levels in 38 preeclamptic women and 72 women with normal pregnancies and found no significant differences between the two groups. Likewise, Hershkovitz et al. [28] found no significant differences in Hb levels of the 21 preeclamptic and 19 normal pregnant women in their study. Similarly, in their study, Monteiro et al. found comparable Hb levels in both preeclamptic and normotensive women [29]. Anderson et al., also reported no significant difference in Hb levels between the study group (n=60) and the control group (n=36), although both groups of women had a high concentration of Hb [15].

The fetal hemoglobin molecule is highly reactive. It can damage cell membranes and lead to vasoconstriction by binding and inactivating nitric oxide [30].The elevated levels of HbF found in the blood of women with preeclampsia highlight the potential of HbF as a predictor of PE [31]. In our study, the mean serum HbF level was significantly higher in women in the PE group (387.76±146.03) as compared to the control group (83.887 ± 31.625), and as the severity of PE increased, HbF levels increased significantly, implying that the level of HbF in maternal circulation plays an important role in prediction of this disorder. Our results are in accordance with previous reports about HbFandPE. In their 2010 study, Olsson et al. [32] found an association between PE and maternal plasma levels of HbF. Levels of HbF were nine times higher in pre-eclamptic women as compared to normal control women. In addition, a strong positive correlation was found between fetal hemoglobin and the severity of the disease. In our study, there was a 5-fold increase of fetal hemoglobin in preeclamptic cases as compared to normal uncomplicated control women. Similarly, Anderson et al. reported significantly higher mean HbF levels in the PE group compared to controls [15]. Our findings, then, are in line with those of previous studies.

Measuring BP is a routine part of antenatal care, and hypertension is the main characteristic of PE and its related symptoms. In the present study, BP measurements taken at sampling time showed higher SBP and DBP in women who later developed PE compared to women with normal pregnancies; however, the difference reached significance only with DBP. Sibai et al. reported an association between elevated SBP and DBP measurements at first antenatal visit and a higher incidence of PE [33].

At the time of PE diagnosis, significant differences between the study and control groups were observed for both SBP and DBP values, which is consistent with the findings of previous studies. Siddiqui et al. (2011) reported that preeclamptic patients had higher mean SBP and DBP values than their counterparts in the control group [23]. In their meta-analysis, Cnossen et al. (2008) examined 34 studies on a total of 60,599 women, 3,341 of whom had PE [34]. They focused on the diastolic average, not on the systolic pressure. Their analysis suggested a predictive relevance for diastolic blood pressure over 75mmHg in the period of 13-20 weeks of gestation [34]. In the study done by Nijdam et al. on 2,334 pregnant women during their antenatal visit prior to 16 weeks of gestation, they found that SBP and DBP were the main predictors for pregnancy-induced hypertension [35]. Similarly, Reiss et al. reported significantly higher SBP and DBP in the first trimester for women with subsequent PE [36].

Some research has indicated an association between higher SBP and DBP and increasing Hb levels [37]. In the present study, we found a positive correlation between SBP and DBP and Hb Flevelsin women with PE. Correlation analysis between HbF and other variables studied indicated a significant positive association between HbF and blood pressure. The higher the blood pressure, either systolic or diastolic, the higher the level of serum fetal Hb in maternal blood. Likewise, Anderson et al. (2015) [31] suggested that HbF levels in combination with blood pressure measurements could play a role in determining PE severity in term pregnancies. On the

other hand, we observed no significant difference between fetal hemoglobin levels with regard to maternal and gestational age, results which are consistent with previous research [15,38,39]. However, other studies reported that as gestational age increased, HbF levels decreased [40]. As expected, this study found a significant difference in gestational length at the time of delivery, with shorter gestation in the preeclamptic women than in the women with normal pregnancies, a finding consistent with previous studies [15,41].

Recommendations:

Based on our findings, we recommend measuring maternal HbFin the first trimester of pregnancy as a screening test to diagnose or predict preeclampsia. More studies on the effectiveness of HbF and other biomarkers in the prediction of preeclampsia should be carried out to improve screening for this serious condition.

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References

1. Carty DM, Delles C, Dominiczak AF. Novel Biomarkers for Predicting Preeclampsia. Trends in Cardiovascular Medicine. 2008;18(5-24):186-194.
2. Redman, C. W. (2011). Hypertension in pregnancy: the NICE guidelines. Heart 97, 1967–1969.
3. Davison JM, Homuth V, Jeyabalan A, Conrad KP, Karumanchi SA, Quaggin S, Dechend R, Luft FC. New aspects in the pathophysiology of Preeclampsia. J Am Soc Nephrol. 2004;15(9):2440–2448.
4. Backes CH, Markham K, Moorehead P, Cordero L, Nankervis CA, Giannone PJ. Maternal Preeclampsia and Neonatal Outcomes. Journal of Pregnancy. 2011;2011:214365.
5. Berg, C. J., Mackay, A. P., Qin, C., and Callaghan, W. M. (2009). Overview of maternal morbidity during hospitalization for labor and delivery in the United States: 1993-1997 and 2001-2005. Obstet. Gynecol. 113, 1075–1081.
6. Osungbade KO, Ige OK. Public health perspectives of preeclampsia in developing countries: implication for health system strengthening. J Pregnancy. 2011;2011:481095.
7. Wagner LK. Diagnosis and management of preeclampsia. American Family Physician. 2004;70(12):2317-2324.
8. Duckitt K, Harrington D. Risk factors for preeclampsia at antenatal booking: systematic review of controlled studies. British Medical Journal. 2005; 330(7491):565.
9. Anderson UD, Olsson MG, Kristensen KH, Akerstorm B, Hansson SR. Review: biochemical markers to predict preeclampsia. Placenta. 2012;33: S42-47.
10. Conde-Agudelo A, Villar J, Lindheimer M. World health organization systematic review of screening tests for preeclamsia. Obstetrics Gynecol. 2004;104(6):1367-1391.
11. Lindheimer MD, Umans JG. Explaining and predicting preeclampsia. The New England Journal of Medicine. 2006; 355(10):1056-1058.
12. Deis S, Rouzier R, Kayem G, Masson C, Haddad B. Development of a nomogram to predict occurrence of preeclamsia. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2008;137(2):146-151.
13. Phaloprakarn C, Tangjitgamol S. Impact of high maternal hemoglobin at first antenatal visit on pregnancy outcomes: a cohort study. *J Perinat Med.* 2008;36(2):115-9.
14. Delic R, Stefanovic M. Optimal laboratory panel for predicting preeclampsia. Journal of Maternal Fetal & Neonatal Medicine. 2010;23(1):96-102.
15. Anderson UD, Olsson MG, Rutardottir S, Centlow M, Kristensen KH, Isberg PE, Thilaganathan B, Akerstrom B, Hansson SR. Fetal hemoglobin and alpha 1-microglubulin as first- and early second-trimester predictive biomarkers for preeclampsia. Am J Obste. 2011; 204(6):520.e l-5.
16. Hansson SR, Gram M, Akerstrom B. Fetal hemoglobin in preeclampsia: a new causative factor, a tool for prediction/diagnosis and a potential target for therapy. Current opinion in obstetrics & gynecology, 2013; 25(6):448-455.
17. Nasiri M, Faghihzadeh S, Alavi Majd H, Zayeri F, Kariman N, Safavi Ardebili N. Longitudinal Discriminant Analysis of Hemoglobin Level for Predicting Preeclampsia. Iranian Red Crescent Medical Journal. 2015;17(3):e19489.
18. Pakniat H, Movahed F, Bahman A, Azoor M. The prediction of preeclampsia and Its association with hemoglobin and hematocrit in the first trimester of pregnancy. Biotechnology and Health Sciences. 2016; 3(3): e36810.
19. Tranquilli AL, Brown MA, Zeeman GG, Dekker G, Sibai BM. The definition of severe and early-onset preeclampsia. Statements from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Pregnancy Hypertens. 2013; 3(1):44-7.
20. Cunningham FG, Leveno KJ, Bloom SL, Hanth JC, Gilstrap LC, Wenstrom KD. Williams Obstetrics.

22nd Edition, McGraw-Hill Professional, New York, 2005,762-763.
21. Hassan B, Almushait M, Mubahar H, Zia S. The role of risk assessment at antenatal care clinics in the prediction of preeclampsia in a high altitude area. International Journal of Clinical Medicine. 2016; 7:10-15.
22. Sobande A, Eskandar M, Bahar A, Abusham A. Severe preeclampsia and eclampsia in Abha, the south west region of Saudi Arabia. Journal of Obstetrics and Gynaecology. 2007;27(2):150-154.
23. Siddiqui IA, Jaleel A, Kadri HM, Saeed WA, Tamimi W. Iron status parameters in preeclamptic women. Archives of Gynecology and Obstetrics. 2011;284(3):587-591.
24. Gupta S, Nanda S, Singh U, Bansal S, Lal H. Evaluation of changes in serum iron levels in pre-eclampsia. Indian J Clin Biochem. 1997; 12(1):91–94
25. Alavi Majd H. The relationship between hemoglobin and hematocrit in the first trimester of pregnancy and preeclampsia. Arak Medical Uni J. 2011;14(4):1-9.
26. Ali E. Early pregnancy hemoglobin levels as prediction of preeclampsia. Inter J Advanced Research (IJAR).2016; 4(10):16-19.
27. 18. Makuyana D, Mahomed K, Shukusho FD, Majoko F. Liverand kidney function tests in normal and pre-eclamptic gestation—a comparison with non-gestational reference values. Cent Afr J Med. 2002; 48(5–6):55–59.
28. 19. Hershkovitz R, Ohel I, Sheizaf B, Nathan I, Erez O, Sheiner E, et al. Erythropoietin concentration among patients with and without preeclampsia. Arch Gynecol Obstet. 2005; 273(3):140–143.
29. Gearaldine M, Subbalakshmi N.K, Pai SR. Relevance of measurement of hematological parameters in subjects with pregnancy induced hypertension. NUJHS. 2014; 4(1):. ISSN 2249-7110.
30. Murji A, Sobel ML, Hasan L, McLeod A, Waye JS, Semer M, Berger H. Pregnancy outcomes in women with elevated levels of fetal hemoglobin. J Matern Fetal Med. 2012;25(2):125-129.
31. Anderson UD, Gram M, Thilaganathan B, Kerstrom B, Hansson SR. Free fetal hemoglobin and hemoglobin-scavenging proteins are predictive first and second trimester biochemical markers for pre-eclampsia. Pregnancy Hypertens. 2015;5:53.
32. Olsson MG, Centlow M, Rutardottir S, Stenfors I, Larsson J, Hosseini-Maaf B, Olsson ML, Hansson SR, Akerstrom B. Increased levels of cell-free hemoglobin, oxidation markers, and the antioxidativeheme scavenger alpha (1)-microglobulin in preeclampsia. Free Radic Biol Med. 2010;48(2):284–291.
33. Sibai BM, Gordon T, Thom E, Caritis SN, Klebanoff M, McNellis D, et al., National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. Risk factors for preeclampsia in healthy nulliparous women: A prospective multicenter study. American Journal of Obstetrics and Gynecology. 1995;172(2):642-648.
34. Cnossen JS, Vollebergt C, De Vrieze N, et al. Accuracy of mean arterial pressure and blood pressure measurements in predicting preeclampsia: systematic review and meta-analysis. BMJ. 2008;336:1117-1120.
35. Nijdam ME, Janssen KJ, Moons KG, Grobbee DE, van der Post JA, Bots ML, Franx A. Prediction model for hypertension in pregnancy in nulliparouswomen using information obtained at the first antenatal visit. J Hypertens. 2010 Jan;28(1):119-26.
36. Reiss RE, O'Shaughnessy RW, Quilligan TJ, Zuspan FP. Retrospective comparison of blood pressure course during preeclamptic and matched control pregnancies. Am J Obstet Gynecol 1987; 156:894–8.
37. Cunningham GF. Severe Preeclampsia and Eclampsia: Systolic Hypertension Is Also Important. Obstetrics & Gynecology. 2005; 105(2): 237-238.
38. Atsma F, Veldhuizen I, de Kort W, van Kraaij M, Pasker-de Jong P, Deinum J, Hemoglobin level is positively associated with blood pressure in a large cohort of healthy individuals. Hypertension. 2012; 60(4):936-941.
39. Shamsi U, Hatcher J, Shamsi A, Zuberi N, Qadri Z, Saleem S. A multicentre matched case control study of risk factors for preeclampsia in healthy women in Pakistan. BMC Womens Health. 2010; 10:14.
40. Ganesh KS, Unnikrishnan B, Nagaraj K, Jayaram S. Determinants of pre-eclampsia: a case-control study in a District Hospital in South India. Indian J Community Med. 2011; 35:502–5.
41. Shyang-Yun Pamela K, Ching-Nan Ou. Accurate Measurements of Fetal Hemoglobin for Neonates with Different Gestational Ages. Int J hemoglobin Res. 2006; 30(4):419-435.

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